TRANSGEN BIOTECH

2015-2016 | PRODUCT CATALOG

Preface

TransGen Biotech, Inc. is a researcher, developer, manufacturer and distributor of more than 200 molecular and cellular biology products and kits for life science research and molecular diagnostics. In 2001, the company was founded by three scientists with a mission to produce innovative and cost-effective products for life science research.

In March 21, 2006, TransGen Biotech was incorporated in Beijing, China. The company's headquarters, R&D, and manufacturing facility are located in Beijing. To date, the company has more than 200 scientists in Beijing, and has more than 30 distribution centers covering all major cities in China. Our extensive R&D experience and state-of-the-art facilities enable us to keep generating the most innovative and the highest quality products. Since 2006, the company was consecutively awarded as one of the "High Tech Corporation in Beijing" by Beijing local government.

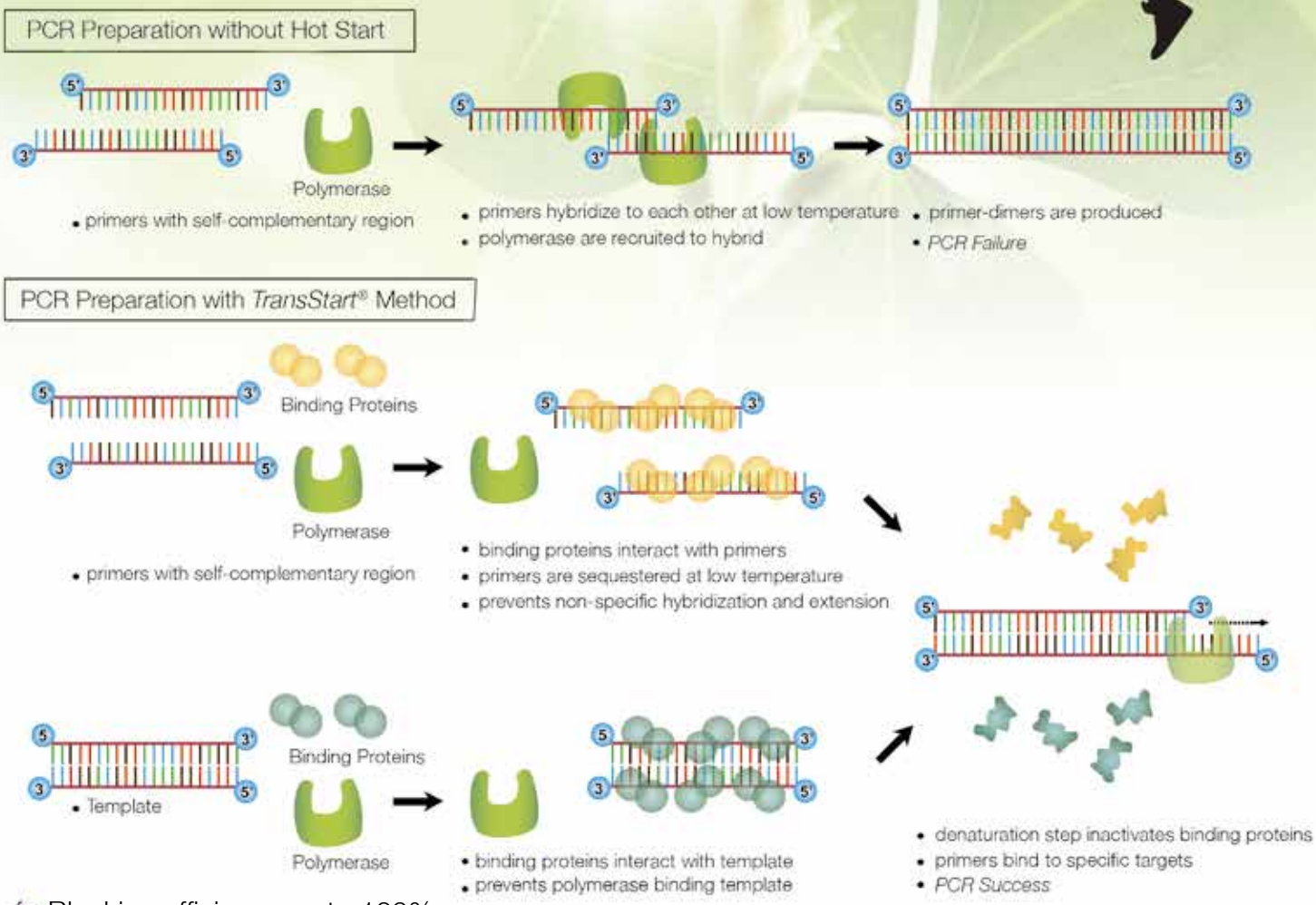
Currently, our products cover: plasmid based DNA markers, high efficiency chemically competent cells, 5 minutes PCR product cloning and expression vectors, a variety of PCR enzymes and SuperMixes, RNase H deficient and high temperature RT enzymes, qPCR and qRT-PCR SuperMixes, 5 minutes fast restriction enzymes the highest efficiency mutagenesis kits, high quality nucleic acid extraction and purification kits, unstained and prestained protein markers, Western blot markers, and protein purification resins, cell culture and transfection reagents, antibodies.

As the leading bioreagent company in China, we are looking forward to partner with you in your quest for ground-breaking life science discoveries.

CEO: 

Certain products will not be sold in some countries. Please contact TransGen for detailed information.

TransStart® Hot Start (Double Blocking)



- Blocking efficiency up to 100%.
- Different from *Taq* antibody blocking, risks of contamination from mammals DNA are avoided.
- Different from chemical modified blocking, long denaturing step is not needed.

- *TransStart® Taq* DNA Polymerase
- *TransStart® TopTaq* DNA Polymerase
- *TransStart® FastPfu* DNA Polymerase
- 2×*TransStart® FastPfu* PCR SuperMix
- *TransStart® FastPfu* Fly DNA Polymerase
- *TransStart® KD* Plus DNA Polymerase
- *TransStart®* Green qPCR SuperMix
- *TransStart®* Green qPCR SuperMix UDG
- *TransStart®* Top Green qPCR SuperMix
- *TransStart®* Tip Green qPCR SuperMix
- *TransStart®* Probe qPCR SuperMix

TransStart® FastPfu DNA Polymerase
TransStart® FastPfu Fly DNA Polymerase

Fast, high fidelity, hot start DNA polymerase

- ***Fast***
- ***Highest Fidelity***
- ***High Sensitivity***

Fast extension rate

TransStart® FastPfu DNA polymerase has an extension rate of up to 4 kb/min.

TransStart® FastPfu Fly DNA polymerase has an extension rate of up to 6 kb/min.

High fidelity

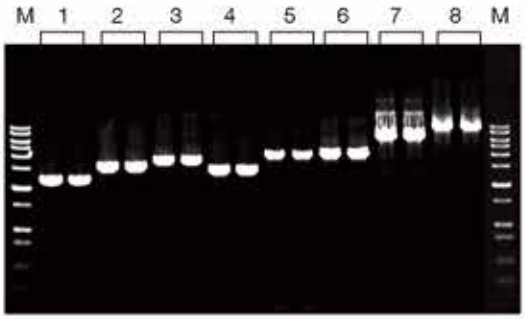
TransStart® FastPfu DNA Polymerase offers 54-fold fidelity as compared to *EasyTaq®* DNA Polymerase.

TransStart® FastPfu Fly DNA Polymerase offers 108-fold fidelity as compared to *EasyTaq®* DNA Polymerase.

Better amplification efficiency

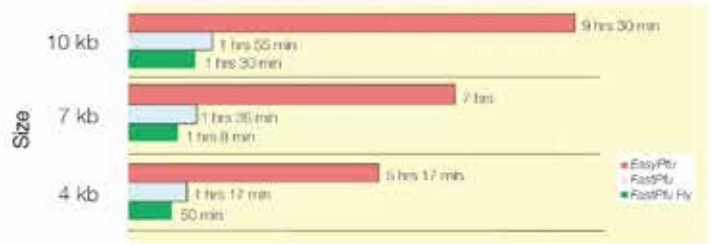
Suitable for long fragment or low copy gene amplification

Amplification using *TransStart® FastPfu* DNA Polymerase



M: 1 Kb Plus DNA Ladder

1: NCBP	2.5 kb	2 h 20 min
2: ACTR	3 kb	2 h 20 min
3: HDP	3.5 kb	2 h 20 min
4: β-globin	3 kb	1 h 27 min
5: Rhod	4.1 kb	1 h 27 min
6: β-globin	4.1 kb	1 h 27 min
7: UDG	7 kb	1 h 36 min
8: LN	10 kb	1 h 55 min



4 kb: Genomic DNA;

7 kb and 10 kb: Plasmid DNA

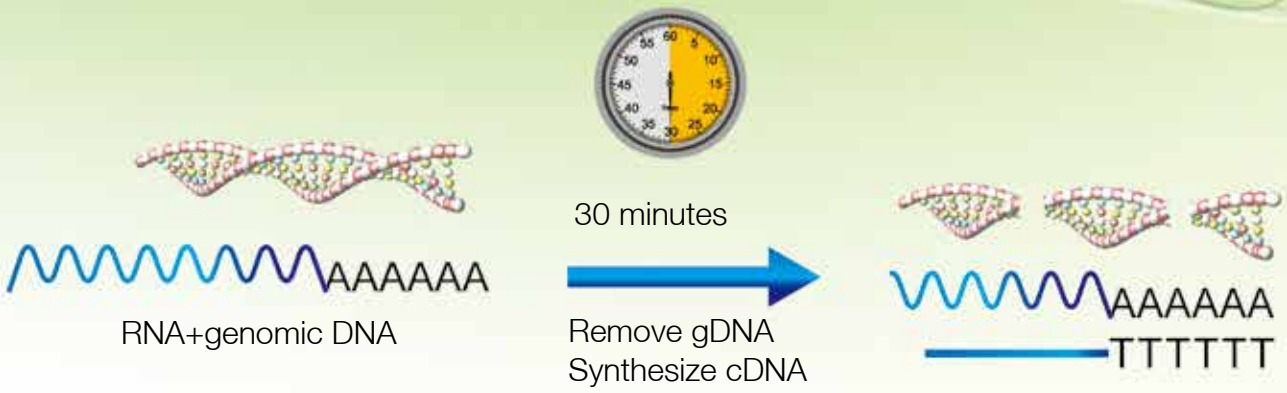
TransDirect® Blood PCR Kit

- High resistance to inhibitors and impurities.
- Direct PCR amplification using the whole blood or cell culture as template without DNA extraction.

TransScript® Fly First-Strand cDNA Synthesis SuperMix

- Fast: 5 minutes reverse transcription.
- cDNA up to 12 kb.

One-Step gDNA Removal and cDNA Synthesis SuperMix



- Simultaneous genomic DNA removal and cDNA synthesis.
- Easy to use SuperMix.

- *TransScript*® -Uni One-Step gDNA Removal and cDNA Synthesis SuperMix (42°C-65°C)
- *EasyScript*® One-Step gDNA Removal and cDNA Synthesis SuperMix (42°C)
- *TransScript*® One-Step gDNA Removal and cDNA Synthesis SuperMix (42°C)
- *TransScript*® II One-Step gDNA Removal and cDNA Synthesis SuperMix (42°C-55°C)

TransScript® -Uni Cell to cDNA Synthesis SuperMix for qPCR

- Resulting lysate without purification can be directly used for reverse transcription.
- Simultaneous genomic DNA removal and cDNA synthesis.
- Suitable for qPCR directly from cells.

Wash cells by PBS

C to C Lysis Buffer
5 minutes lysis at room temperature

Incubate at 75 °C for 5 minutes

Add RT/RI Mix and gDNA Remover
Reverse transcription for 30 minutes

cDNA

RT "All-in-One" SuperMix

Primers dNTPs

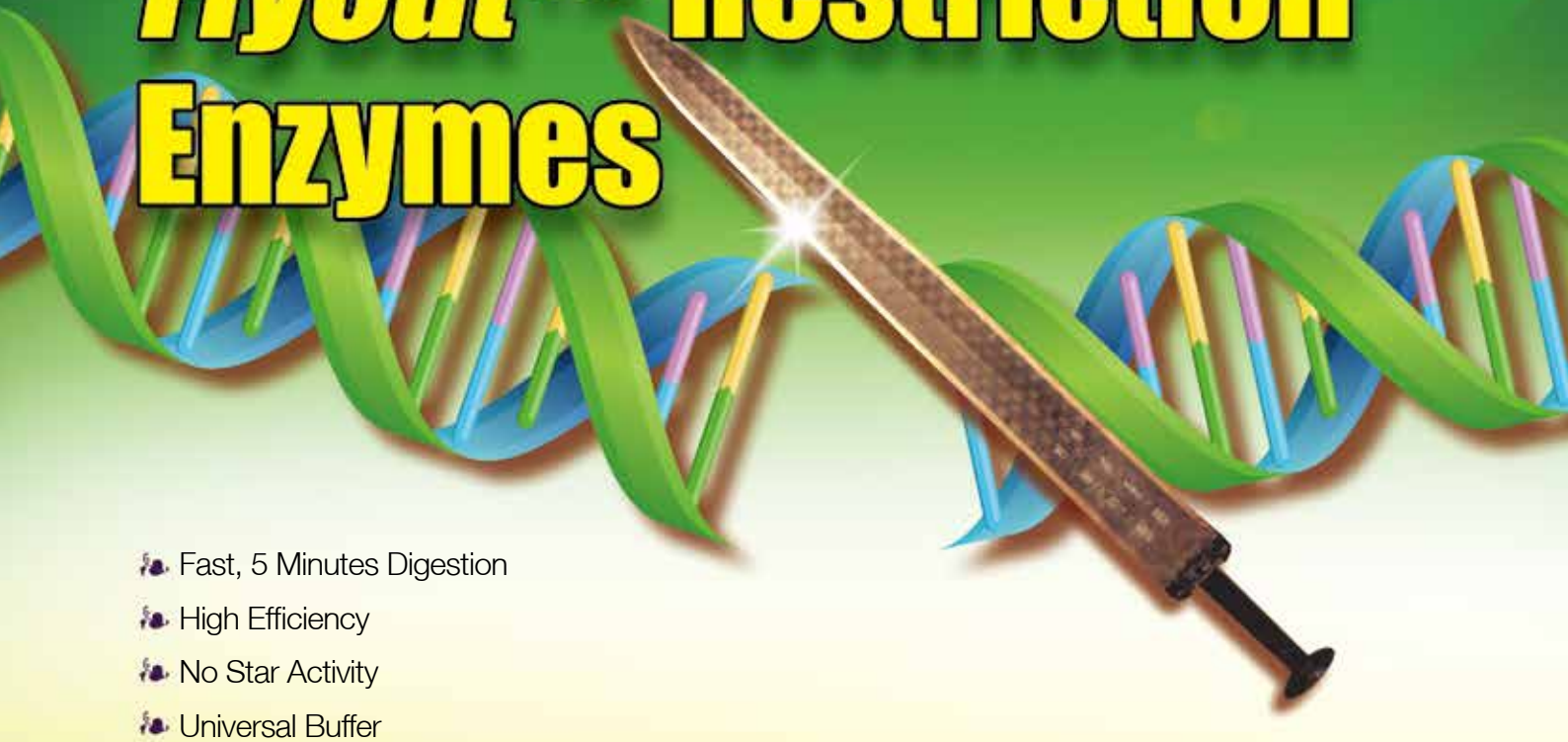
RI **All-Mix** RTase

Buffer

- Easy: All components (except RNA template) are premixed.
- Fast: 30 minutes RT reaction for PCR template; 15 minutes one step gDNA removal and RT reaction for qPCR template.

- *TransScript*® All-in-One First-Strand cDNA Synthesis SuperMix for qPCR (One-Step gDNA Removal)
- *TransScript*® II All-in-One First-Strand cDNA Synthesis SuperMix for qPCR (One-Step gDNA Removal)
- *TransScript*® All-in-One First-Strand cDNA Synthesis SuperMix for PCR
- *TransScript*® II All-in-One First-Strand cDNA Synthesis SuperMix for PCR

FlyCut™ Restriction Enzymes



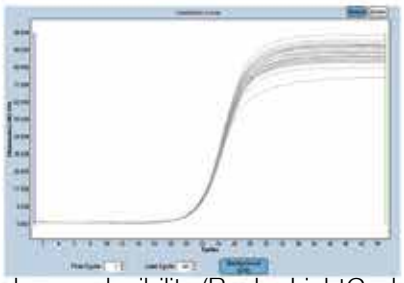
- Fast, 5 Minutes Digestion
- High Efficiency
- No Star Activity
- Universal Buffer

- *FlyCut*™ Avr II-HF
- *FlyCut*™ Bam HI-HF
- *FlyCut*™ Bgl II-HF
- *FlyCut*™ Bsg I-HF
- *FlyCut*™ Eag I-HF
- *FlyCut*™ EcoR I-HF
- *FlyCut*™ EcoR V-HF
- *FlyCut*™ Hind III-HF
- *FlyCut*™ Kpn I-HF
- *FlyCut*™ Nco I-HF
- *FlyCut*™ Nde I-HF
- *FlyCut*™ Nhe I-HF
- *FlyCut*™ Not I-HF
- *FlyCut*™ Pst I-HF
- *FlyCut*™ Pvu I-HF
- *FlyCut*™ Sac I-HF
- *FlyCut*™ Sac II-HF
- *FlyCut*™ Sal I-HF
- *FlyCut*™ Sca I-HF
- *FlyCut*™ Sma I-HF
- *FlyCut*™ Spe I-HF
- *FlyCut*™ Sph I-HF
- *FlyCut*™ Xba I-HF
- *FlyCut*™ Xho I-HF
- *FlyCut*™ Xma I-HF

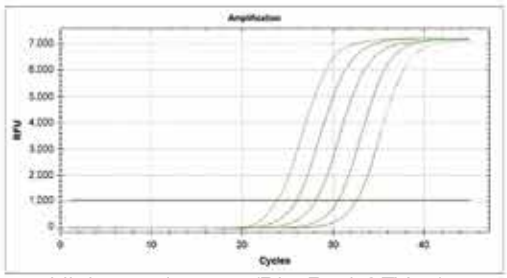
TransStart® Tip Green qPCR SuperMix

- ***High specificity***
- ***High sensitivity***

- A combination of chemical blocking technique and *TransStart*® hot start technique to achieve complete blocking. Compared with double blocking *TransStart*® *TopTaq*, this method provides higher sensitivity, and better amplification.
- Double cation (K^+, NH_4^+) buffer enhances specificity and reduces primer-dimers formation.
- Passive reference dyes for different qPCR instruments.

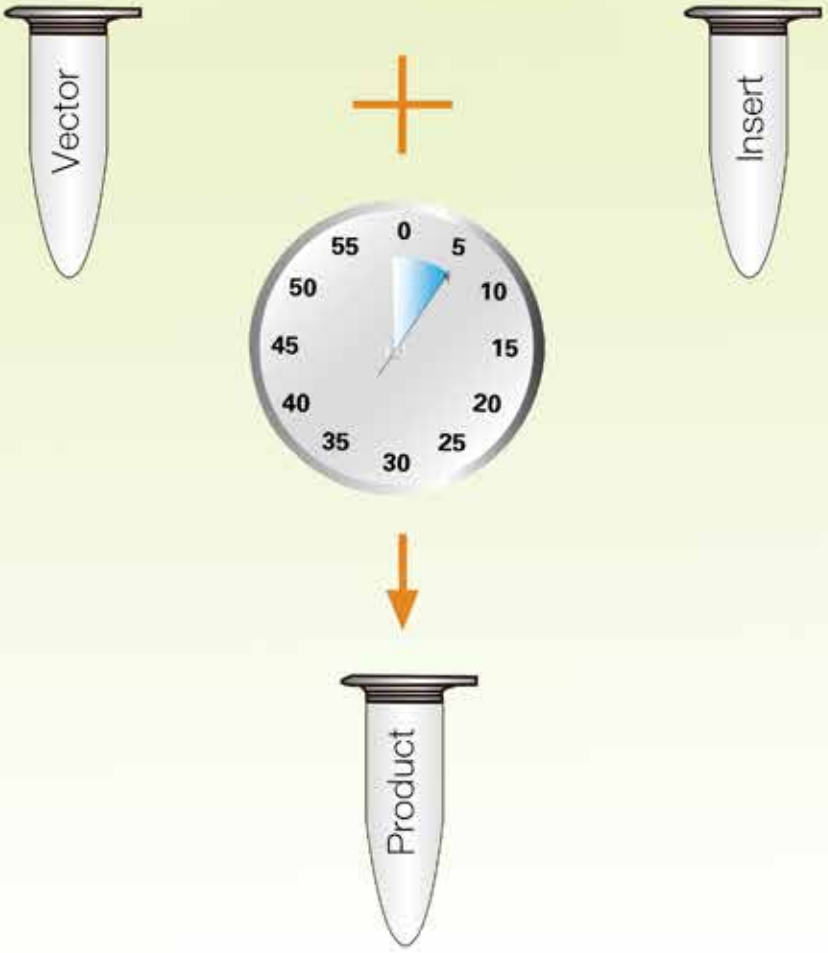


High reproducibility (Roche LightCycler480)



High consistency (Bio-Rad CFX96)

pEASY® Cloning
Room Temperature
5 Minutes Fast Cloning

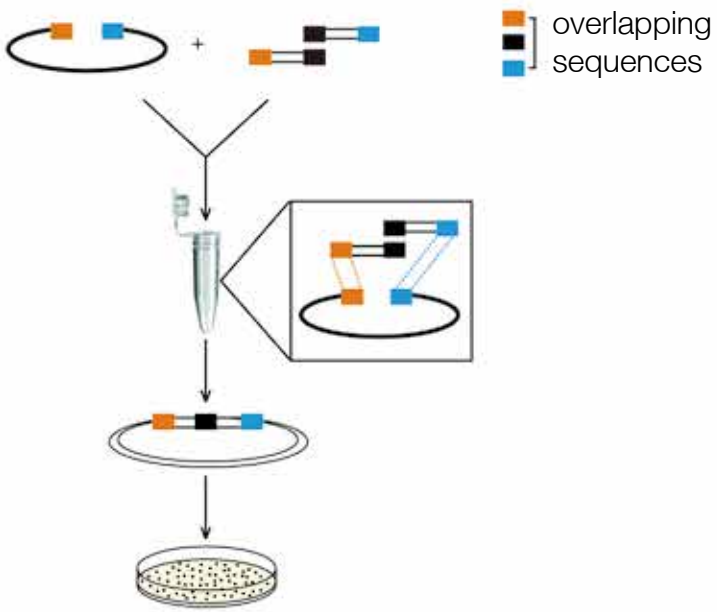


- *pEASY*®-T1 Cloning Kit
- *pEASY*®-Blunt Cloning Kit
- *pEASY*®-T1 Simple Cloning Kit
- *pEASY*®-Blunt Simple Cloning Kit
- *pEASY*®-T3 Cloning Kit
- *pEASY*®-Blunt3 Cloning Kit
- *pEASY*®-T5 Zero Cloning Kit
- *pEASY*®-Blunt Zero Cloning Kit
- *pEASY*®-Blunt E1 Expression Kit
- *pEASY*®-Blunt E2 Expression Kit
- *pEASY*®-Blunt M2 Expression Kit
- *pEASY*®-Blunt M3 Expression Kit

pEASY®-Uni Seamless Cloning and Assembly Kit

- **Fast:** 15 minutes.
- **Broad:** no restriction enzyme digestions. Can be cloned into any sites.
- **High efficiency:** up to 95% cloning efficiency.
- **Seamless:** no extra sequences introduced; up to 5 fragments assembly.

1. Prepare linearized vector by PCR/Enzyme digestion
2. PCR amplify inserts with 15-25 bp overlapping sequences
3. Mix vector, DNA fragments and Assembly Mix together, incubate at 50°C for 15 minutes
4. Transformation



Fast Mutagenesis System

High fidelity and fast amplification

2×*TransStart*® *FastPfu* PCR SuperMix improves the fidelity and shorts the amplification time.

Visible

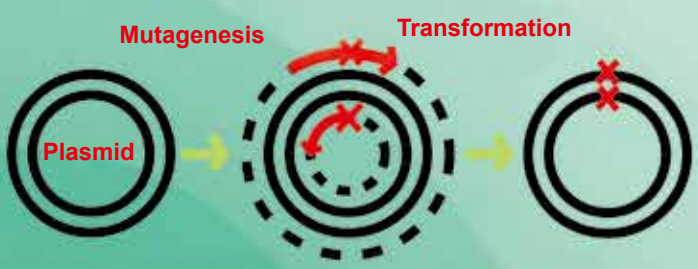
Amplification products can be visualized on agarose gel.

High efficiency

Both primers have the desired mutation sites providing higher mutation efficiency. DMT enzyme digests parental plasmids *in vitro* and DMT competent cell digests parental plasmids *in vivo* providing much lower background.

- ***Fast***
- ***Convenient***
- ***High Performance***

Fast Mutagenesis System



1

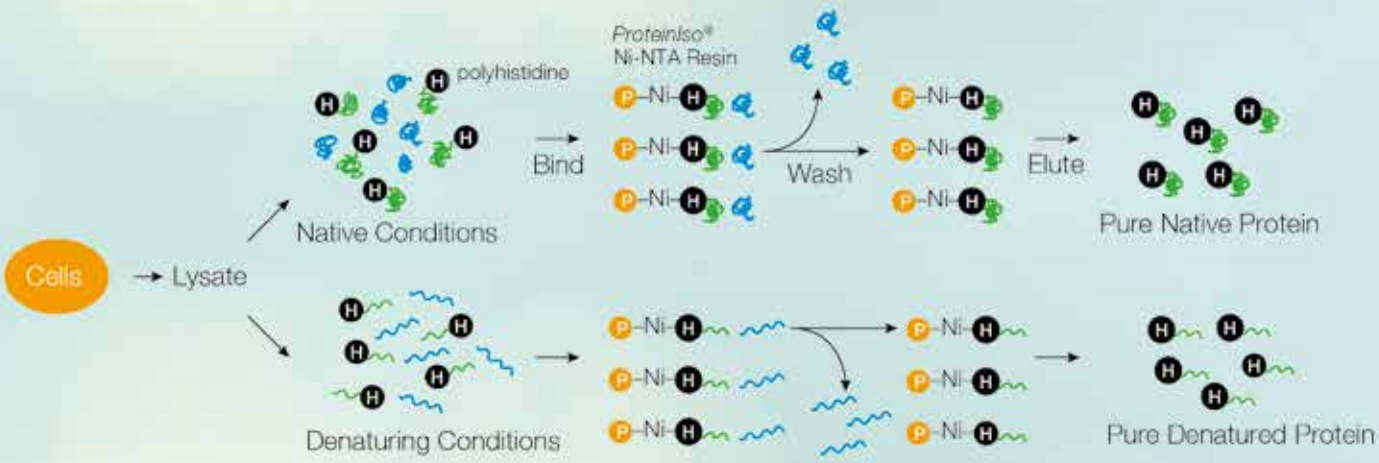
PCR amplification
Mutagenesis by PCR amplification with two overlapping primers. Both primers contain the target mutations.

2

Digestion of parental plasmid
DMT enzyme digests and DMT competent cell further digests parental plasmids.

X= mutation

ProteinIso® Ni-NTA Resin



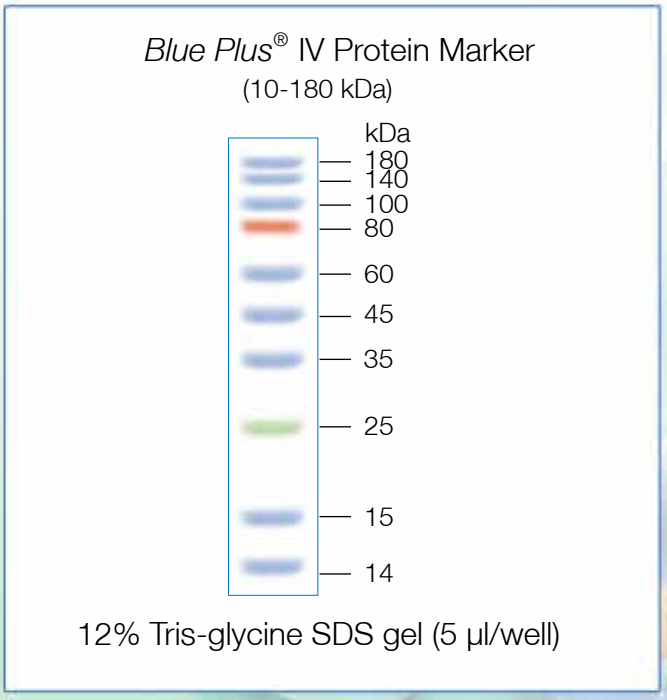
- High selectivity for high purity.
- Binding under denaturing and non-denaturing conditions.
- Easy to regenerate.

- ***High adsorption capacity***
- ***Good selectivity***
- ***Strong permeability***
- ***Easy regeneration***

Blue Plus® IV Protein Marker

Visible estimation of protein electrophoresis and membrane transfer efficiency

It is composed of prestained proteins from 10 kDa to 180 kDa. Different color bands are favorable to monitor electrophoresis and estimate membrane transfer efficiency.



Convenience

Ready-to-use format.

EasySee® Western Marker

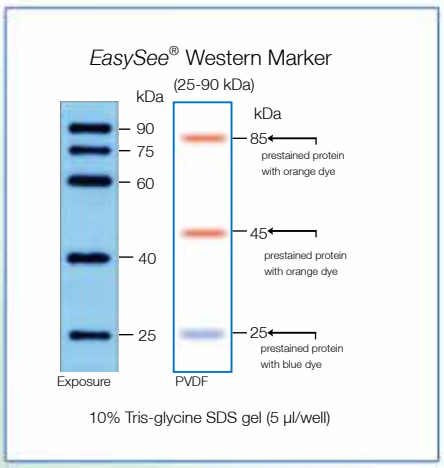
Visible estimation of protein electrophoresis and membrane transfer efficiency

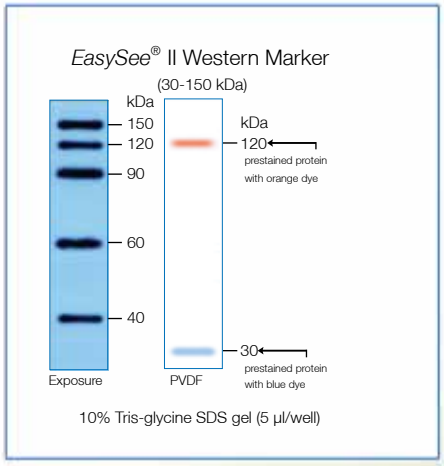
It is composed of prestained and unstained proteins from 25 kDa to 150 kDa. Different color bands are favorable to monitor electrophoresis, estimate membrane transfer efficiency and determine direction of membrane transfer.

- ***Visible Western Blot***
- ***High sensitivity***

Real visualization and accuracy

Bands from unstained proteins are visible by alkaline phosphatase and horseradish peroxidase chemiluminescence detection, providing more accurate molecular weight estimation than dye-detached protein markers.



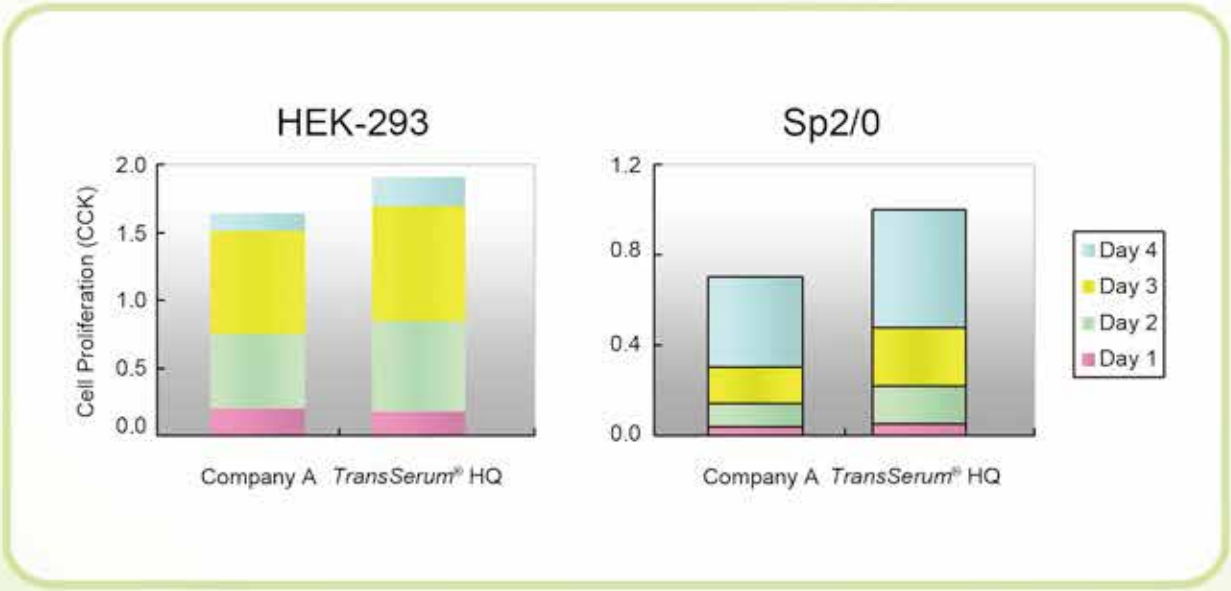


Convenience

Ready-to-use format.

TransSerum® HQ Fetal Bovine Serum

- Low toxicity
- Better cell growth
- Suitable for a broad range of cells

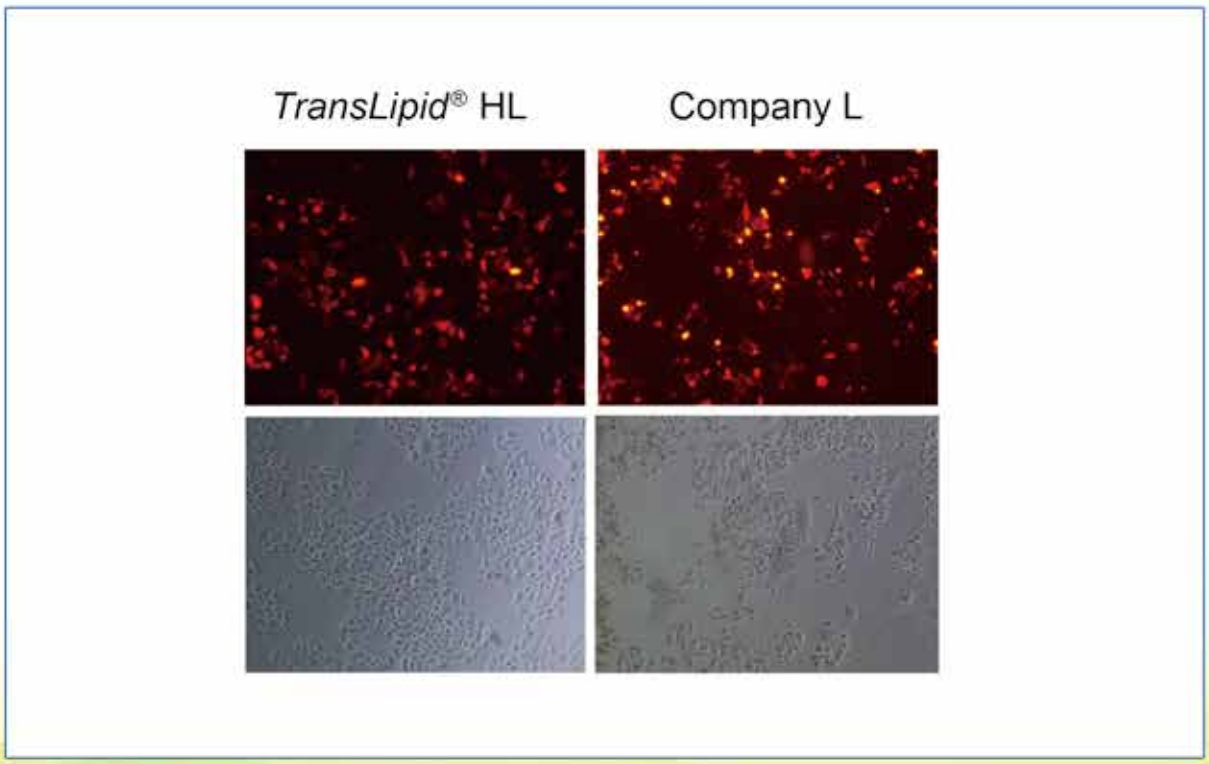


- ***Fast growth***
- ***Short doubling time***

TransLipid® HL Transfection Reagent

High efficiency and Low cytoxicity

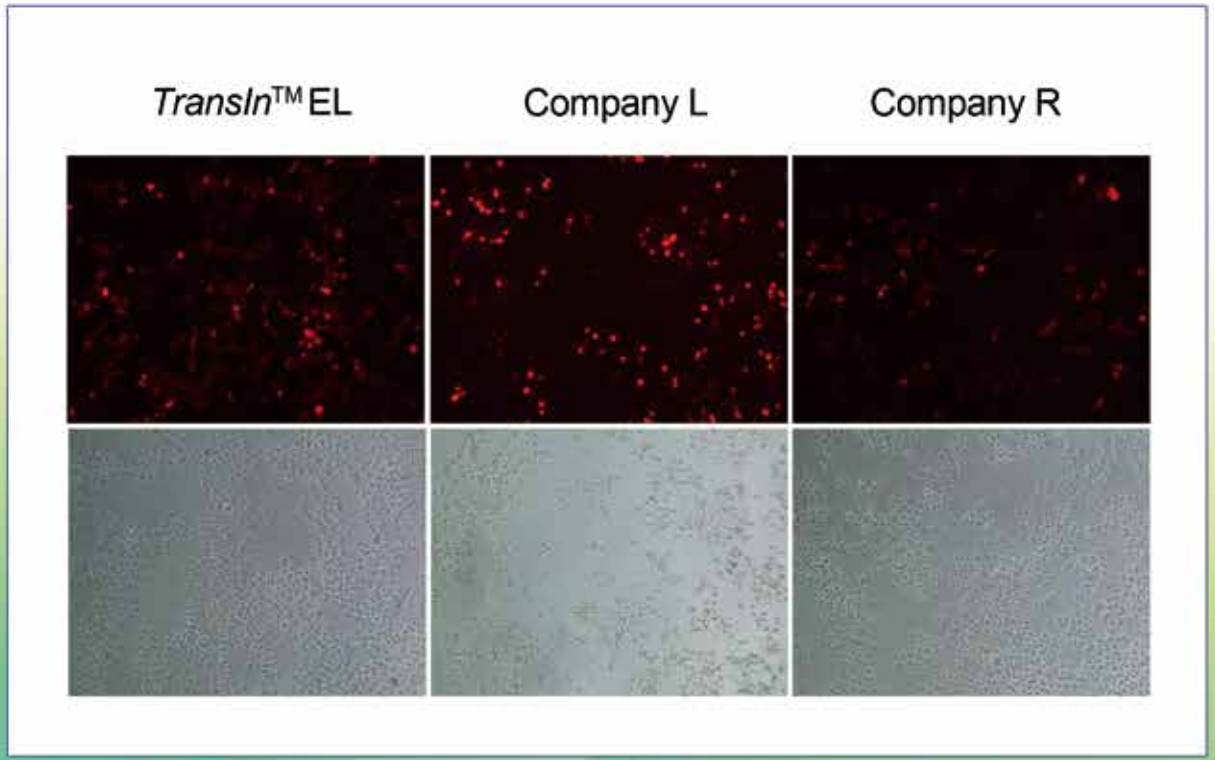
- Transfect DNA, RNA, siRNA.
- Adherent or suspension cells.
- Can be used in the presence of serum and antibiotics.



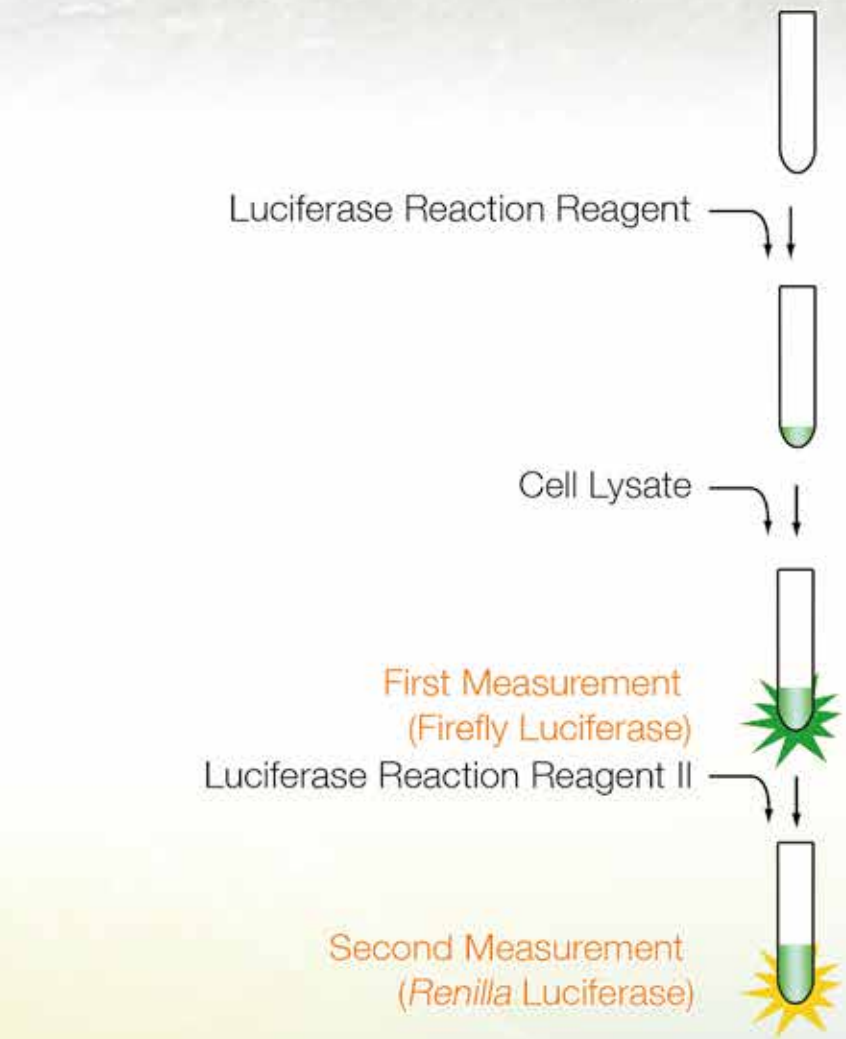
TransIn™ EL Transfection Reagent

High efficiency and Low cytoxicity

- Non-liposomal Transfection Reagent.
- Adherent or suspension cells.
- Can be used in the presence of serum and antibiotics.

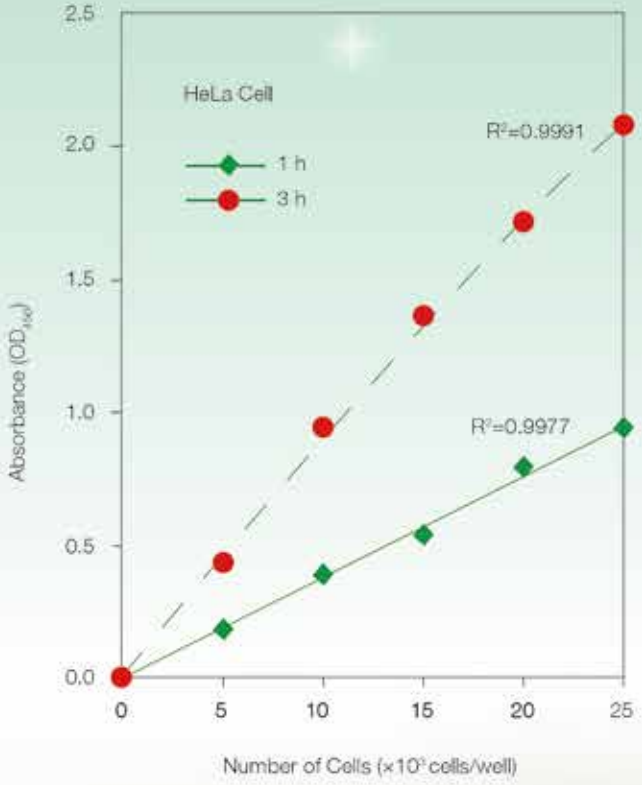


TransDetect® Double-Luciferase Reporter Assay Kit



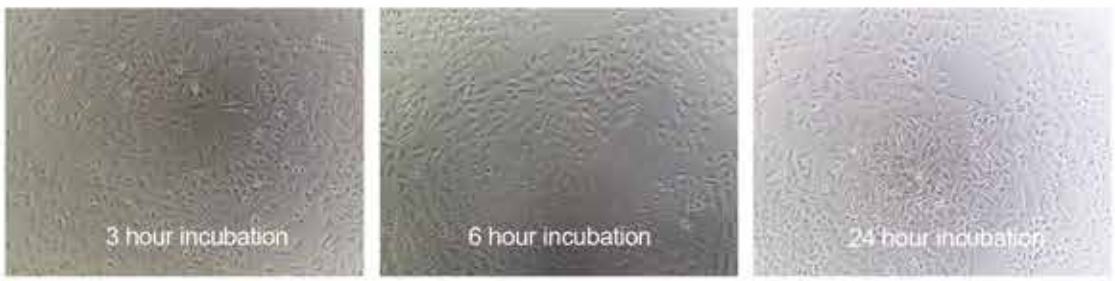
- ***Fast detection***
- ***High sensitivity***
- ***Broad detection range***
- ***No endogenous activity***

TransDetect® Cell Counting Kit (CCK)



Detection of CCK

- *Fast and sensitive*
- *Minimal cytotoxicity*
- *Broad linear range*
- *High reproducibility*



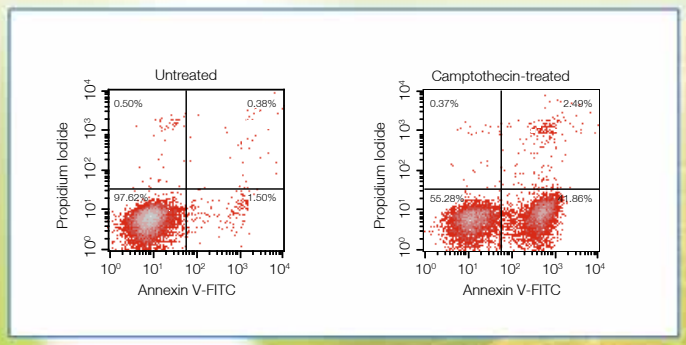
Low cytotoxicity

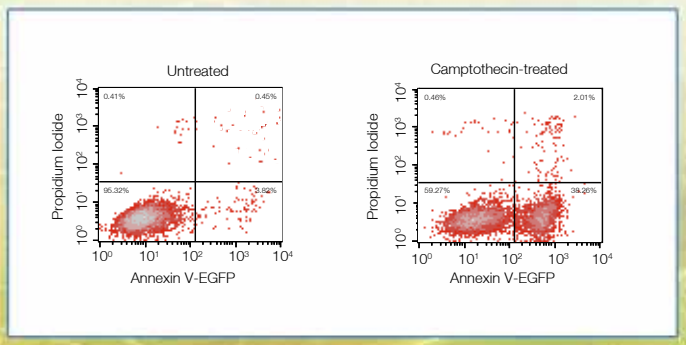
TransDetect® Annexin V -FITC/PI Cell Apoptosis Detection Kit

TransDetect® Annexin V -EGFP/PI Cell Apoptosis Detection Kit

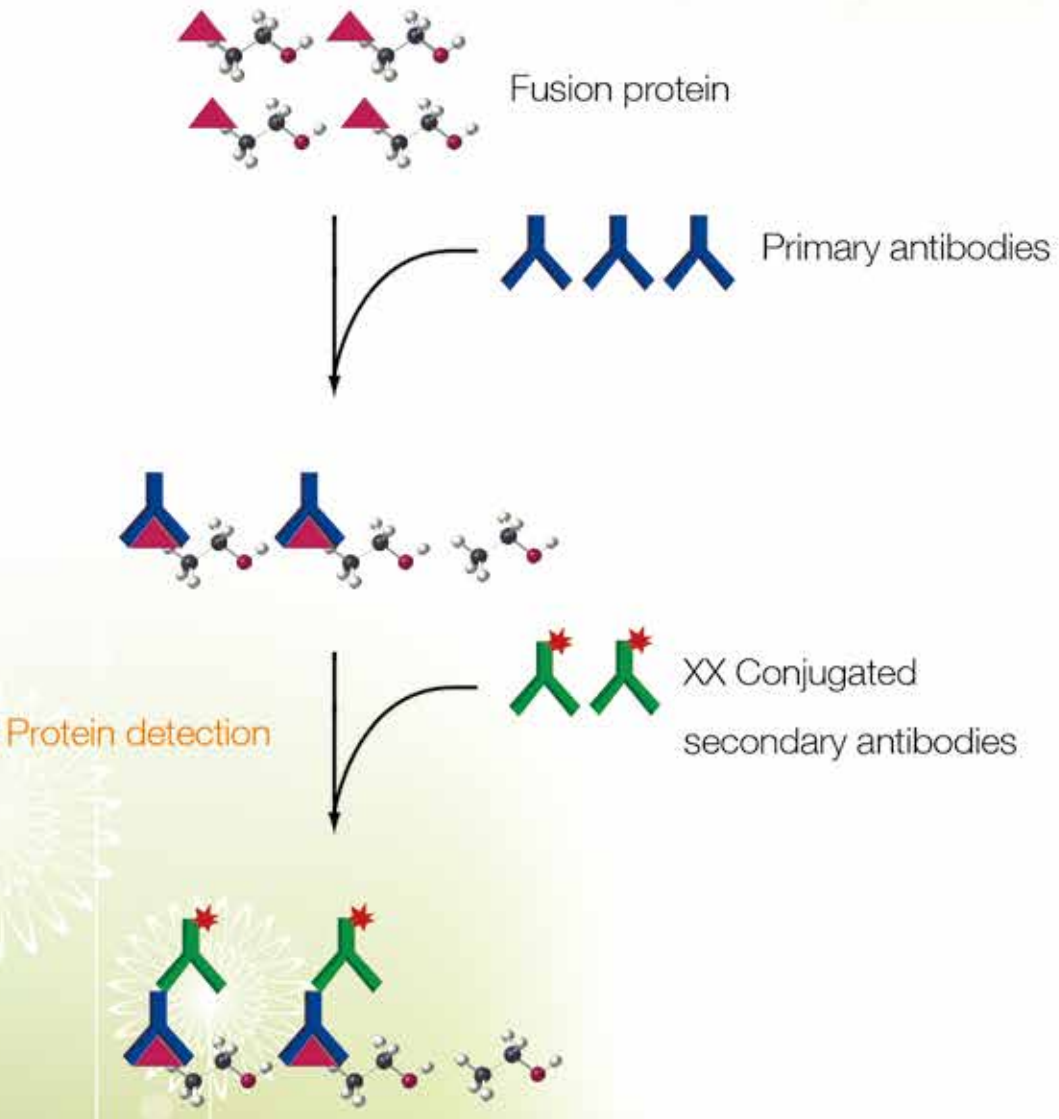
- ***High sensitivity***
- ***High specificity***

- Rapid fluorescent detection of annexin V bound cells.
- No cell fixation, the cells can be used for futher study after this assay.
- Propidium iodide provided to differentiate apoptotic cells from viable and necrotic cells.





Primary Antibodies Fluorescent-labeled Secondary Antibodies



- ***High sensitivity***
- ***High specificity***

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Chapter 1 PCR, RT-PCR, qPCR and qRT-PCR

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PCR SuperMix

Direct PCR

RT-PCR

Chapter 1 PCR, RT-PCR, qPCR and qRT-PCR

RT-PCR

qPCR and qRT-PCR SuperMix

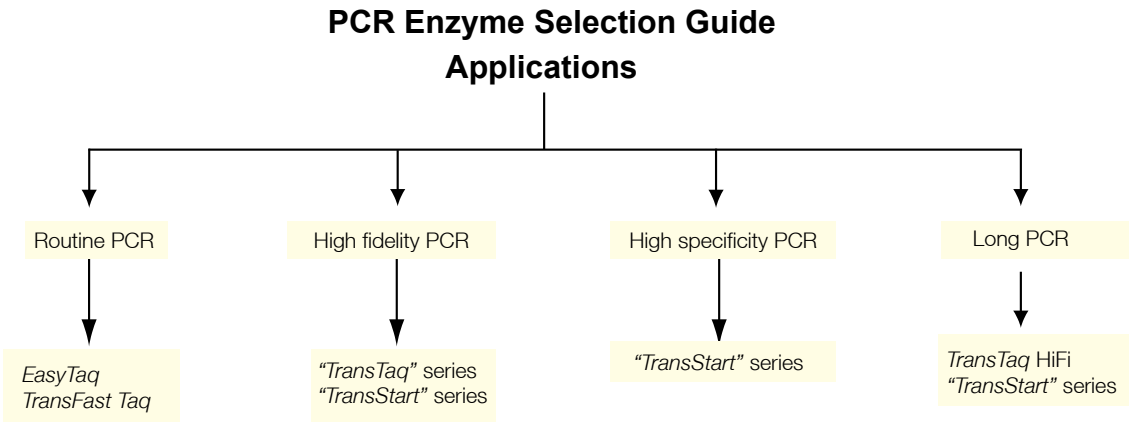
Chapter 1 PCR, RT-PCR, qPCR and qRT-PCR

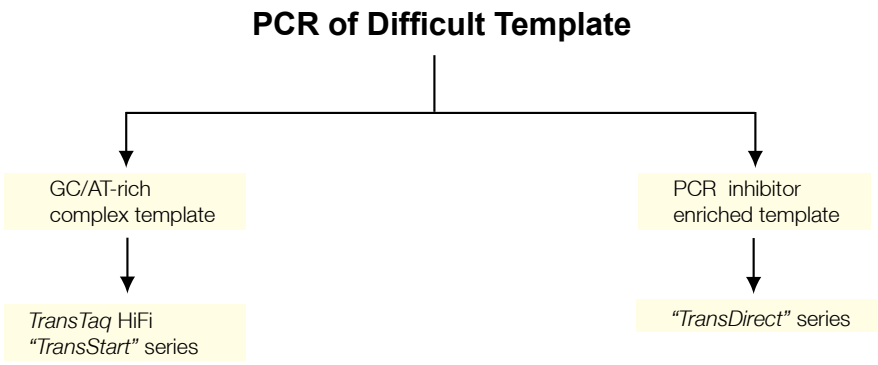
qPCR and qRT-PCR SuperMix

High Pure dNTPs

PCR Enzymes

PCR enzymes are purified from *E.coli* strains carrying genes for specific DNA polymerase. The choice of DNA polymerase for PCR application highly depends on the characteristics of the system as well as the desired results. The following is PCR Enzyme Selection Guide.





Feature of PCR Enzymes

PCR Enzyme	*TransFast® Taq*	*EasyTaq®*	*TransTaq®-T*	*TransTaq® HiFi*	*TransStart® Taq*	*TransStart® TopTaq*
Amplification Efficiency	*TransFast® Taq =EasyTaq®<TransTaq®-T<TransTaq® HiFi<TransStart® Taq<TransStart® TopTaq*					
Specificity	*TransFast® Taq =EasyTaq®<TransTaq®-T<TransTaq® HiFi<TransStart® Taq<TransStart® TopTaq*					
Fidelity (vs. *EasyTaq®*)	1×	1×	18×	18×	18×	18×
Extension Rate	6 kb/min	1-2 kb/min	1-2 kb/min	1-2 kb/min	1-2 kb/min	1-2 kb/min
Hot Start	-	-	+	+	+	+
"A" at 3' end	+	+	+	+	+	+
Product Size (human genomic DNA as template)	≤4 kb	≤4 kb	≤8 kb	≤15 kb	≤15 kb	≤15 kb

PCR Enzyme	*EasyPfu*	*TransStart® KD* Plus	*TransStart® FastPfu*	*TransStart® FastPfu* Fly
Amplification Efficiency	*EasyPfu* < *TransStart® KD* Plus = *TransStart® FastPfu* < *TransStart® FastPfu* Fly			
Specificity	*EasyPfu* < *TransStart® KD* Plus = *TransStart® FastPfu* < *TransStart® FastPfu* Fly			
Fidelity (vs. *EasyTaq®*)	18×	108×	54×	108×
Extension Rate	0.5 kb/min	1 kb/min	2-4 kb/min	2-6 kb/min
Hot Start	+	+	+	+
"A" at 3' end	-	-	-	-
Product Size (human genomic DNA as template)	≤6 kb	≤15 kb	≤15 kb	≤15 kb
Product Size (plasmid DNA as template)	≤10 kb	≤20 kb	≤20 kb	≤20 kb

Applications

PCR Enzyme	Application
TransFast® Taq DNA Polymerase	routine PCR, fast PCR, colony PCR
EasyTaq® DNA Polymerase	routine PCR, colony PCR
EasyTaq® DNA Polymerase for PAGE	short fragment PCR
TransTaq®-T DNA Polymerase	complex templates, TA cloning
TransTaq® HiFi DNA Polymerase *TransStart® Taq* DNA Polymerase *TransStart® TopTaq* DNA Polymerase	GC/AT-rich templates, complex templates, long PCR, TA cloning
TransStart® Taq DNA Polymerase *TransStart® TopTaq* DNA Polymerase	GC/AT-rich templates, complex templates, qPCR, multiplex PCR, TA cloning
EasyPfu DNA Polymerase *TransStart® KD* Plus DNA Polymerase	high fidelity PCR, blunt cloning; site-directed mutagenesis
TransStart® FastPfu DNA Polymerase *TransStart® FastPfu* Fly DNA Polymerase	high fidelity PCR, fast PCR, complex templates, blunt cloning, site-directed mutagenesis

TransFast® *Taq* DNA Polymerase

dNTPs-free	AP101-01	500 units
	AP101-02	6×500 units
dNTPs (2.5 mM)	AP101-11	500 units
	AP101-12	6×500 units

Concentration
5 units/μl

Contents
- *TransFast*® *Taq* DNA Polymerase
- 10×*TransFast*® *Taq* Buffer
  (200 mM Tris-HCl pH 8.4; 100 mM KCl;
  100 mM $(NH_4)_2SO_4$; 20 mM $MgSO_4$; others)
- 6×DNA Loading Buffer

Storage
at -20°C for two years

Description

TransFast® *Taq* DNA Polymerase is an engineered version of *Taq* DNA Polymerase. The enzyme consists of a single polypeptide with a molecular weight of approximately 94 kDa. *TransFast*® *Taq* DNA Polymerase has 5'-3' DNA polymerase activity and 5'-3' exonuclease activity. It lacks 3'-5' exonuclease activity.

- Extension rate is about 6 kb/min.
- Template-independent "A" can be generated at the 3' end of the PCR product. PCR products can be directly cloned into *pEASY*®-T vectors.
- Amplification of genomic DNA fragment up to 4 kb.

Applications

- Routine PCR
- Fast PCR
- Colony PCR

Unit Definition

One unit of *TransFast*® *Taq* DNA Polymerase incorporates 10 nmol of deoxyribonucleotide into acid-precipitable material in 30 minutes at 74°C.

Quality Control

TransFast® *Taq* DNA Polymerase has passed the following quality control assays: functional absence of double- and single-strand endonuclease activity; >99% homogeneous measured by SDS-PAGE. Each batch of *TransFast*® *Taq* DNA Polymerase has been assayed for amplification efficiency to amplify p53 gene from 10 ng of human genomic DNA.

PROTOCOL

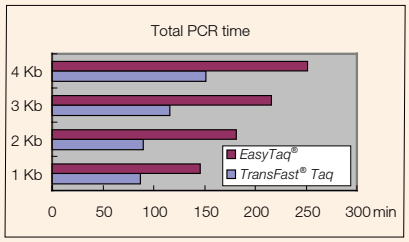
Reaction Components

Component	Volume	Final Concentration
Template	Variable	as required
Forward Primer (10 μM)	1 μl	0.2 μM
Reverse Primer (10 μM)	1 μl	0.2 μM
10×*TransFast*® *Taq* Buffer	5 μl	1×
2.5 mM dNTPs	4 μl	0.2 mM
TransFast® *Taq* DNA Polymerase	0.5 -1 μl	2.5-5 units
ddH_2O	Variable	-
Total volume	50 μl	-

Thermal cycling conditions

94°C	2-5 min	
94°C	5 sec	30-35 cycles
50-60°C	15 sec	
72°C	x sec	
72°C	5-10 min	

Target	Extension time
0-2 kb	10 sec/kb
2-3 kb	20 sec/kb
>3 kb	30 sec/kb



M: 1Kb Plus DNA Ladder
E: *EasyTaq*® DNA Polymerase
F: *TransFast*® *Taq* DNA Polymerase

EasyTaq® DNA Polymerase

dNTPs-free	AP111-01	500 units
	AP111-02	6×500 units
	AP111-03	4×2,500 units
	AP111-04	10×5,000 units
dNTPs (2.5 mM)	AP111-11	500 units
	AP111-12	6×500 units
	AP111-13	4×2,500 units

Concentration
5 units/μl

Contents
- *EasyTaq*® DNA Polymerase
- 10×*EasyTaq*® Buffer
  (200 mM Tris-HCl pH 8.3; 200 mM KCl; 100 mM $(NH_4)_2SO_4$; 20 mM $MgSO_4$; others)
- 6×DNA Loading Buffer

Storage
at -20°C for two years

Description
EasyTaq® DNA Polymerase is purified from *E. coli* expressing a cloned DNA polymerase from *Thermus aquaticus*. The enzyme consists of a single polypeptide with a molecular weight of approximately 94 kDa. *EasyTaq*® DNA Polymerase has 5'-3' DNA polymerase activity and 5'-3' exonuclease activity. It lacks 3'-5' exonuclease activity. *EasyTaq*® DNA Polymerase is suitable for routine amplification. PCR products are not suitable for PAGE.

- Extension rate is about 1-2 kb/min.
- Template-independent "A" can be generated at the 3' end of the PCR product. PCR products can be directly cloned into *pEASY*®-T vectors.
- Amplification of genomic DNA fragment up to 4 kb.

Applications
- Routine PCR
- Colony PCR

Unit Definition
One unit of *EasyTaq*® DNA Polymerase incorporates 10 nmol of

deoxyribonucleotide into acid-precipitable material in 30 minutes at 74°C.

Quality Control

EasyTaq® DNA Polymerase has passed the following quality control assays: functional absence of double- and single-strand endonuclease activity; >99% homogeneous measured by SDS-PAGE. Each batch of *EasyTaq*® DNA Polymerase has been assayed for amplification efficiency to amplify p53 gene from 10 ng of human genomic DNA.

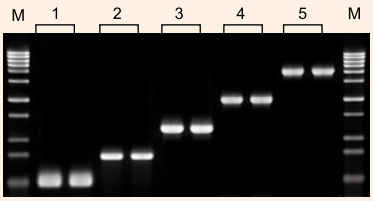
PROTOCOL

Reaction Components

Component	Volume	Final Concentration
Template	Variable	as required
Forward Primer (10 μM)	1 μl	0.2 μM
Reverse Primer (10 μM)	1 μl	0.2 μM
10×*EasyTaq*® Buffer	5 μl	1×
2.5 mM dNTPs	4 μl	0.2 mM
EasyTaq® DNA Polymerase	0.5-1 μl	2.5-5 units
ddH_2O	Variable	-
Total volume	50 μl	-

Thermal cycling conditions

94°C	2-5 min	
94°C	30 sec	30-35 cycles
50-60°C	30 sec	
72°C	1-2 kb/min	
72°C	5-10 min	



M: 1Kb Plus DNA Ladder
1: CCRD 0.5 kb; 2: BDNF 0.8 kb;
3: Rhod 1.2 kb; 4: Rhod 2 kb;
5: Rhod 4.17 kb.
50 ng of Human Genomic DNA as templates

EasyTaq® DNA Polymerase for PAGE

dNTPs-free	AP112-01	2,500 units
	AP112-02	4×2,500 units
dNTPs (2.5 mM)	AP112-11	2,500 units
	AP112-12	4×2,500 units

Concentration
5 units/μl

Contents
- *EasyTaq*® DNA Polymerase for PAGE
- 10×*EasyTaq*® Buffer for PAGE (200 mM Tris-HCl pH 8.3; 200 mM KCl; 100 mM $(NH_4)_2SO_4$; 20 mM $MgSO_4$; others)
- 6×DNA Loading Buffer

Storage
at -20°C for two years

Description
EasyTaq® DNA Polymerase for PAGE is purified from *E. coli* expressing a cloned DNA polymerase from *Thermus aquaticus*. The enzyme consists of a single polypeptide with a molecular weight of approximately 94 kDa. *EasyTaq*® DNA Polymerase for PAGE has 5'-3' DNA polymerase activity and 5'-3' exonuclease activity. It lacks 3'-5' exonuclease activity.
- Extension rate is about 1-2 kb/min.
- Unique buffer system compatible with PAGE.
- Template-independent "A" can be generated at the 3' end of the PCR product. PCR products can be directly cloned into *pEASY*®-T vectors.
- Amplification of genomic DNA fragment up to 3 kb.

Application
Short fragment PCR

Unit Definition
One unit of *EasyTaq*® DNA Polymerase for PAGE incorporates 10 nmol of deoxyribonucleotide into acid-precipitable material in 30 minutes at 74°C.

Quality Control
EasyTaq® DNA Polymerase for PAGE has passed the following quality control assays: functional absence of double- and single-strand endonuclease activity; >99% homogeneous measured by SDS-PAGE. Each batch of *EasyTaq*® DNA Polymerase for PAGE has been assayed for amplification efficiency to amplify p53 gene from 10 ng of human genomic DNA.

PROTOCOL

Reaction Components

Component	Volume	Final Concentration
Template	Variable	as required
Forward Primer (10 μM)	1 μl	0.2 μM
Reverse Primer (10 μM)	1 μl	0.2 μM
10×*EasyTaq*® Buffer for PAGE	5 μl	1×
2.5 mM dNTPs	4 μl	0.2 mM
EasyTaq® DNA Polymerase for PAGE	0.5-1 μl	2.5-5 units
ddH_2O	Variable	-
Total volume	50 μl	-

Thermal cycling conditions

94°C	2-5 min	
94°C	30 sec	30-35 cycles
50-60°C	30 sec	
72°C	1-2 kb/min	
72°C	5-10 min	

TransTaq®-T DNA Polymerase

dNTPs-free	AP122-01	250 units
	AP122-02	500 units
	AP122-03	6×500 units
dNTPs (2.5 mM)	AP122-11	250 units
	AP122-12	500 units
	AP122-13	6×500 units

Concentration
5 units/μl

Contents
- *TransTaq*®-T DNA Polymerase
- 10×*TransTaq*®-T Buffer (200 mM Tris-HCl pH 9.0; 100 mM KCl; 100 mM $(NH_4)_2SO_4$; 20 mM $MgSO_4$; others)
- 6×DNA Loading Buffer

Storage
at -20°C for two years

Description
TransTaq®-T DNA Polymerase is a mixture of *EasyTaq*® DNA Polymerase with a proofreading 3'-5' exonuclease. The fidelity is equal to *EasyPfu* DNA Polymerase. The yield is equal to that from *EasyTaq*® DNA Polymerase. It is more suitable for high fidelity TA cloning.
- *TransTaq*®-T DNA Polymerase offers 18-fold fidelity as compared to *EasyTaq*® DNA Polymerase.
- Extension rate is about 1-2 kb/min.
- Template-independent "A" can be generated at the 3' end of the PCR product. PCR products can be directly cloned into *pEASY*®-T vectors.
- Amplification of genomic DNA fragment up to 8 kb.

Applications
- Complex templates
- TA cloning

Unit Definition
One unit of *TransTaq*®-T DNA Polymerase incorporates 10 nmol of deoxyribonucleotide into acid-precipitable material in 30 minutes at 74°C.

Quality Control
TransTaq®-T DNA Polymerase has passed the following quality control assays: functional absence of double- and single-strand endonuclease activity; >99% homogeneous measured by SDS-PAGE. Each batch of *TransTaq*®-T DNA Polymerase has been assayed for amplification efficiency to amplify p53 gene from 10 ng of human genomic DNA.

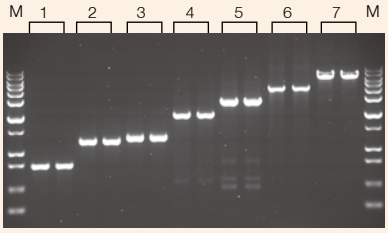
PROTOCOL

Reaction Components

Component	Volume	Final Concentration
Template	Variable	as required
Forward Primer (10 μM)	1 μl	0.2 μM
Reverse Primer (10 μM)	1 μl	0.2 μM
10×*TransTaq*®-T Buffer	5 μl	1×
2.5 mM dNTPs	4 μl	0.2 mM
TransTaq®-T DNA Polymerase	0.5-1 μl	2.5-5 units
ddH_2O	Variable	-
Total volume	50 μl	-

Thermal cycling conditions

94°C	2-5 min	
94°C	30 sec	30-35 cycles
50-60°C	30 sec	
72°C	1-2 kb/min	
72°C	5-10 min	



M: 1Kb Plus DNA Ladder
1: BDNF 0.8 kb; 2: Rhod 1.2 kb;
3: β-globin 1.3 kb; 4: Rhod 2.0 kb ;
5: β-globin 3.0 kb; 6: Rhod 4.17 kb;
7: Factor IX 7.5 kb
50 ng of Human Genomic DNA as templates

TransTaq® DNA Polymerase High Fidelity (HiFi)

dNTPs-free	AP131-01	250 units
	AP131-02	500 units
	AP131-03	6×500 units
dNTPs (2.5 mM)	AP131-11	250 units
	AP131-12	500 units
	AP131-13	6×500 units

Concentration
5 units/μl

Contents
- *TransTaq*® HiFi DNA Polymerase
- 10×*TransTaq*® HiFi Buffer I, II
  (200 mM Tris-HCl pH 9.0; 100 mM KCl; 100 mM $(NH_4)_2SO_4$; 20 mM $MgSO_4$; 10% glycerol; others)
- GC Enhancer
- 6×DNA Loading Buffer

Storage
at -20°C for two years

Description
TransTaq® DNA Polymerase High Fidelity (*TransTaq*® HiFi DNA Polymerase) contains *TransTaq*®-T DNA Polymerase and a proofreading 3'-5' exonuclease. *TransTaq*® HiFi DNA Polymerase provides higher specificity and higher amplification efficiency than *TransTaq*®-T DNA Polymerase. Two different buffers are provided in the kit. *TransTaq*® HiFi Buffer I is optimized for the amplification of genomic DNA and *TransTaq*® HiFi Buffer II is optimized for the amplification of λDNA, cDNA or plasmid DNA.
- *TransTaq*® HiFi DNA Polymerase offers 18-fold fidelity as compared to *EasyTaq*® DNA Polymerase.
- Extension rate is about 1-2 kb/min.
- Template-independent "A" can be generated at the 3' end of the PCR product. PCR products can be directly cloned into *pEASY*®-T vectors.
- Amplification of genomic DNA fragment up to 15 kb.

Applications
- Complex templates
- GC/AT rich templates
- Long PCR
- High yield PCR

Unit Definition
One unit of *TransTaq*® HiFi DNA Polymerase incorporates 10 nmol of deoxyribonucleotide into acid-precipitable material in 30 minutes at 74°C.

Quality Control

TransTaq® HiFi DNA Polymerase has passed the following quality control assays: functional absence of double- and single-strand endonuclease activity; >99% homogeneous measured by SDS-PAGE. Each batch of *TransTaq*® HiFi DNA Polymerase has been assayed for amplification efficiency to amplify p53 gene from 10 ng of human genomic DNA.

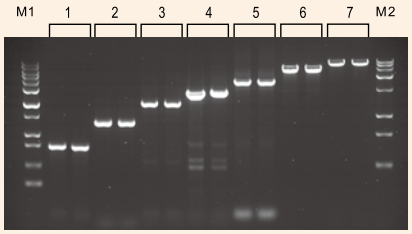
PROTOCOL

Reaction Components

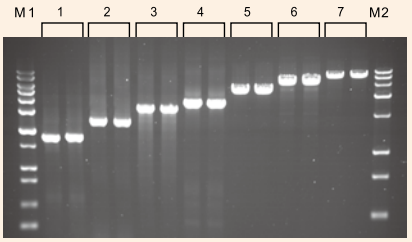
Component	Volume	Final Concentration
Template	Variable	as required
Forward Primer (10 μM)	1 μl	0.2 μM
Reverse Primer (10 μM)	1 μl	0.2 μM
10×*TransTaq*® HiFi Buffer I/II	5 μl	1×
2.5 mM dNTPs	4 μl	0.2 mM
TransTaq® HiFi DNA Polymerase	0.5-1 μl	2.5-5 units
ddH_2O	Variable	-
Total volume	50 μl	-

Thermal cycling conditions

94°C	2-5 min	
94°C	30 sec	30-35 cycles
50-60°C	30 sec	
72°C	1-2 kb/min	
72°C	5-10 min	

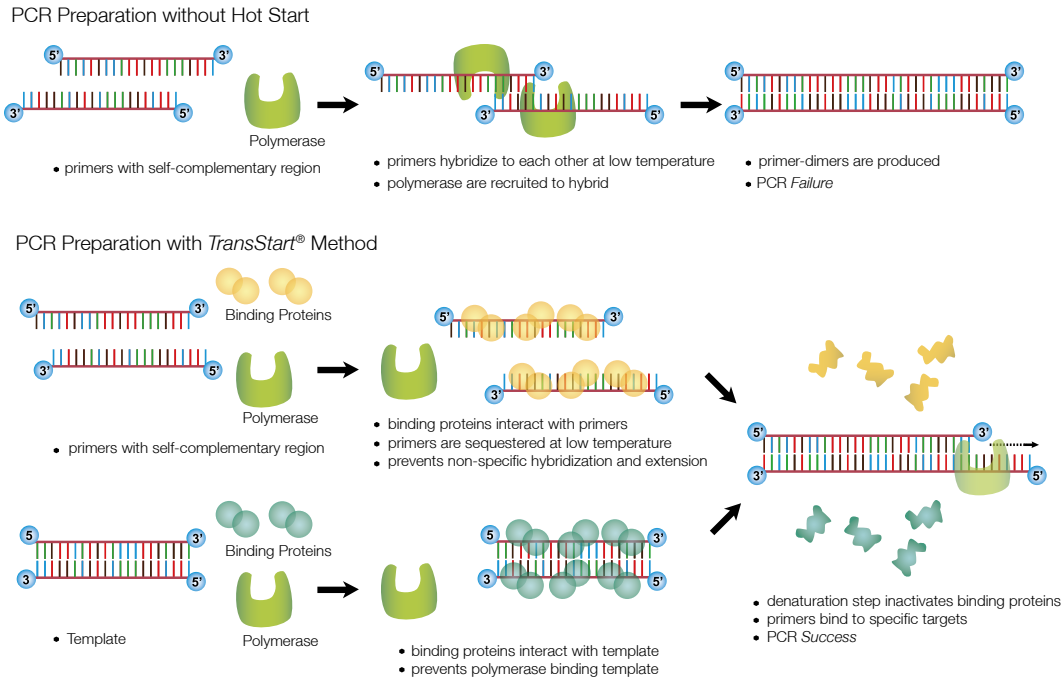


TransTaq® HiFi Buffer I
M1: 1Kb Plus DNA Ladder
M2: *Trans*15K DNA Marker
1: BDNF 0.8 kb;
2: Rhod 1.2 kb;
3: Rhod 2.0 kb;
4: β-globin 3.0 kb;
5: Rhod 4.17 kb;
6: Factor IX 7.5 kb;
7: Serum albumin 12.4 kb
50 ng of Human Genomic DNA as templates



TransTaq® HiFi Buffer II
M1: 1Kb Plus DNA Ladder
M2: *Trans*15K DNA Marker
1: REPA 1.8 kb; 2: NCBP 2.5 kb;
3: HDP 3.5 kb; 4: VIN 4.6 kb;
5: Pol 6.8 kb; 6: APC 8.5 kb;
7: Dynein 12.3 kb
100 ng of Human total RNA as templates

TransStart® Hot Start (Double Blocking)



At room temperature, one binding protein binds to double-strand DNA template and another binding protein binds to primer. These unique formulations effectively neutralize the DNA polymerase activity at room temperature. Blocking proteins are released from templates and primers during the initial denaturation. This double blocking method has higher efficiency than antibody based, or chemically modified hot start PCR.

TransStart® *Taq* DNA Polymerase

dNTPs-free	AP141-01	250 units
	AP141-02	500 units
	AP141-03	6×500 units
dNTPs (2.5 mM)	AP141-11	250 units
	AP141-12	500 units
	AP141-13	6×500 units

Concentration
2.5 units/μl

Contents
- *TransStart*® *Taq* DNA Polymerase
- 10×*TransStart*® *Taq* Buffer (500 mM Tris-HCl pH 9.0; 200 mM $(NH_4)_2SO_4$; 20 mM $MgSO_4$; 10% glycerol; others)
- GC Enhancer
- 6×DNA Loading Buffer

Storage
at -20°C for two years

Description
TransStart® *Taq* DNA Polymerase is a hot start *Taq* DNA polymerase containing *Taq* DNA polymerase and two proprietary DNA binding proteins. At room temperature, one binding protein binds to double-strand DNA template and another binding protein binds to primer. These unique formulations effectively neutralize the DNA polymerase activity at room temperature. Blocking proteins are released from templates and primers during the initial denaturation. This double blocking method has higher efficiency than antibody based, or chemically modified hot start PCR.
- *TransStart*® *Taq* DNA Polymerase offers 18-fold fidelity as compared to *EasyTaq*® DNA Polymerase.
- Extension rate is about 1-2 kb/min.
- Template-independent "A" can be generated at the 3' end of the PCR product. PCR products can be directly cloned into *pEASY*®-T vectors.
- Reduced nonspecific amplification and primer dimer formation.
- Different from *Taq* antibody, no risk of contamination from mammalian DNA.
- Different from chemical modification, long denaturing step is not needed.
- Amplification of genomic DNA fragment up to 15 kb.

Applications
- Complex templates
- GC/AT-rich templates
- Multiplex PCR
- High yield PCR

Unit Definition
One unit of *TransStart*® *Taq* DNA Polymerase incorporates 10 nmol of deoxyribonucleotide into acid-precipitable material in 30 minutes at 74°C.

Quality Control
TransStart® *Taq* DNA Polymerase has passed the following quality control assays: functional absence of double- and single-strand endonuclease activity; >99% homogeneous measured by SDS-PAGE. Each batch of *TransStart*® *Taq* DNA Polymerase has been assayed for amplification efficiency to amplify p53 gene from 10 ng of human genomic DNA.

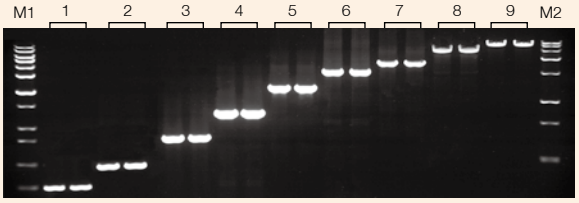
PROTOCOL

Reaction Components

Component	Volume	Final Concentration
Template	Variable	as required
Forward Primer (10 μM)	1 μl	0.2 μM
Reverse Primer (10 μM)	1 μl	0.2 μM
10×*TransStart® Taq* Buffer	5 μl	1×
2.5 mM dNTPs	4 μl	0.2 mM
TransStart® Taq DNA Polymerase	0.5-1 μl	1.25-2.5 units
ddH_2O	Variable	-
Total volume	50 μl	-

Thermal cycling conditions

94°C	2-5 min	
94°C	30 sec	30-35 cycles
50-60°C	30 sec	
72°C	1-2 kb/min	
72°C	5-10 min	



M1: 1Kb Plus DNA Ladder
M2: *Trans*15K DNA Marker
1: Numb 0.3 kb; 2: CCRD 0.5 kb;
3: BDNF 0.8 kb; 4: Rhod 1.2 kb;
5: Rhod 2.0 kb; 6: β-globin 3.0 kb;
7: Rhod 4.17 kb; 8: Factor IX 7.5 kb;
9: Serum albumin 12.4 kb
50 ng of Human Genomic DNA as templates

TransStart® *TopTaq* DNA Polymerase

dNTPs-free	AP151-01	250 units
	AP151-02	500 units
	AP151-03	6×500 units
dNTPs (2.5 mM)	AP151-11	250 units
	AP151-12	500 units
	AP151-13	6×500 units

Concentration

2.5 units/μl

Contents

- *TransStart*® *TopTaq* DNA Polymerase
- 10×*TransStart*® *TopTaq* Buffer (500 mM Tris-HCl (pH 9.0); 200 mM $(NH_4)_2SO_4$; 20 mM $MgSO_4$; 10% glycerol others)
- GC Enhancer
- 6×DNA Loading Buffer

Storage

at -20°C for two years

Description

TransStart® *TopTaq* DNA Polymerase is an engineered version of *Taq* DNA Polymerase combined with *TransStart*® technique. One binding protein binds to double-strand DNA template, preventing polymerase activity at room temperature. Other two binding proteins bind primers, preventing primer-dimer formation. Blocking proteins are released from primers and templates during the initial denaturation. This double blocking method has higher efficiency than antibody based, or chemically modified hot start PCR.

- Compared with *TransStart*® *Taq* DNA Polymerase, *TransStart*® *TopTaq* DNA Polymerase has higher amplification efficiency, specificity and sensitivity.
- *TransStart*® *TopTaq* DNA Polymerase offers 18-fold fidelity as compared to *EasyTaq*® DNA Polymerase.
- The specificity is higher than antibody based or chemically modified hot start DNA polymerases.
- Template-independent "A" can be generated at the 3' end of the PCR product. PCR products can be directly cloned into *pEASY*®-T vectors.
- Reduced nonspecific amplification and primer dimer formation.
- Different from *Taq* antibody, no risk of contamination from mammalian DNA.
- Different from chemical modification, long denaturing step is not needed.
- Amplification of genomic DNA fragment up to 15 kb.

Applications

- Complex templates
- GC/AT-rich templates
- Multiplex PCR
- High yield PCR

Unit Definition

One unit of *TransStart*® *TopTaq* DNA Polymerase incorporates 10 nmol of deoxyribonucleotide into acid-precipitable material in 30 minutes at 74°C.

Quality Control

TransStart® *TopTaq* DNA Polymerase has passed the following quality control assays: functional absence of double- and single-strand endonuclease activity; >99% homogeneous measured by SDS-PAGE. Each batch of *TransStart*® *TopTaq* DNA Polymerase has been assayed for amplification efficiency to amplify p53 gene from 10 ng of human genomic DNA.

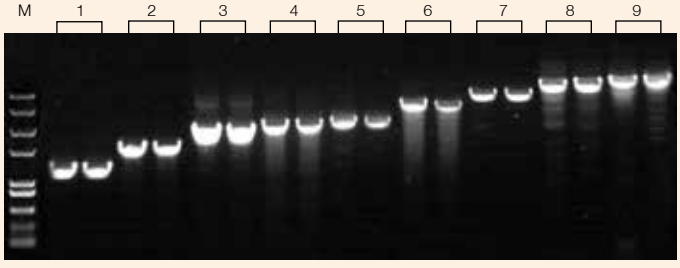
PROTOCOL

Reaction Components

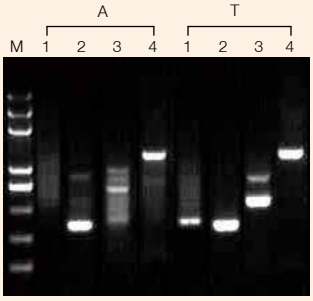
Component	Volume	Final Concentration
Template	Variable	as required
Forward Primer (10 μM)	1 μl	0.2 μM
Reverse Primer (10 μM)	1 μl	0.2 μM
10×*TransStart*® *TopTaq* Buffer	5 μl	1×
2.5 mM dNTPs	4 μl	0.2 mM
TransStart® *TopTaq* DNA Polymerase	0.5-1 μl	1.25-2.5 units
ddH_2O	Variable	-
Total volume	50 μl	-

Thermal cycling conditions

94°C	2-5 min	
94°C	30 sec	30-35 cycles
50-60°C	30 sec	
72°C	1-2 kb/min	
72°C	5-10 min	



M: *Trans*2K® Plus II DNA Marker
1: Rhod 1.2 kb 2: Rhod 2.0 kb 3: β-globin 3.0 kb
4: β-globin 4.1 kb 5: Rhod 4.17 kb 6: β-globin 6.1 kb
7: Factor IX 7.5 kb 8: IGF2R 8.9 kb 9: Serum albumin 12.4 kb
50 ng Human Genomic DNA as templates



M: *Trans*2K® Plus DNA Marker
1: DMD1 0.3 kb 2: Numb 0.3 kb
3: P53 0.5 kb 4: P1P2 1.2 kb
A: Competitor A Hot Start DNA Polymerase
T: *TransStart*® *TopTaq* DNA Polymerase

EasyPfu DNA Polymerase

dNTPs-free	AP211-01	250 units
	AP211-02	500 units
	AP211-03	6×500 units

dNTPs (2.5 mM)	AP211-11	250 units
	AP211-12	500 units
	AP211-13	6×500 units

Concentration

2.5 units/μl

Contents

- *EasyPfu* DNA Polymerase
- 10×*EasyPfu* Buffer (200 mM Tris-HCl pH 8.8; 100 mM $(NH_4)_2SO_4$; 100 mM KCl; 20 mM $MgSO_4$; others)
- 50 mM $MgSO_4$
- 6×DNA Loading Buffer

Storage

at -20°C for two years

Description

EasyPfu DNA Polymerase is an engineered version of *pfu* DNA Polymerase with enhanced yield and higher fidelity. *EasyPfu* DNA Polymerase possesses a proofreading 3'-5' exonuclease activity.

- *EasyPfu* DNA Polymerase offers 18-fold fidelity as compared to *EasyTaq*® DNA Polymerase.
- Extension rate is about 0.5 kb/min.
- PCR products can be directly cloned into *pEASY*®-Blunt vectors.
- Amplification of genomic DNA fragment up to 6 kb.
- Amplification of plasmid DNA fragment up to 10 kb.

Applications

- High fidelity PCR
- Blunt-end cloning
- Site-directed mutagenesis

Unit Definition

One unit of *EasyPfu* DNA Polymerase incorporates 10 nmol of deoxyribonucleotide into acid-precipitable material in 30 minutes at 74°C.

Quality Control

EasyPfu DNA Polymerase has passed the following quality control assays: functional absence of double- and single-strand endonuclease activity, >99% homogeneous measured by SDS-PAGE. Each batch of *EasyPfu* DNA Polymerase has been assayed for amplification efficiency to amplify p53 gene from 10 ng of human genomic DNA.

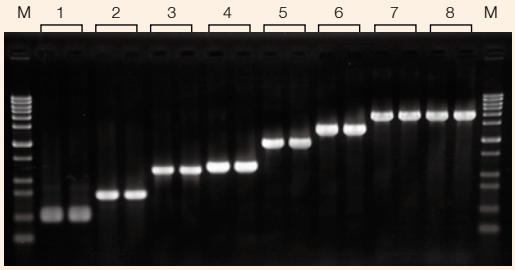
PROTOCOL

Reaction Components

Component	Volume	Final Concentration
Template	Variable	as required
Forward Primer (10 μM)	1 μl	0.2 μM
Reverse Primer (10 μM)	1 μl	0.2 μM
10×*EasyPfu* Buffer	5 μl	1×
2.5 mM dNTPs	4 μl	0.2 mM
EasyPfu DNA Polymerase	1 μl	2.5 units
ddH_2O	Variable	-
Total volume	50 μl	-

Thermal cycling conditions

94°C	2-5 min	
94°C	30 sec	30-35 cycles
50-60°C	30 sec	
72°C	0.5 kb/min	
72°C	5-10 min	



M: 1Kb Plus DNA Ladder
1: CCRD 0.5 kb; 2: BDNF 0.8 kb; 3: Rhod 1.2 kb; 4: β-globin 1.3 kb;
5: Rhod 2 kb; 6: β-globin 3 kb; 7: Rhod 4.17 kb; 8: β-globin 4.1 kb
50 ng of Human Genomic DNA as templates

TransStart® *FastPfu* DNA Polymerase

dNTPs-free	AP221-01	250 units
	AP221-02	500 units
	AP221-03	6×500 units
dNTPs (2.5 mM)	AP221-11	250 units
	AP221-12	500 units
	AP221-13	6×500 units

Concentration
2.5 units/μl

Contents
- *TransStart*® *FastPfu* DNA Polymerase
- 5×*TransStart*® *FastPfu* Buffer (100 mM Tris-SO_4 pH 9.2; 50 mM $(NH_4)_2SO_4$; 200 mM KCl; 10 mM $MgSO_4$; 10% glycerol; others)
- 50 mM $MgSO_4$
- PCR Stimulant
- 6×DNA Loading Buffer

Storage
at -20°C for two years

Description
TransStart® *FastPfu* DNA Polymerase is a fast, high fidelity and high processivity hot start DNA polymerase.
- Extension rate is about 2-4 kb/min.
- *TransStart*® *FastPfu* DNA Polymerase offers 54-fold fidelity as compared to *EasyTaq*® DNA Polymerase.
- PCR products can be directly cloned into *pEASY*® -Blunt vectors.
- Amplification of genomic DNA fragment up to 15 kb.
- Amplification of plasmid DNA fragment up to 20 kb.

Applications
- High fidelity PCR
- High yield and fast PCR
- Blunt end cloning
- Site-directed mutagenesis
- Complex templates

Unit Definition
One unit of *TransStart*® *FastPfu* DNA Polymerase incorporates 10 nmol of deoxyribonucleotide into acid-precipitable material in 30 minutes at 74°C.

Quality Control
TransStart® *FastPfu* DNA Polymerase has passed the following quality control assays: functional absence of double- and single-strand endonuclease activity; >99% homogeneous measured by SDS-PAGE. Each batch of *TransStart*® *FastPfu* DNA Polymerase has been assayed for amplification efficiency to amplify p53 gene from 10 ng of human genomic DNA.

PROTOCOL

Reaction Components

Component	Volume	Final Concentration
Template	Variable	as required
Forward Primer (10 μM)	1 μl	0.2 μM
Reverse Primer (10 μM)	1 μl	0.2 μM
5×*TransStart® FastPfu* Buffer	10 μl	1×
2.5 mM dNTPs	4 μl	0.2 mM
TransStart® FastPfu DNA Polymerase	1 μl	2.5 units
ddH_2O	Variable	-
Total volume	50 μl	-

Suggested conditions

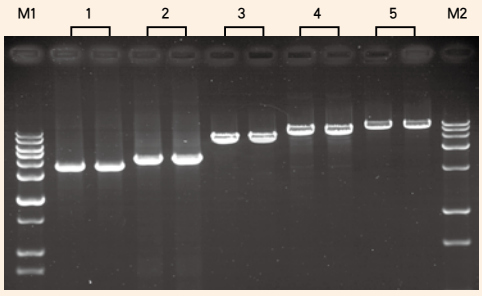
(50 μl reaction volume)

Parameter	Targets≤10 kb	Targets≥10 kb	cDNA
Template	100 ng Genomic DNA 5-30 ng Plasmid DNA	200-500 ng Genomic DNA 5-30 ng Plasmid DNA	1-2 μl cDNA from RT reaction (50-500 ng RNA for RT reaction)
$MgSO_4$	Add extra 1-2 μl of 50 mM $MgSO_4$ to a final concentration of 3-4 mM if the amplified product is larger than 5 kb		

Thermal cycling conditions

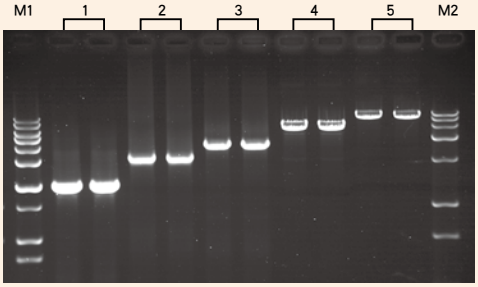
Number of cycles	Temperature	Plasmid or Genomic DNA	cDNA
1 cycle	95°C	2 min	1 min
Plasmid or Genomic DNA: 30-35 cycles cDNA: 35-40 cycles	95°C	20 sec	20 sec
	Tm-5°C	20 sec	20 sec
	72°C	4 kb/min for targets≤1 kb 2-4 kb/min for targets>1 kb	2 kb/min
1 cycle	72°C	5 min	5 min

Amplification from cDNA templates with *TransStart® FastPfu* DNA Polymerase



M1: 1Kb Plus DNA Ladder
M2: *Trans*15K DNA Marker
1: ACTR 3.5 kb; 2 hrs 14 min
2: VIN 4.6 kb; 2 hrs 34 min
3: Pol 6.8 kb; 3 hrs 09 min
4: APC 8.5 kb; 3 hrs 41 min
5: Dynein 12.3 kb; 4 hrs 48 min

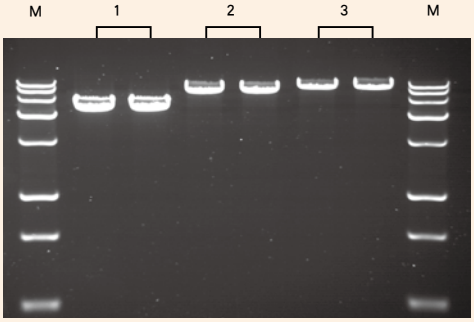
Amplification from genomic DNA templates with *TransStart*® *FastPfu* DNA Polymerase



M1: 1Kb Plus DNA Ladder
M2: *Trans*15K DNA Marker

1: Rhod 2.0 kb;	1 hrs 19 min
2: β-globin 3.0 kb;	1 hrs 27 min
3: Rhod 4.17 kb;	1 hrs 29 min
4: Factor IX 7.5 kb;	3 hrs 25 min
5: Serum albumin 12.4 kb;	4 hrs 48 min

Amplification from plasmid DNA templates with *TransStart*® *FastPfu* DNA Polymerase



M: *Trans*15K DNA Marker

1: UDG 7.0 kb;	1 hrs 36 min
2: LN 10.0 kb;	1 hrs 55 min
3: Fang 14.7 kb;	2 hrs 26 min

TransStart® *FastPfu* Fly DNA Polymerase

dNTPs-free	AP231-01	250 units
	AP231-02	500 units
	AP231-03	6×500 units
dNTPs (2.5 mM)	AP231-11	250 units
	AP231-12	500 units
	AP231-13	6×500 units

Concentration

2.5 units/μl

Contents

- *TransStart*® *FastPfu* Fly DNA Polymerase
- 5×*TransStart*® *FastPfu* Fly Buffer (100 mM Tris-SO_4 pH 9.2; 50 mM $(NH_4)_2SO_4$; 200 mM KCl; 10 mM $MgSO_4$; 10% glycerol; others)
- 50 mM $MgSO_4$
- PCR Stimulant
- 6×DNA Loading Buffer

Storage

at -20°C for two years

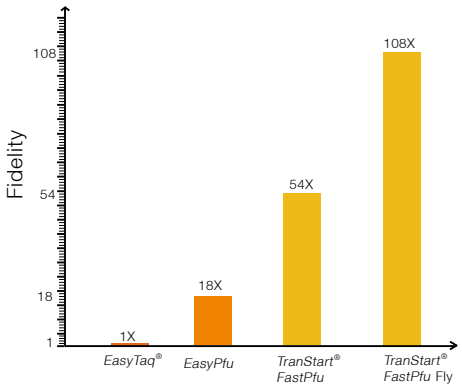
Description

TransStart® *FastPfu* Fly DNA Polymerase is a hot start, high fidelity and high processivity DNA Polymerase. *TransStart*® *FastPfu* Fly DNA Polymerase has an extension rate of up to 6 kb/min. Compared with *TransStart*® *FastPfu* DNA Polymerase, *TransStart*® *FastPfu* Fly DNA Polymerase has higher extension rate, higher fidelity, and higher amplification efficiency.

- *TransStart*® *FastPfu* Fly DNA Polymerase offers 108-fold fidelity as compared to *EasyTaq*® DNA Polymerase.
- Extension rate is about 2-6 kb/min.
- PCR products can be directly cloned into *pEASY*®-Blunt vectors.
- Amplification of genomic DNA fragment up to 15 kb.
- Amplification of plasmid DNA fragment up to 20 kb.

Applications

- High fidelity PCR
- High yield and fast PCR
- Blunt end cloning
- Site-directed mutagenesis
- Complex templates



Unit Definition

One unit of *TransStart® FastPfu* Fly DNA Polymerase incorporates 10 nmol of deoxyribonucleotide into acid-precipitable material in 30 minutes at 74°C.

Quality Control

TransStart® FastPfu Fly DNA Polymerase has passed the following quality control assays: functional absence of double- and single-strand endonuclease activity; >99% homogeneous measured by SDS-PAGE. Each batch of *TransStart® FastPfu* Fly DNA Polymerase has been assayed for amplification efficiency to amplify p53 gene from 10 ng of human genomic DNA.

Reaction Components

Component	Volume	Final Concentration
Template	Variable	as required
Forward Primer (10 μM)	1 μl	0.2 μM
Reverse Primer (10 μM)	1 μl	0.2 μM
5×*TransStart® FastPfu* Fly Buffer	10 μl	1×
2.5 mM dNTPs	4 μl	0.2 mM
TransStart® FastPfu Fly DNA Polymerase	1 μl	2.5 units
ddH_2O	Variable	-
Total volume	50 μl	-

Suggested conditions

(50 μl reaction volume)

Parameter	Targets≤10 kb	Targets≥10 kb	cDNA
Template	100 ng Genomic DNA 5-30 ng Plasmid DNA	200-500 ng Genomic DNA 5-30 ng Plasmid DNA	1-2 μl cDNA from RT reaction (50-500 ng RNA for RT reaction)
$MgSO_4$	Add extra 1-2 μl of 50 mM $MgSO_4$ to a final concentration of 3-4 mM if the amplified product is larger than 5 kb		

Thermal cycling conditions

Number of cycles	Temperature	cDNA or Genomic DNA	Plasmid DNA
1 cycle	95°C	2 min	2 min
Plasmid or Genomic DNA: 30-35 cycles cDNA: 35-40 cycles	95°C	20 sec	20 sec
	Tm-5°C	20 sec	20 sec
	72°C	6 kb/min for targets≤2 kb 2-4 kb/min for targets>2 kb	6 kb/min for targets≤6 kb 2-4 kb/min for targets>6 kb
1 cycle	72°C	5 min	5 min

Total PCR Time

4 kb: Genomic DNA; 7 kb and 10 kb: Plasmid DNA

TransStart® *KD* Plus DNA Polymerase

dNTPs-free	AP301-01	100 units
	AP301-02	200 units
	AP301-03	6×200 units
dNTPs (2.5 mM)	AP301-11	100 units
	AP301-12	200 units
	AP301-13	6×200 units

Concentration

1 unit/μl

Contents

- *TransStart*® *KD* Plus DNA Polymerase
- 5×*TransStart*® *KD* Plus Buffer (100 mM Tris-HCl pH 9.2; 50 mM $(NH_4)_2SO_4$; 200 mM KCl; 5 mM $MgSO_4$;10% Glycerol; others)
- 50 mM $MgSO_4$
- 6×DNA Loading Buffer

Storage

at -20°C for two years

Description

TransStart® *KD* Plus DNA Polymerase is a genetically modified high fidelity DNA polymerase. This enzyme provides higher amplification capability than traditional *Pfu* DNA polymerase and fast amplification speed equal to *Taq* DNA polymerase (1 kb/min). Due to strong 3'-5' exonuclease activity, this enzyme offers 108-fold fidelity as compared to *EasyTaq*® DNA Polymerase.

- PCR products can be directly cloned into *pEASY*®-Blunt vectors.
- Amplification of genomic DNA fragment up to 15 kb.
- Amplification of plasmid DNA fragment up to 20 kb.

Applications

- Fast, high specificity amplification
- High fidelity, high yield amplification

Unit Definition

One unit of *TransStart*® *KD* Plus DNA Polymerase incorporates 10 nmol of deoxyribonucleotide into acid-precipitable material in 30 minutes at 74°C.

Quality Control

TransStart® *KD* Plus DNA Polymerase has passed the following quality control assays: functional absence of double- and single-strand endonuclease activity, >99% homogeneous measured by SDS-PAGE. Each batch of *TransStart*® *KD* Plus DNA Polymerase has been assayed for amplification efficiency to amplify p53 gene from 10 ng of human genomic DNA.

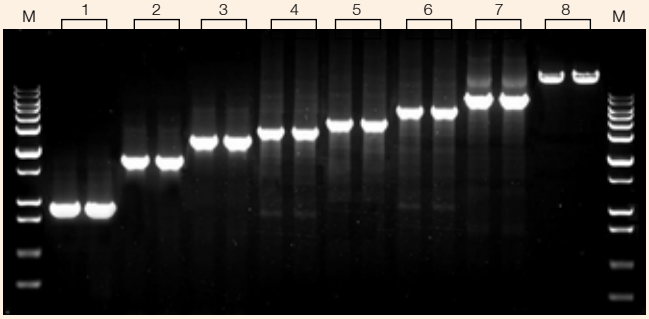
PROTOCOL

Reaction Components

Component	Volume	Final Concentration
Template	Variable	as required
Forward Primer (10 μM)	1 μl	0.2 μM
Reverse Primer (10 μM)	1 μl	0.2 μM
5×*TransStart® KD* Plus Buffer	10 μl	1×
2.5 mM dNTPs	4 μl	0.2 mM
TransStart® KD Plus DNA Polymerase	1 μl	1 unit
ddH_2O	Variable	-
Total volume	50 μl	-

Thermal cycling conditions

94°C	2-5 min	
94°C	30 sec	30-35 cycles
50-60°C	30 sec	
68°C	1 kb/min	
68°C	5-10 min	



M: 1Kb Plus DNA Ladder

Human cDNA as templates
1: GAPDH 0.9 kb;
2: REPA 1.8 kb;
3: NCBP 2.5 kb;
4: ACTR 3 kb;

Human Genomic DNA as templates
5: Rhod 4.17 kb;
6: β-globin 6.1 kb;
7: Factor IX 7.5 kb;

Plasmid DNA as templates
8: Fang 14.7 kb

GC Enhancer

AG101-01	200 µl

Storage
at -20°C for two years

Description

GC Enhancer can be used to increase sensitivity and specificity for GC/AT-rich template or complex template. The stock concentration is 10×, and the working concentration can be varied between 0.5× to 5×.

Applications

- Complex templates
- GC/AT-rich templates

(50 µl reaction volume)

Volume of GC Enhancer (µl)	Final Concentration
2.5	0.5×
5	1×
10	2×
15	3×
20	4×
25	5×

PCR Stimulant

AG111-01	200 µl

Storage
at -20°C for two years

Description

PCR Stimulant can be used to increase sensitivity and specificity for GC/AT-rich template or complex template. It is especially suitable for *Pfu* enzymes. The stock concentration is 5×, and the working concentration can be varied between 0.5× to 2.5×.

Applications

- Complex templates
- GC/AT-rich templates

(50 µl reaction volume)

Volume of PCR Stimulant (µl)	Final Concentration
5	0.5×
10	1×
20	2×
25	2.5×

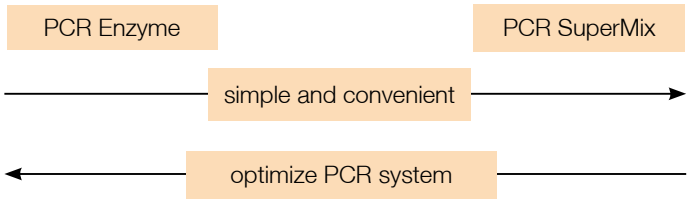
PCR SuperMix

PCR SuperMix is a ready-to-use mixture of DNA polymerase, salt, magnesium, dNTPs and other components for efficient PCR amplification. Only add template, primers and ddH_2O to the SuperMix for PCR. If PCR SuperMix with dye is used, dye needs to be removed before cloning or sequencing.

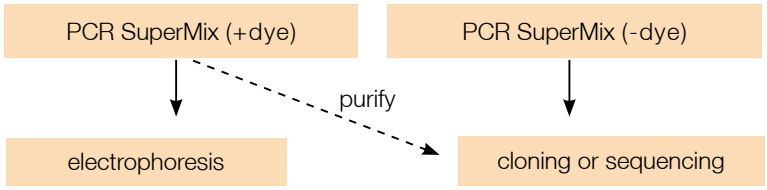
Applications

PCR SuperMix	Application
2×*EasyTaq*® PCR SuperMix	routine PCR
2×*EasyTaq*® PCR SuperMix for PAGE	short fragment PCR
2×*TransTaq*®-T PCR SuperMix	complex templates, TA cloning
2×*TransTaq*® High Fidelity (HiFi) PCR SuperMix I	GC/AT-rich templates, complex templates, long PCR, genomic DNA amplification (<15 kb), TA cloning
2×*TransTaq*® High Fidelity (HiFi) PCR SuperMix II	GC/AT-rich templates, complex templates, long PCR, λDNA, cDNA, plasmid DNA amplification, TA cloning
2×*EasyPfu* PCR SuperMix	high fidelity PCR, blunt cloning, site-directed mutagenesis
2×*TransStart*® *FastPfu* PCR SuperMix	high fidelity PCR, fast PCR, blunt cloning, site-directed mutagenesis

PCR Enzyme vs. PCR SuperMix



PCR SuperMix Selection Chart



2×*EasyTaq*® PCR SuperMix

Mix (-dye)	AS111-01	1 ml
	AS111-02	5×1 ml
	AS111-03	15×1 ml
Mix (+dye)	AS111-11	1 ml
	AS111-12	5×1 ml
	AS111-13	15×1 ml
	AS111-14	6×80 ml

Storage
at -20°C for two years

Description

EasyTaq® PCR SuperMix is a ready-to-use mixture of *EasyTaq*® DNA Polymerase, dNTPs and optimized buffer. The SuperMix is provided at 2× concentration and used at 1× concentration by adding template, primers and H_2O. PCR products are not suitable for PAGE.

- Extension rate is about 1-2 kb/min.
- Template-independent "A" can be generated at the 3' end of the PCR product. PCR products can be cloned into *pEASY*®-T vectors.
- Amplification of genomic DNA fragment up to 4 kb.

Application

Routine PCR

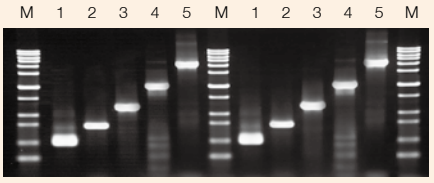
PROTOCOL

Reaction Components

Component	Volume	Final Concentration
Template	Variable	as required
Forward Primer (10 μM)	1 μl	0.2 μM
Reverse Primer (10 μM)	1 μl	0.2 μM
2×*EasyTaq*® PCR SuperMix	25 μl	1×
ddH_2O	Variable	-
Total volume	50 μl	-

Thermal cycling conditions

94°C	2-5 min	
94°C	30 sec	30-35 cycles
50-60°C	30 sec	
72°C	1-2 kb/min	
72°C	5-10 min	



M: 1Kb Plus DNA Ladder
Lane 1: CCRD 0.5 kb
Lane 2: BDNF 0.8 kb
Lane 3: Rhod 1.2 kb
Lane 4: Rhod 2 kb
Lane 5: Rhod 4.17 kb
50 ng of Human Genomic DNA as templates

2×*EasyTaq*® PCR SuperMix for PAGE

	AS112-11	1 ml
Mix (+dye)	AS112-12	5×1 ml
	AS112-13	15×1 ml

Storage

at -20°C for two years

Description

EasyTaq® PCR SuperMix for PAGE is a ready-to-use mixture of *EasyTaq*® DNA Polymerase for PAGE, dNTPs and optimized buffer. The SuperMix for PAGE is provided at 2× concentration and used at 1× concentration by adding template, primers and H_2O.

- Extension rate is about 1-2 kb/min.
- Unique buffer system compatible with PAGE.
- Template-independent "A" can be generated at the 3' end of the PCR product. PCR products can be cloned into *pEASY*®-T vectors.
- Amplification of genomic DNA fragment up to 3 kb.

Application

Short fragment PCR

PROTOCOL

Reaction Components

Component	Volume	Final Concentration
Template	Variable	as required
Forward Primer (10 μM)	1 μl	0.2 μM
Reverse Primer (10 μM)	1 μl	0.2 μM
2×*EasyTaq*® PCR SuperMix for PAGE	25 μl	1×
ddH_2O	Variable	-
Total volume	50 μl	-

Thermal cycling conditions

94°C	2-5 min	
94°C	30 sec	30-35 cycles
50-60°C	30 sec	
72°C	1-2 kb/min	
72°C	5-10 min	

2×*TransTaq*®-T PCR SuperMix

Mix (-dye)	AS122-01	1 ml
	AS122-02	5×1 ml
Mix (+dye)	AS122-11	1 ml
	AS122-12	5×1 ml

Storage
at -20°C for two years

Description

TransTaq®-T PCR SuperMix is a ready-to-use mixture of *TransTaq*®-T DNA Polymerase, dNTPs and optimized buffer. The SuperMix is provided at 2× concentration and used at 1× concentration by adding template, primers and H_2O. Efficiency of PCR products with "A" is equal to *EasyTaq*® DNA polymerase. It is more suitable for high fidelity TA cloning.

- *TransTaq*®-T PCR SuperMix offers 18-fold fidelity as compared to *EasyTaq*® DNA Polymerase.
- Extension rate is about 1-2 kb/min.
- Template-independent "A" can be generated at the 3' end of the PCR product. PCR products can be cloned into *pEASY*®-T vectors.
- Amplification of genomic DNA fragment up to 8 kb.

Applications

- Complex templates
- TA cloning

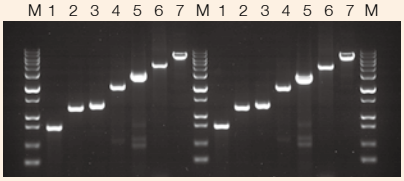
PROTOCOL

Reaction Components

Component	Volume	Final Concentration
Template	Variable	as required
Forward Primer (10 μM)	1 μl	0.2 μM
Reverse Primer (10 μM)	1 μl	0.2 μM
2×*TransTaq*®-T PCR SuperMix	25 μl	1×
ddH_2O	Variable	-
Total volume	50 μl	-

Thermal cycling conditions

94°C	2-5 min	
94°C	30 sec	30-35 cycles
50-60°C	30 sec	
72°C	1-2 kb/min	
72°C	5-10 min	



M: 1Kb Plus DNA Ladder
Lane 1: BDNF 0.8 kb; Lane 2: Rhod 1.2 kb;
Lane 3: β-globin 1.3 kb; Lane 4: Rhod 2.0 kb;
Lane 5: β-globin 3.0 kb; Lane 6: Rhod 4.17 kb;
Lane 7: Factor IX 7.5 kb
50 ng of Human Genomic DNA as templates

2×*TransTaq*® High Fidelity (HiFi) PCR SuperMix

Mix I (-dye)	AS131-01	1 ml
	AS131-02	5×1 ml
Mix II (-dye)	AS131-21	1 ml
	AS131-22	5×1 ml

Storage
at -20°C for two years

Description

TransTaq® High Fidelity (HiFi) PCR SuperMix I or II is a ready-to-use mixture of *TransTaq*® High Fidelity (HiFi) DNA polymerase, dNTPs and optimized buffer. *TransTaq*® High Fidelity (HiFi) PCR SuperMix I is optimized for the amplification of genomic DNA and PCR SuperMix II is optimized for the amplification of λDNA, cDNA or plasmid DNA. The SuperMix is provided at 2× concentration and can be used at 1× concentration by adding template, primers and H_2O.

- *TransTaq*® High Fidelity (HiFi) PCR SuperMix offers 18-fold fidelity as compared to *EasyTaq*® DNA Polymerase.
- Extension rate is about 1-2 kb/min.
- Template-independent "A" can be generated at the 3' end of the PCR product. PCR products can be directly cloned into *pEASY*®-T vectors.
- Amplification of genomic DNA fragment up to 15 kb.

Applications

- Complex templates
- GC/AT-rich templates
- Long PCR
- High yield PCR

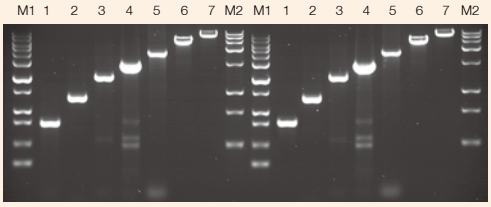
PROTOCOL

Reaction Components

Component	Volume	Final Concentration
Template	Variable	as required
Forward Primer (10 μM)	1 μl	0.2 μM
Reverse Primer (10 μM)	1 μl	0.2 μM
2×*TransTaq*® HiFi PCR SuperMix	25 μl	1×
ddH_2O	Variable	-
Total volume	50 μl	-

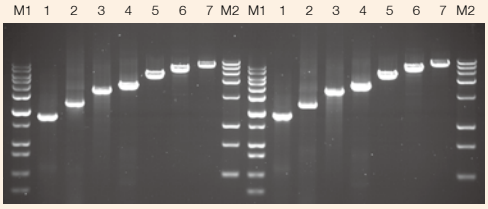
Thermal cycling conditions

94°C	2-5 min	
94°C	30 sec	30-35 cycles
50-60°C	30 sec	
72°C	1-2 kb/min	
72°C	5-10 min	



TransTaq® HiFi DNA Polymerase 2×*TransTaq*® HiFi PCR SuperMix I

M1: 1Kb Plus DNA Ladder
M2: *Trans*15K DNA Marker
Lane 1: BDNF 0.8 kb;
Lane 2: Rhod 1.2 kb;
Lane 3: Rhod 2.0 kb;
Lane 4: β-globin 3.0 kb;
Lane 5: Rhod 4.17 kb;
Lane 6: Factor IX 7.5 kb;
Lane 7: Serum albumin 12.4 kb
50 ng of Human Genomic DNA as templates



TransTaq® HiFi DNA Polymerase 2×*TransTaq*® HiFi PCR SuperMix II

M1: 1Kb Plus DNA Ladder
M2: *Trans*15K DNA Marker
Lane 1: REPA 1.8 kb;
Lane 2: NCBP 2.5 kb;
Lane 3: HDP 3.5 kb;
Lane 4: VIN 4.6 kb;
Lane 5: Pol 6.8 kb;
Lane 6: APC 8.5 kb;
Lane 7: Dynein 12.3 kb
Human cDNA as templates

2×*EasyPfu* PCR SuperMix

Mix (-dye)	AS211-01	1 ml
	AS211-02	5×1 ml

Storage
at -20°C for two years

Description

EasyPfu PCR SuperMix is a ready-to-use mixture of *EasyPfu* DNA Polymerase, dNTPs and optimized buffer. The SuperMix is provided at 2× concentration and used at 1× concentration by adding template, primers and H_2O.

- *EasyPfu* PCR SuperMix offers 18-fold fidelity as compared to *EasyTaq*® DNA Polymerase.
- Extension rate is about 0.5 kb/min.
- PCR products can be directly cloned into *pEASY*®-Blunt vectors.
- Amplification of genomic DNA fragment up to 6 kb.
- Amplification of plasmid DNA fragment up to 10 kb.

Applications

- High fidelity PCR
- Blunt end cloning
- Site-directed mutagenesis

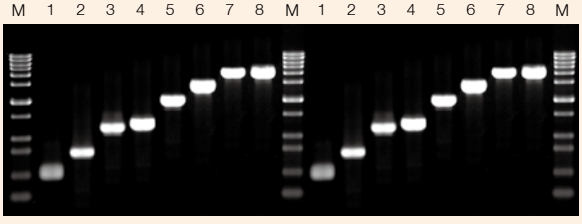
PROTOCOL

Reaction Components

Component	Volume	Final Concentration
Template	Variable	as required
Forward Primer (10 μM)	1 μl	0.2 μM
Reverse Primer (10 μM)	1 μl	0.2 μM
2×*EasyPfu* PCR SuperMix	25 μl	1×
ddH_2O	Variable	-
Total volume	50 μl	-

Thermal cycling conditions

94°C	2-5 min	
94°C	30 sec	30-35 cycles
50-60°C	30 sec	
72°C	0.5 kb/min	
72°C	5-10 min	



EasyPfu DNA Polymerase 2x*EasyPfu* PCR SuperMix

M: 1Kb Plus DNA Ladder
Lane 1: CCRD 0.5 kb;
Lane 2: BDNF 0.8 kb;
Lane 3: Rhod 1.2 kb;
Lane 4: β-globin 1.3 kb;
Lane 5: Rhod 2 kb;
Lane 6: β-globin 3 kb;
Lane 7: Rhod 4.17 kb;
Lane 8: β-globin 4.1 kb
50 ng of Human Genomic DNA as templates

2×*TransStart*® *FastPfu* PCR SuperMix

Mix (-dye)	AS221-01	1 ml
	AS221-02	5×1 ml

Storage
at -20℃ for two years

Description

TransStart® *FastPfu* PCR SuperMix is a ready-to-use mixture of *TransStart*® *FastPfu* DNA polymerase, dNTPs, and optimized buffer. The SuperMix is provided at 2× concentration and can be used at 1× concentration by adding template, primers and H_2O.

- *TransStart*® *FastPfu* PCR SuperMix offers 54-fold fidelity as compared to *EasyTaq*® DNA Polymerase.
- Extension rate is about 2-4 kb/min.
- PCR products can be directly cloned into *pEASY*®-Blunt vectors.
- Amplification of genomic DNA fragment up to 15 kb.
- Amplification of plasmid DNA fragment up to 20 kb.

Applications

- High fidelity PCR
- High yield PCR
- Fast PCR
- Blunt end cloning
- Site-directed mutagenesis
- Complex templates

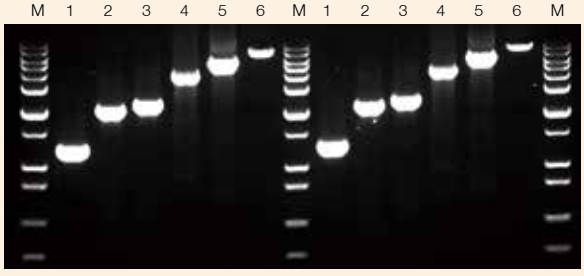
PROTOCOL

Reaction Components

Component	Volume	Final Concentration
Template	Variable	as required
Forward Primer (10 μM)	1 μl	0.2 μM
Reverse Primer (10 μM)	1 μl	0.2 μM
2×*TransStart*® *FastPfu* PCR SuperMix	25 μl	1×
ddH_2O	Variable	-
Total volume	50 μl	-

Thermal cycling conditions

94℃	2-5 min	
94℃	20 sec	30-35 cycles
50-60℃	20 sec	
72℃	2-4 kb/min	
72℃	5-10 min	



TransStart® *FastPfu* DNA Polymerase 2×*TransStart*® *FastPfu* PCR SuperMix

M: 1Kb Plus DNA Ladder
Lane 1: β-globin 1.3 kb Human genomic DNA
Lane 2: Rhod 2.0 kb Human genomic DNA
Lane 3: NCBP 2.5 kb Human cDNA
Lane 4: VIN 4.6 kb Human cDNA
Lane 5: Pol 6.8 kb Human cDNA
Lane 6: LN 10.0 kb Plasmid DNA

Direct PCR

TransDirect® PCR uses a proprietary formulated lysis reagent to release nucleic acids from a variety of fresh or frozen animal cells/tissues and plant tissues. Unpurified DNA is used as template for PCR using 2×*TransDirect*® PCR SuperMix which has extremely high resistance to PCR inhibitors found in animal tissues, plant tissues and blood.

Direct PCR Kit	Application
TransDirect® Animal Tissue PCR Kit	Mammalian cell cultures, saliva, hair shaft, animal tissues, blood
TransDirect® Plant Tissue PCR Kit	Low polysaccharides, low polyphenols plant tissues
TransDirect® Blood PCR Kit	Fresh or frozen blood stored in EDTA, heparin, or citric acid Fresh or dried blood without anticoagulant Human oral epithelial cells

TransDirect® Animal Tissue PCR Kit

AD201-01	100 rxns (20 µl per reaction)
AD201-02	500 rxns (20 µl per reaction)

Storage
at -20°C for two years

Description

TransDirect® Animal Tissue PCR Kit uses a unique lysis buffer to lyse animal tissues (fresh or frozen) and blood. The resulting lysate without purification can be directly used as PCR template. 2×*TransDirect*® PCR SuperMix (+dye) is highly resistant to various PCR inhibitors present in animal tissues. PCR product can be directly used for gel electrophoresis.

Applications

- Direct amplification from unpurified lysate. Suitable for high throughput applications.
- Suitable for mammalian cells, saliva, hair shaft, animal tissues and blood.
- Amplification of genomic DNA fragment up to 3 kb.

Kit Contents

Component	AD201-01	AD201-02
AD1 Buffer	4 ml	20 ml
AD2 Buffer	1 ml	5 ml
AD3 Buffer	4 ml	2×10 ml
2×*TransDirect*® PCR SuperMix (+dye)	1 ml	5×1 ml
ddH_2O	5 ml	25 ml

Materials

Material	Amount
Mammalian Cells	≤10^6 cell
Hair shaft	≤10 mg
Mouse Tail	≤0.5 cm
Mouse Ear	≤0.5 cm^2
Saliva	≤10 µl
Animal Tissues	≤10 mg
Blood	≤10 µl

PROTOCOL

Genomic DNA extraction

1. Mix 40 µl of AD1 buffer with 10 µl of AD2 buffer. For more samples, premix AD1 buffer with AD2 buffer at a ratio of 4:1. The mixture can be stored up to 2 hours at room temperature.
2. Sample treatment

- Mammalian Cells
  Pellet the cells by centrifugation and remove the supernatant. Add the mixture of AD1/AD2, mix thoroughly by pipetting up and down.
- Saliva
  Directly add saliva into the mixture of AD1/AD2, mix thoroughly by pipetting up and down.
- Hair Shafts
  Cut hair into pieces, add the mixture of AD1/AD2, mix thoroughly by pipetting up and down.
- Animal Tissues
  Cut up tissues with sterile scissors or blade, add the mixture of AD1/AD2, mix thoroughly by pipetting up and down.
- Blood
  Directly add blood into the mixture of AD1/AD2, mix thoroughly by pipetting up and down.

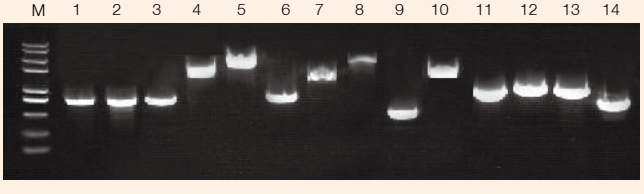
3. Incubate at room temperature for 10 minutes, followed by at 95°C for 3 minutes (for hard-to-lyse tissues, like hair, we suggest incubating at 55°C for 10 minutes, followed by at 95°C for 3 minutes).
4. Add 40 µl of AD3 buffer, mix well. The lysate can be used as PCR template or stored at 4°C for three months or at -20°C for six months.

PCR

Component	Volume	Final Concentration
Unpurified Lysate	Variable (≤4 µl)	as required
Forward Primer (10 µM)	0.4 µl	0.2 µM
Reverse Primer (10 µM)	0.4 µl	0.2 µM
2×*TransDirect*® PCR SuperMix (+dye)	10 µl	1×
ddH_2O	Variable	-
Total volume	20 µl	-

Thermal cycling conditions

94°C	5-10 min	
94°C	30 sec	35-40 cycles
50-60°C	30 sec	
72°C	1-2 kb/min	
72°C	5-10 min	



M: *Trans*2K® Plus II DNA Marker
Lane 1: Hair 0.8 kb
Lane 2: Saliva 0.8 kb
Lane 3: HeLa cell 0.8 kb
Lane 4: HeLa cell 2 kb
Lane 5: HeLa cell 3 kb
Lane 6: Mouse ear 0.86 kb
Lane 7: Mouse ear 1.8 kb
Lane 8: Mouse ear 3 kb
Lane 9: Drosophila 0.42 kb
Lane 10: Drosophila 2 kb
Lane 11: Nematode 0.9 kb
Lane 12: Shrimp 1.1 kb
Lane 13: Crab 1 kb
Lane 14: Razor clam 0.56 kb

TransDirect® Plant Tissue PCR Kit

AD301-01	100 rxns (20 µl per reaction)
AD301-02	500 rxns (20 µl per reaction)

Storage
at -20°C for two years

Description

TransDirect® Plant Tissue PCR Kit uses a unique lysis buffer to lyse plant tissues (fresh or frozen). The resulting lysate without purification can be directly used as PCR template. 2×*TransDirect*® PCR SuperMix (+dye) is highly resistant to various PCR inhibitors present in plant tissues. PCR product can be directly used for gel electrophoresis.

Applications

- Direct amplification from unpurified lysate. Suitable for high throughput applications.
- Amplification of genomic DNA fragment up to 2 kb.

Kit Contents

Component	AD301-01	AD301-02
PD1 Buffer	4 ml	20 ml
PD2 Buffer	4 ml	20 ml
2×*TransDirect*® PCR SuperMix (+dye)	1 ml	5×1 ml
ddH_2O	5 ml	25 ml

PROTOCOL

Genomic DNA Extraction

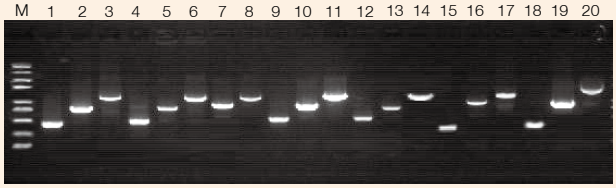
1. Cut 5 mg or 0.5 cm^2 plant tissues and add it to a tube containing 40 µl of PD1 buffer, vortex.
2. Incubate at 95°C for 10 minutes (for hard-to-lyse tissues, we suggest incubating at 95°C for 30 minutes).
3. Add 40 µl of PD2 buffer and vortex to mix. The lysate can be used as PCR template or stored at 4°C for three months or at -20°C for six months.

PCR

Component	Volume	Final Concentration
Unpurified Lysate	Variable (≤4 µl)	as required
Forward Primer (10 µM)	0.4 µl	0.2 µM
Reverse Primer (10 µM)	0.4 µl	0.2 µM
2×*TransDirect*® PCR SuperMix (+dye)	10 µl	1×
ddH_2O	Variable	-
Total volume	20 µl	-

Thermal cycling conditions

94°C	5-10 min	
94°C	30 sec	35-40 cycles
50-60°C	30 sec	
72°C	1-2 kb/min	
72°C	5-10 min	



M: *Trans*2K® Plus II DNA Marker

Lane 1: Corn 0.4 kb	Lane 8: Soybean 1.5 kb	Lane 15: Cotton 0.3 kb
Lane 2: Corn 0.8 kb	Lane 9: Arabidopsis 0.5 kb	Lane 16: Cotton 1 kb
Lane 3: Corn 1.5 kb	Lane 10: Arabidopsis 0.9 kb	Lane 17: Cotton 1.6 kb
Lane 4: Wheat 0.4 kb	Lane 11: Arabidopsis 1.5 kb	Lane 18: Rice 0.3 kb
Lane 5: Wheat 0.9 kb	Lane 12: Tobacco 0.5 kb	Lane 19: Rice 0.9 kb
Lane 6: Wheat 1.5 kb	Lane 13: Tobacco 0.9 kb	Lane 20: Rice 1.9 kb
Lane 7: Soybean 0.9 kb	Lane 14: Tobacco 1.5 kb	

TransDirect® Blood PCR Kit

AD401-01	100 rxns (20 μl per reaction)
AD401-02	500 rxns (20 μl per reaction)

Storage
at -20°C for two years

Description

TransDirect® Blood PCR Kit is designed for DNA amplification from whole blood without DNA extraction. 2×*TransDirect*® PCR SuperMix (+dye) is highly resistant to various PCR inhibitors present in blood.

Applications

- Fresh or frozen blood stored in EDTA, heparin or citric acid
- Fresh or dried blood without anticoagulant
- Human oral epithelial cells
- Amplification of genomic DNA fragment up to 4 kb

Kit Contents

Component	AD401-01	AD401-02
2×*TransDirect*® PCR SuperMix (+dye)	1 ml	5×1 ml
ddH_2O	5 ml	25 ml

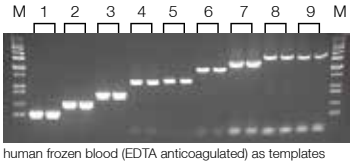
PROTOCOL

PCR

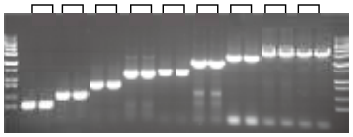
Component	Volume	Final Concentration
Blood	Variable (≤1 µl)	as required
Forward Primer (10 µM)	0.4 µl	0.2 µM
Reverse Primer (10 µM)	0.4 µl	0.2 µM
2×*TransDirect*® PCR SuperMix (+dye)	10 µl	1×
ddH_2O	Variable	-
Total volume	20 µl	-

Thermal cycling conditions

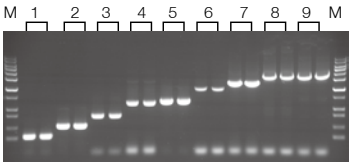
94°C	5 min	
94°C	30 sec	30-40 cycles
50-60°C	30 sec	
72°C	1-2 kb/min	
72°C	5-10 min	



human frozen blood (EDTA anticoagulated) as templates



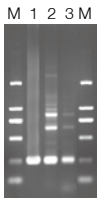
human frozen blood (heparin anticoagulated) as templates



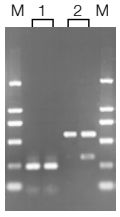
human fresh blood (without anticoagulant) as templates

0.5 µl of blood in 20 µl reaction.

M: 1Kb Plus DNA Ladder
1: Hdt gene 0.32 kb
2: Hmt gene 0.5 kb
3: BDNF 0.8 kb
4: Rhod 1.2 kb
5: β-globin 1.3 kb
6: Rhod 2.0 kb
7: β-globin 3.0 kb
8: Rhod 4.17 kb
9: β-globin 4.1 kb



0.5 µl of diluted chicken blood (sodium citrate anticoagulated) as templates to amplify a 0.25 kb fragment in 20 µl reaction.
M: *Trans*2K® DNA Marker
Lane 1: Blood
Lane 2: 1:10 dilution
Lane 3: 1:100 dilution

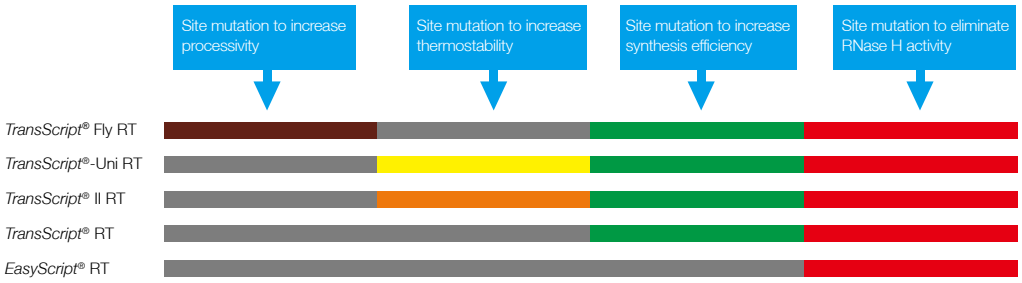


0.5 µl of 1:40 diluted mouse blood (heparin anticoagulated) as templates to amplify Neo and MAP genes in 20 µl reaction.
M: *Trans*2K® DNA Marker
1: Neo 0.25 kb
2: MAP 0.6 kb

Human oral epithelial cells as templates to amplify Hdt gene (0.32 kb) in 20 µl reaction.
M: *Trans*2K® DNA Marker

RT-PCR

Trans reverse transcriptases



Products	Size of cDNA products	Temperature	Sensitivity	Fidelity	GC-rich or Complex template
EasyScript® RT	≤8 kb	42°C	•	•	•
TransScript® RT	≤12 kb	42°C	• •	• •	• •
TransScript® II RT	≤15 kb	42°C-55°C	• • •	• •	• • •
EasyScript® First-Strand cDNA Synthesis SuperMix	≤8 kb	42°C	•	•	•
EasyScript® One-Step gDNA Removal and cDNA Synthesis SuperMix	≤8 kb	42°C	•	•	•
TransScript® First-Strand cDNA Synthesis SuperMix	≤12 kb	42°C	• •	• •	• •
TransScript® One-Step gDNA Removal and cDNA Synthesis SuperMix	≤12 kb	42°C	• •	• •	• •
TransScript® Fly First-Strand cDNA Synthesis SuperMix	≤12 kb	42°C	• •	• •	• •
TransScript®-Uni One-Step gDNA Removal and cDNA Synthesis SuperMix	≤20 kb	42°C-65°C	• • •	• •	• • •
TransScript®-Uni Cell to cDNA Synthesis SuperMix for qPCR	≤ 250 bp	42°C	• • •	• •	• • •
TransScript® miRNA First-Strand cDNA Synthesis SuperMix	≤ 250 bp	42°C	• •	• •	• •
TransScript® II First-Strand cDNA Synthesis SuperMix	≤15 kb	42°C-55°C	• • •	• •	• • •
TransScript® II One-Step gDNA Removal and cDNA Synthesis SuperMix	≤15 kb	42°C-55°C	• • •	• •	• • •
TransScript® All-in-One First-Strand cDNA Synthesis SuperMix for PCR	≤12 kb	42°C	• •	• •	• •
TransScript® All-in-One First-Strand cDNA Synthesis SuperMix for qPCR (One-Step gDNA Removal)	≤ 250 bp	42°C	• •	• •	• •
TransScript® II All-in-One First-Strand cDNA Synthesis SuperMix for PCR	≤15 kb	42°C-55°C	• • •	• •	• • •
TransScript® II All-in-One First-Strand cDNA Synthesis SuperMix for qPCR (One-Step gDNA Removal)	≤ 250 bp	42°C-55°C	• • •	• •	• • •
TransScript® Two-Step RT-PCR SuperMix	≤12 kb	42°C	• •	• •	• •
TransScript® II Two-Step RT-PCR SuperMix	≤15 kb	42°C-55°C	• • •	• •	• • •
EasyScript® One-Step RT-PCR SuperMix	≤4 kb	45°C	•	•	•
TransScript® One-Step RT-PCR SuperMix	≤8 kb	45°C	• •	• •	• •
TransScript® II One-Step RT-PCR SuperMix	≤8 kb	50°C	• • •	• •	• • •

Trans RT
no heating or ice bath needed

Add RNA, primers, buffer, RNase inhibitor, dNTPs, *Trans* RTase

↓

Incubate 30 min at 42°C/50°C, then 5 sec at 85°C

Trans First-Strand cDNA Synthesis SuperMix
simple and fast

Primer+RNA
+
2×RT Reaction Mix (Buffer+dNTPs)
+
RT/RI Enzyme Mix
(RT Enzyme+RNase Inhibitor)

↓

Incubate 30 min/ 5 min at 42°C/50°C, then 5 sec at 85°C

gDNA Removal and cDNA Synthesis
simultaneous genomic DNA removal and cDNA synthesis

RNA+gDNA Remover and cDNA Synthesis SuperMix

↓

Incubate 30 min at 42°C/50°C, then 5 sec at 85°C

All-in-One for PCR/qPCR
simple and fast

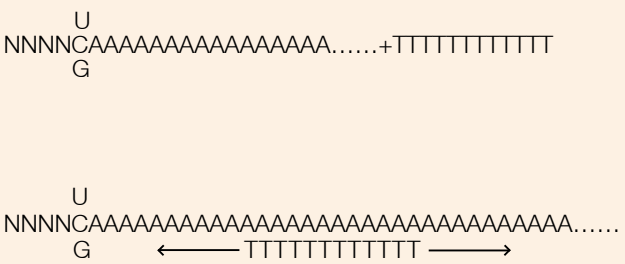
RNA+All-in-One SuperMix for PCR/qPCR

↓

Incubate 30 min/15 min at 42°C/50°C, then 5 sec at 85°C

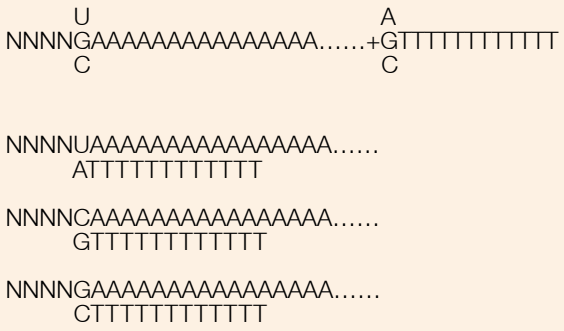
- *Trans* RT (except one-step kits) use Anchored Oligo(dT) to increase cDNA yield and full length cDNA products.

Traditional Oligo(dT)$_{12\text{-}18}$ Primer



- Poly(A) tail can be a few hundreds nt long and Oligo(dT) Primer binds randomly.
- Lower efficiency because of long poly(A) tail.
- Less full length cDNA products.

Anchored Oligo(dT) Primer



- Anchored Oligo(dT) Primer only anneals at 5' end of the Poly(A) tail of mRNA.
- Higher efficiency because of Anchored Oligo(dT) Primer.
- More full length cDNA products.

EasyScript® Reverse Transcriptase[M-MLV, RNase H$^-$]

AE101-02	10,000 units
AE101-03	5×10,000 units

Concentration
200 units/μl

Contents
- *EasyScript*® RT
- 5×ES RT Buffer (375 mM KCl; 15 mM $MgCl_2$; 100 mM Tris-HCl pH 8.4)
- Anchored Oligo$(dT)_{18}$ Primer

Storage
at -20°C for one year

Description
EasyScript® Reverse Transcriptase is an engineered version of M-MLV reverse transcriptase with deficient RNase H activity. The enzyme is purified to near homogeneity from *E.coli* containing the modified M-MLV RT gene.
- Deficient RNase H activity to reduce RNA template degradation during the first-strand cDNA synthesis.
- Anchored Oligo$(dT)_{18}$ Primer for higher yield and more full length cDNA.
- cDNA up to 8 kb.

Applications
- First-strand cDNA synthesis
- Multiple copy gene detection

Unit Definition
One unit of *EasyScript*® RT incorporates 1 nmol of deoxyribonucleotide into acid precipitable material in 10 minutes at 37°C using Poly(A)/Oligo(dT) as template/primer.

PROTOCOL

First-Strand cDNA synthesis

1. Reaction Components

Component	Volume
Total RNA/mRNA	50 ng-5 μg/5-500 ng
Anchored Oligo$(dT)_{18}$ Primer (0.5 μg/μl)	1 μl
or Random Primer(N9) (0.1 μg/μl)	1 μl
or GSP	2 pmol
10 mM dNTPs	1 μl
5×ES RT Buffer	4 μl
Ribonuclease Inhibitor (50 units/μl)	0.5 μl
EasyScript® RT	1 μl
RNase-free Water	to 20 μl

2. Incubation
- For anchored oligo$(dT)_{18}$ primer or GSP, incubate at 42°C for 30 minutes.
- For random primer, incubate at 25°C for 10 minutes, then at 42°C for 30 minutes.

3. Incubate at 85°C for 5 seconds to inactivate enzymes.

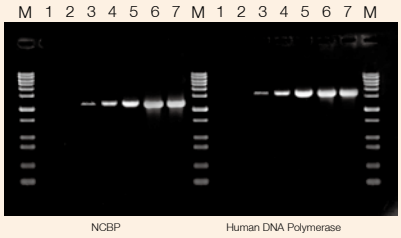
RT-PCR

Reaction Components

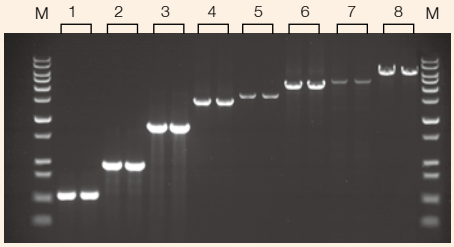
Component	Volume	Final Concentration
Template	Variable	as required
Forward Primer (10 μM)	1 μl	0.2 μM
Reverse Primer (10 μM)	1 μl	0.2 μM
2×*TransTaq*® HiFi PCR SuperMix II	25 μl	1×
ddH_2O	Variable	-
Total volume	50 μl	-

Thermal cycling conditions

94°C	2-5 min	
94°C	30 sec	35-40 cycles
50-60°C	30 sec	
72°C	1-2 kb/min	
72°C	5-10 min	



M: 1Kb Plus DNA Ladder
Lanes 1-7: 0, 0.01, 0.1, 1, 10, 100,
1,000 pg human total RNA as templates



M: 1Kb Plus DNA Ladder
1: GAPDH 0.5 kb; 2: GAPDH 0.9 kb;
3: REPA 1.8 kb; 4: ACTR 3 kb;
5: ACTR 3.5 kb; 6: VIN 4.6 kb;
7: TSC 5.3 kb; 8: Pol 6.8 kb
Human cDNA as templates

TransScript® Reverse Transcriptase[M-MLV, RNase H$^-$]

AT101-02	10,000 units
AT101-03	5×10,000 units

Concentration
200 units/μl

Contents
- *TransScript*® RT
- 5×TS RT Buffer (250 mM KCl; 15 mM $MgCl_2$; 100 mM Tris-HCl pH 8.4)
- Anchored Oligo$(dT)_{18}$ Primer

Storage
at -20°C for one year

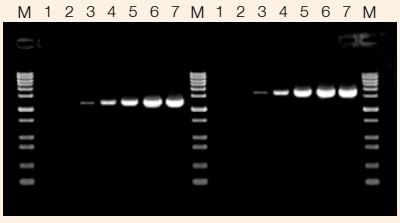
Description
TransScript® Reverse Transcriptase is a recombinant M-MLV reverse transcriptase with deficient RNase H activity.
- Deficient RNase H activity to reduce RNA template degradation during the first-strand cDNA synthesis.
- Anchored Oligo$(dT)_{18}$ Primer for higher yield and more full length cDNA.
- cDNA up to 12 kb.

Applications
- First-strand cDNA synthesis
- Multiple copy and low copy gene detection

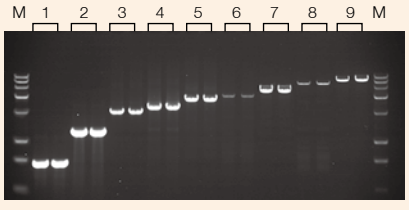
Unit Definition
One unit of *TransScript*® RT incorporates 1 nmol of deoxyribonucleotide into acid-precipitable material in 10 minutes at 37°C using Poly(A)/Oligo(dT) as template/primer.

PROTOCOL

The suggested condition for the first-strand cDNA synthesis and RT-PCR are the same as described on pages 41-42.



M: 1Kb Plus DNA Ladder
Lanes 1-7: 0, 0.01, 0.1, 1, 10, 100, 1,000 pg human total RNA as templates



M: *Trans*15K DNA Marker
1: GAPDH 0.9 kb; 2: REPA 1.8 kb;
3: ACTR 3 kb; 4: HDP 3.5 kb;
5: VIN 4.6 kb; 6: TSC 5.3 kb;
7: Pol 6.8 kb; 8: FIB 9.4 kb;
9: Dynein 12.3 kb
Human cDNA as templates

TransScript® II Reverse Transcriptase[M-MLV, RNase H$^-$] (High Temperature RT)

AH101-02	10,000 units

Concentration
200 units/μl

Contents
- *TransScript*® II RT
- 10×TS II RT Buffer (500 mM KCl; 30 mM $MgCl_2$; 200 mM Tris-HCl pH 8.4)
- Anchored Oligo(dT)$_{20}$ Primer

Storage
at -20°C for one year

Description
TransScript® II Reverse Transcriptase is a recombinant M-MLV reverse transcriptase with deficient RNase H activity and increased thermostability. The enzyme is active at up to 55°C. It provides higher specificity, higher yield and more full-length cDNA products.
- Increased thermostability for more full-length cDNA products.
- Reaction temperature at 42°C-55°C.
- Deficient RNase H activity to reduce RNA template degradation during the first-strand cDNA synthesis.
- Anchored Oligo(dT)$_{20}$ Primer for higher yield and more full length cDNA.
- cDNA up to 15 kb.

Applications
- First-strand cDNA synthesis, cDNA library construction, 3' and 5' RACE
- Multiple copy and low copy gene detection
- GC-rich or complex secondary structure RNA template

Unit Definition
One unit of *TransScript*® II RT incorporates 1 nmol of deoxyribonucleotide into acid-precipitable material in 10 minutes at 37°C using Poly(A)/Oligo(dT) as template/primer.

PROTOCOL

First-Strand cDNA synthesis

1. Reaction Components

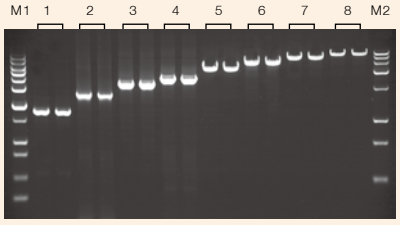
Component	Volume
Total RNA/mRNA	50 ng-5 μg/5-500 ng
Anchored Oligo(dT)$_{20}$ Primer (0.5 μg/μl)	1 μl
or Random Primer(N9) (0.1 μg/μl)	1 μl
or GSP	2 pmol
10 mM dNTPs	1 μl
10×TS II RT Buffer	2 μl
Ribonuclease Inhibitor (50 units/μl)	0.5 μl
TransScript® II RT	1 μl
RNase-free Water	to 20 μl

2. Incubation
- For anchored oligo(dT)$_{20}$ primer or GSP, incubate at 50°C for 30 minutes.
- For random primer, incubate at 25°C for 10 minutes, then at 50°C for 30 minutes.
- For GC-rich or complex secondary structure RNA template, incubate at 55°C for 30 minutes.

3. Incubate at 85°C for 5 seconds to inactivate enzymes.

RT-PCR

The suggested reaction condition is the same as described on page 42.



M1: 1Kb Plus DNA Ladder
M2: *Trans*15K DNA Marker
1: REPA 1.8 kb; 2: NCBP 2.5 kb;
3: HDP 3.5 kb; 4: VIN 4.6 kb;
5: Pol 6.8 kb; 6: APC 8.5 kb;
7: Dynein 12.3 kb; 8: FAL 15.1 kb
Human cDNA as templates

EasyScript® First-Strand cDNA Synthesis SuperMix

AE301-02	50 rxns (20 µl per reaction)
AE301-03	100 rxns (20 µl per reaction)

Storage

at -20°C for one year

Description

EasyScript® First-Strand cDNA Synthesis SuperMix provides all the necessary components for cDNA synthesis from total RNA or mRNA. The cDNA is efficiently synthesized by *EasyScript*® RT/RI Enzyme Mix and 2×ES Reaction Mix.

- Deficient RNase H activity to reduce RNA template degradation during the first-strand cDNA synthesis.
- The product obtained from 15 minutes reaction is used for qPCR; the product obtained from 30 minutes reaction is used for PCR.
- Anchored Oligo(dT)$_{18}$ Primer for higher yield and more full length cDNA.
- cDNA up to 8 kb.

Application

Multiple copy gene detection

Kit Contents

Component	AE301-02	AE301-03
EasyScript® RT/RI Enzyme Mix	50 µl	100 µl
2×ES Reaction Mix	500 µl	1 ml
Random Primer(N9) (0.1 µg/µl)	50 µl	100 µl
Anchored Oligo(dT)$_{18}$ Primer (0.5 µg/µl)	50 µl	100 µl
RNase-free Water	500 µl	1 ml

PROTOCOL

First-Strand cDNA synthesis

1. Reaction Components

Component	Volume
Total RNA/mRNA	50 ng -5 µg/5-500 ng
Anchored Oligo(dT)$_{18}$ Primer (0.5 µg/µl)	1 µl
or Random Primer(N9) (0.1 µg/µl)	1 µl
or GSP	2 pmol
2×ES Reaction Mix	10 µl
EasyScript® RT/RI Enzyme Mix	1 µl
RNase-free Water	to 20 µl

2. Incubation

- For anchored oligo(dT)$_{18}$ primer or GSP, incubate at 42°C for 15 minutes (for qPCR) or incubate at 42°C for 30 minutes (for PCR).
- For random primer, incubate at 25°C for 10 minutes. After that, incubate at 42°C for 15 minutes (for qPCR) or incubate at 42°C for 30 minutes (for PCR).

3. Incubate at 85°C for 5 seconds to inactivate enzymes.

RT-PCR

The suggested reaction condition is the same as described on page 42.

EasyScript® One-Step gDNA Removal and cDNA Synthesis SuperMix

AE311-02	50 rxns (20 µl per reaction)
AE311-03	100 rxns (20 µl per reaction)

Storage
at -20°C for one year

Description

Unique genomic DNA remover is combined with *EasyScript*® First-Strand cDNA Synthesis SuperMix to achieve simultaneous genomic DNA removal and cDNA synthesis. After cDNA synthesis, gDNA remover and reverse transcriptase are inactivated by heating at 85°C for 5 seconds.

- Simultaneous genomic DNA removal and cDNA synthesis in one tube to minimize RNA contamination.
- The product obtained from 15 minutes reaction is used for qPCR; the product obtained from 30 minutes reaction is used for PCR.
- cDNA up to 8 kb.

Application

Multiple copy gene detection

Kit Contents

Component	AE311-02	AE311-03
EasyScript® RT/RI Enzyme Mix	50 µl	100 µl
gDNA Remover	50 µl	100 µl
2×ES Reaction Mix	500 µl	1 ml
Random Primer(N9) (0.1 µg/µl)	50 µl	100 µl
Anchored Oligo(dT)$_{18}$ Primer (0.5 µg/µl)	50 µl	100 µl
RNase-free Water	500 µl	1 ml

PROTOCOL

First-Strand cDNA synthesis

1. Reaction Components

Component	Volume
Total RNA/mRNA	50 ng -5 µg/5-500 ng
Anchored Oligo(dT)$_{18}$ Primer (0.5 µg/µl)	1 µl
or Random Primer(N9) (0.1 µg/µl)	1 µl
or GSP	2 pmol
2×ES Reaction Mix	10 µl
EasyScript® RT/RI Enzyme Mix	1 µl
gDNA Remover	1 µl
RNase-free Water	to 20 µl

2. Incubation

- For anchored oligo(dT)$_{18}$ primer or GSP, incubate at 42°C for 15 minutes (for qPCR) or incubate at 42°C for 30 minutes (for PCR).
- For random primer, incubate at 25°C for 10 minutes. After that, incubate at 42°C for 15 minutes (for qPCR) or incubate at 42°C for 30 minutes (for PCR).

3. Incubate at 85°C for 5 seconds to inactivate enzymes.

RT-PCR

The suggested reaction condition is the same as described on page 42.

TransScript® First-Strand cDNA Synthesis SuperMix

AT301-02	50 rxns (20 µl per reaction)
AT301-03	100 rxns (20 µl per reaction)

Storage
at -20°C for one year

Description

TransScript® First-Strand cDNA Synthesis SuperMix provides all the necessary components for cDNA synthesis from total RNA or mRNA. The cDNA is efficiently synthesized by *TransScript*® RT/RI Enzyme Mix and 2×TS Reaction Mix.

- Deficient RNase H activity to reduce RNA template degradation during the first-strand cDNA synthesis.
- The product obtained from 15 minutes reaction is used for qPCR; the product obtained from 30 minutes reaction is used for PCR.
- Anchored Oligo(dT)$_{18}$ Primer for higher yield and more full length cDNA.
- cDNA up to 12 kb.

Application

Multiple copy and low copy gene detection

Kit Contents

Component	AT301-02	AT301-03
TransScript® RT/RI Enzyme Mix	50 µl	100 µl
2×TS Reaction Mix	500 µl	1 ml
Random Primer(N9) (0.1 µg/µl)	50 µl	100 µl
Anchored Oligo(dT)$_{18}$ Primer (0.5 µg/µl)	50 µl	100 µl
RNase-free Water	500 µl	1 ml

PROTOCOL

First-Strand cDNA synthesis

1. Reaction Components

Component	Volume
Total RNA/mRNA	50 ng -5 µg/5-500 ng
Anchored Oligo(dT)$_{18}$ Primer (0.5 µg/µl)	1 µl
or Random Primer(N9) (0.1 µg/µl)	1 µl
or GSP	2 pmol
2×TS Reaction Mix	10 µl
TransScript® RT/RI Enzyme Mix	1 µl
RNase-free Water	to 20 µl

2. Incubation

- For anchored oligo(dT)$_{18}$ primer or GSP, incubate at 42°C for 15 minutes (for qPCR) or incubate at 42°C for 30 minutes (for PCR).
- For random primer, incubate at 25°C for 10 minutes. After that, incubate at 42°C for 15 minutes (for qPCR) or incubate at 42°C for 30 minutes (for PCR).

3. Incubate at 85°C for 5 seconds to inactivate enzymes.

RT-PCR

The suggested reaction condition is the same as described on page 42.

TransScript® One-Step gDNA Removal and cDNA Synthesis SuperMix

AT311-02	50 rxns (20 µl per reaction)
AT311-03	100 rxns (20 µl per reaction)

Storage
at -20°C for one year

Description

Unique genomic DNA remover is combined with *TransScript*® First-Strand cDNA Synthesis SuperMix to achieve simultaneous genomic DNA removal and cDNA synthesis. After cDNA synthesis, gDNA remover and reverse transcriptase are inactivated by heating at 85°C for 5 seconds.

- Simultaneous genomic DNA removal and cDNA synthesis in one tube to minimize RNA contamination.
- The product obtained from 15 minutes reaction is used for qPCR; the product obtained from 30 minutes reaction is used for PCR.
- cDNA up to 12 kb.

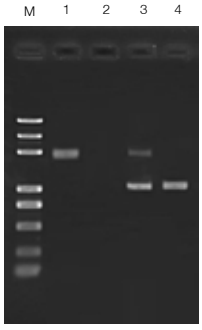
Application

Multiple copy and low copy gene detection

Kit Contents

Component	AT311-02	AT311-03
TransScript® RT/RI Enzyme Mix	50 µl	100 µl
gDNA Remover	50 µl	100 µl
2×TS Reaction Mix	500 µl	1 ml
Random Primer(N9) (0.1 µg/µl)	50 µl	100 µl
Anchored Oligo(dT)$_{18}$ Primer (0.5 µg/µl)	50 µl	100 µl
RNase-free Water	500 µl	1 ml

PROTOCOL



M: *Trans*2K® Plus DNA Marker
PCR +/-gDNA remover
Lane 1: 200 ng Human Genomic DNA (-gDNA remover);
Lane 2: 200 ng Human Genomic DNA (+gDNA remover);
RT-PCR +/- gDNA remover
Lane 3: 100 ng Human total RNA (-gDNA remover);
Lane 4: 100 ng Human total RNA (+gDNA remover);
cDNA as template, 1 kb
Genomic DNA as template, 2 kb

First-Strand cDNA synthesis

1. Reaction Components

Component	Volume
Total RNA/mRNA	50 ng -5 µg/5-500 ng
Anchored Oligo(dT)$_{18}$ Primer (0.5 µg/µl)	1 µl
or Random Primer(N9) (0.1 µg/µl)	1 µl
or GSP	2 pmol
2×TS Reaction Mix	10 µl
TransScript® RT/RI Enzyme Mix	1 µl
gDNA Remover	1 µl
RNase-free Water	to 20 µl

2. Incubation

- For anchored oligo(dT)$_{18}$ primer or GSP, incubate at 42°C for 15 minutes (for qPCR) or incubate at 42°C for 30 minutes (for PCR).
- For random primer, incubate at 25°C for 10 minutes. After that, incubate at 42°C for 15 minutes (for qPCR) or incubate at 42°C for 30 minutes (for PCR).

3. Incubate at 85°C for 5 seconds to inactivate enzymes.

RT-PCR

The suggested reaction condition is the same as described on page 42.

TransScript® Fly First-Strand cDNA Synthesis SuperMix

AF301-02	50 rxns (20 µl per reaction)
AF301-03	100 rxns (20 µl per reaction)

Storage
at -20°C for one year

Description

TransScript® Fly RT is generated by site mutations. It provides high affinity to RNA template with fast extension rate. The cDNA is efficiently synthesized by *TransScript*® Fly RT/RI Enzyme Mix and 2×TS Fly Reaction Mix. The entire reverse transcription can be completed within 5 minutes.

- 5 minutes reverse transcription.
- Deficient RNase H activity to reduce RNA template degradation during the first-strand cDNA synthesis.
- Anchored Oligo(dT)$_{18}$ Primer for higher yield and more full length cDNA.
- cDNA up to 12 kb.

Application

Multiple copy and low copy gene fast detection

Kit Contents

Component	AF301-02	AF301-03
TransScript® Fly RT/RI Enzyme Mix	50 µl	100 µl
2×TS Fly Reaction Mix	500 µl	1 ml
Random Primer(N9) (0.1 µg/µl)	50 µl	100 µl
Anchored Oligo(dT)$_{18}$ Primer (0.5 µg/µl)	50 µl	100 µl
RNase-free Water	500 µl	1 ml

PROTOCOL

First-Strand cDNA synthesis

1. Reaction Components

Component	Volume
Total RNA/mRNA	50 ng -5 µg/5-500 ng
Anchored Oligo(dT)$_{18}$ Primer (0.5 µg/µl)	1 µl
or Random Primer(N9) (0.1 µg/µl)	1 µl
or GSP	2 pmol
2×TS Fly Reaction Mix	10 µl
TransScript® Fly RT/RI Enzyme Mix	1 µl
RNase-free Water	to 20 µl

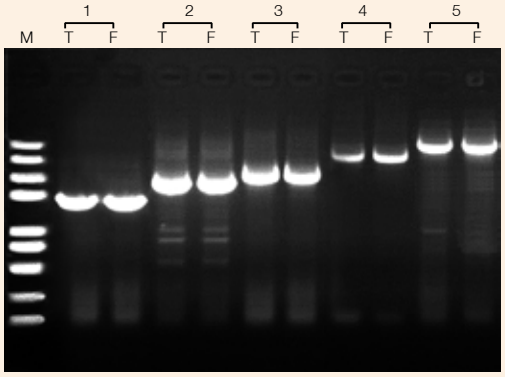
2. Incubation

- For anchored oligo(dT)$_{18}$ primer or GSP, incubate at 42°C for 5 minutes.
- For random primer, incubate at 25°C for 10 minutes, then at 42°C for 5 minutes.

3. Incubate at 85°C for 5 seconds to inactivate enzymes.

RT-PCR

The suggested reaction condition is the same as described on page 42.



M: *Trans*2K® Plus II DNA Marker
1: REPA 1.8 kb
2: NCBP 2.5 kb
3: ACTR 3.5 kb
4: Pol 6.8 kb
5: APC 8.5 kb
T: *TransScript*® RT (35 minutes RT reaction time)
F: *TransScript*® Fly (5 minutes RT reaction time)
Human cDNA as templates

TransScript®-Uni One-Step gDNA Removal and cDNA Synthesis SuperMix

AU311-02	50 rxns (20 µl per reaction)
AU311-03	100 rxns (20 µl per reaction)

Storage
at -20°C for one year

Description

TransScript®-Uni RT is an improved version of M-MLV reverse transcriptase with broad range of reaction temperature (42°C-65°C) and higher thermostability. The suggested reaction temperature is 50°C. The SuperMix contains reagents for simultaneous genomic DNA removal and cDNA synthesis. After cDNA synthesis, gDNA remover and reverse transcriptase are inactivated by heating at 85°C for 5 seconds.

- Broad range reaction temperature (42°C-65°C) .
- Simultaneous genomic DNA removal and cDNA synthesis in one tube to minimize RNA contamination.
- The product obtained from 15 minutes reaction is used for qPCR; the product obtained from 30 minutes reaction is used for PCR.
- cDNA up to 20 kb.

Applications

- Multiple copy and low copy gene detection
- GC-rich or complex secondary structure RNA template
- cDNA library construction, primer extension, 3' and 5' RACE

Kit Contents

Component	AU311-02	AU311-03
TransScript®-Uni RT/RI Enzyme Mix	50 µl	100 µl
gDNA Remover	50 µl	100 µl
2×TS-Uni Reaction Mix	500 µl	1 ml
Random Primer(N9) (0.1 µg/µl)	50 µl	100 µl
Anchored Oligo(dT)$_{20}$ Primer (0.5 µg/µl)	50 µl	100 µl
RNase-free Water	500 µl	1 ml

PROTOCOL

First-Strand cDNA synthesis

1. Reaction Components

Component	Volume
Total RNA/mRNA	50 ng -5 µg/5-500 ng
Anchored Oligo(dT)$_{20}$ Primer (0.5 µg/µl)	1 µl
or Random Primer(N9) (0.1 µg/µl)	1 µl
or GSP	2 pmol
2×TS-Uni Reaction Mix	10 µl
gDNA Remover	1 µl
TransScript®-Uni RT/RI Enzyme Mix	1 µl
RNase-free Water	to 20 µl

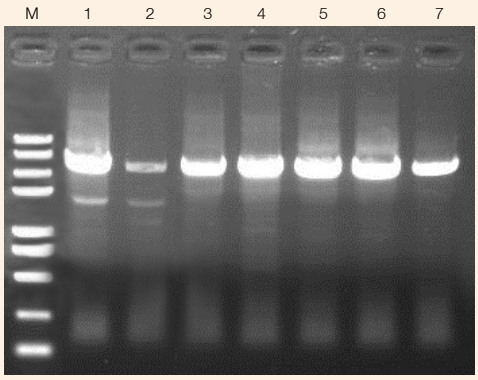
2. Incubation

- For anchored oligo(dT)$_{20}$ primer or GSP, incubate at 50°C for 15 minutes (for qPCR) or incubate at 50°C for 30 minutes (for PCR).
- For random primer, incubate at 25°C for 10 minutes. After that, at incubate 50°C for 15 minutes (for qPCR) or incubate at 50°C for 30 minutes (for PCR).
- For GC-rich or complex secondary structure RNA template, better yield can be obtained by optimizing the reaction temperature.

3. Incubate at 85°C for 5 seconds to inactivate enzymes.

RT-PCR

The suggested reaction condition is the same as described on page 42.



M: *Trans*2K® Plus II DNA Marker
Lane 1: Company A kit, 42°C
Lane 2: Company B kit, 42°C
Lane 3: *TransScript*®-Uni, 42°C
Lane 4: *TransScript*®-Uni, 50°C
Lane 5: *TransScript*®-Uni, 55°C
Lane 6: *TransScript*®-Uni, 60°C
Lane 7: *TransScript*®-Uni, 65°C
Human cDNA as template, VIN 4.6 kb

TransScript®-Uni Cell to cDNA Synthesis SuperMix for qPCR

AC301-01	25 rxns

Storage
at -20°C for one year

Description

TransScript®-Uni Cell to cDNA Synthesis SuperMix for qPCR uses an unique lysis buffer to lyse cells. The resulting lysate (without purification) can be directly used as template for reverse transcription. Unique genomic DNA remover is combined with *TransScript*®-Uni RT/RI Enzyme Mix to achieve simultaneous genomic DNA removal and cDNA synthesis in one tube. This kit is suitable to generate qPCR-ready cDNA directly from cells.

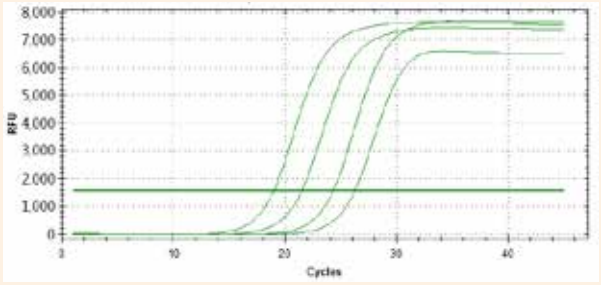
Application

Multiple copy and low copy gene detection

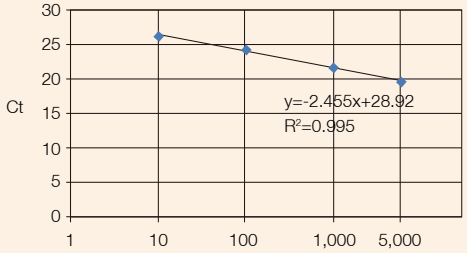
Kit Contents

Component	AC301-01
C to C Lysis Buffer	2×1.25 ml
TransScript®-Uni RT/RI Enzyme Mix	12.5 µl
gDNA Remover	12.5 µl
2×TS-Uni Reaction Mix	250 µl
Oligo(dT)/RP Mix	25 µl
RNase-free Water	250 µl

Amplification Plots



Standard Curve



Cell Types Tested with the Kit

A549	Hep G2	SGC-7901
CHO-K1	K-562	Sp2/0-Ag14
HEK-293	MCF7	Vero
HEK-293T	MDA-MB-231	WI-38
HeLa	P815	

PROTOCOL

Cell Lysis

1. Add 50 µl of C to C Lysis Buffer to each well (5×10^2-5×10^4 cells), incubate at room temperature (22°C-25°C) for 5 minutes.
2. Mix by pipetting up and down. Transfer the lysate into a microcentrifuge tube. Incubate at 75°C for 5 minutes, then place the tube on ice.

First-Strand cDNA synthesis

1. Reaction Components

Component	Volume
Cell Lysate	2 µl
Oligo(dT)/RP Mix	1 µl
2×TS-Uni Reaction Mix	10 µl
gDNA Remover	0.5 µl
TransScript®-Uni RT/RI Enzyme Mix	0.5 µl
RNase-free Water	to 20 µl

2. Gently mix and incubate at 42°C for 15 minutes.
3. Incubate at 85°C for 5 seconds to inactivate *TransScript*®-Uni RT/RI Enzyme Mix and gDNA Remover.

Suggested qPCR conditions (20 µl reaction volume)

Component	Volume	Final Concentration
Template	Variable	as required
Forward Primer (10 µM)	0.4 µl	0.2 µM
Reverse Primer (10 µM)	0.4 µl	0.2 µM
2×*TransStart*® Top/Tip Green qPCR SuperMix	10 µl	1×
Passive Reference Dye (50×) (optional)	0.4 µl	1×
ddH_2O	Variable	-
Total Volume	20 µl	-

Thermal cycling conditions (three-step)

94°C	30 sec	
94°C	5 sec	40-45 cycles
50-60°C	15 sec*	
72°C	10 sec*	

Dissociation Stage

Thermal cycling conditions (two-step)

94°C	30 sec	
94°C	5 sec	40-45 cycles
60°C	30 sec*	

Dissociation Stage

Fluorescent signals can be collected during the annealing or extension stage. For ABI qPCR instrument, we suggest using the following signal collecting time:

* For ABI Prism7700/7900, the time is 30 seconds.
* For ABI Prism7000/7300, the time is 31 seconds.
* For ABI Prism7500, the time is 34 seconds.
* For ABI ViiA 7, the time is at least 19 seconds.

Two-step qPCR is more suitable for higher specificity assay.

Three-step qPCR is more suitable for higher sensitivity assay.

TransScript® miRNA First-Strand cDNA Synthesis SuperMix

AT351-01	20 rxns (20 µl per reaction)

Storage
at -20°C for one year

Description

TransScript® miRNA First-Strand cDNA Synthesis SuperMix provides all the necessary components for cDNA synthesis from miRNA template. High efficient poly(A) tail addition and first-strand cDNA synthesis are performed by *TransScript*® miRNA RT Enzyme Mix (containing tailing enzyme and RT enzyme) and 2×TS miRNA Reaction Mix.

- Optimized enzyme and buffer system for high efficient cDNA synthesis.
- One-step Poly(A) tailing and cDNA synthesis.

Application

miRNA synthesis

Kit Contents

Component	AT351-01
TransScript® miRNA RT Enzyme Mix	20 µl
2×TS miRNA Reaction Mix	200 µl
Universal miRNA qPCR Primer (10 µM)	200 µl
RNase-free Water	1 ml

PROTOCOL

Tail addition and First-Strand cDNA synthesis

1. Reaction Components

Component	Volume
Total RNA/miRNA*	x µl
TransScript® miRNA RT Enzyme Mix	1 µl
2×TS miRNA Reaction Mix	10 µl
RNase-free Water	to 20 µl

* Total RNA ≤5 µg. Since miRNA cannot be directly quantified by spectrophotometer, we suggest using 1-9 µl for 20 µl reaction.

2. Mix gently, and incubate at 37°C for 1 hour.

3. Incubate at 85°C for 5 seconds to inactivate RT Enzyme Mix.

Suggested qPCR conditions (20 µl reaction volume)

Component	Volume	Final Concentration
cDNA*[1]	Variable	as required
Forward Primer (10 µM)*[2]	0.4 µl	0.2 µM
Universal miRNA qPCR Primer (10 µM)	0.4 µl	0.2 µM
2×*TransStart*® Tip/Top Green qPCR SuperMix	10 µl	1×
Passive Reference Dye (50×) (optional)	0.4 µl	1×
ddH_2O	Variable	-
Total volume	20 µl	-

*1. We suggest diluting the synthesized cDNA 5-10 folds.

*2. Upstream primer is target miRNA specific primer, which will be designed by customers according to target miRNA.

Thermal cycling conditions (three-step)

94°C	30 sec	
94°C	5 sec	40-45 cycles
50-60°C	15 sec*	
72°C	10 sec*	

Dissociation Stage

Thermal cycling conditions (two-step)

94°C	30 sec	
94°C	5 sec	40-45 cycles
60°C	30 sec*	

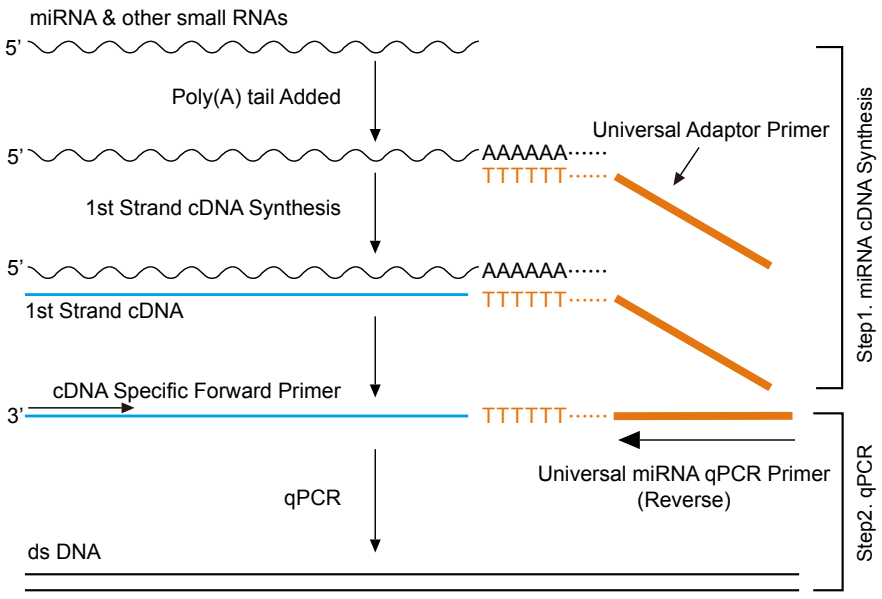
Dissociation Stage

Fluorescent signals can be collected during the annealing or extension stage. For ABI qPCR instrument, we suggest using the following signal collecting time:

* For ABI Prism7700/7900, the time is 30 seconds.
* For ABI Prism7000/7300, the time is 31 seconds.
* For ABI Prism7500, the time is 34 seconds.
* For ABI ViiA 7, the time is at least 19 seconds.

Two-step qPCR is more suitable for higher specificity assay.

Three-step qPCR is more suitable for higher sensitivity assay.



Principle of miRNA Detection

TransScript® II First-Strand cDNA Synthesis SuperMix

AH301-02	50 rxns (20 µl per reaction)
AH301-03	100 rxns (20 µl per reaction)

Storage
at -20°C for one year

Description

TransScript® II First-Strand cDNA Synthesis SuperMix provides all the necessary components for cDNA synthesis from total RNA or mRNA. The cDNA is efficiently synthesized by *TransScript*® II RT/RI Enzyme Mix and 2×TS II Reaction Mix.

- Deficient RNase H activity to reduce RNA template degradation during the first-strand cDNA synthesis.
- The product obtained from 15 minutes reaction is used for qPCR; the product obtained from 30 minutes reaction is used for PCR.
- Anchored Oligo(dT)$_{20}$ Primer for higher yield and more full length cDNA.
- cDNA up to 15 kb.

Applications

- cDNA library construction, 3' and 5' RACE
- Multiple copy and low copy gene detection
- GC-rich or complex secondary structure RNA template

Kit Contents

Component	AH301-02	AH301-03
TransScript® II RT/RI Enzyme Mix	50 µl	100 µl
2×TS II Reaction Mix	500 µl	1 ml
Random Primer(N9) (0.1 µg/µl)	50 µl	100 µl
Anchored Oligo(dT)$_{20}$ Primer (0.5 µg/µl)	50 µl	100 µl
RNase-free Water	500 µl	1 ml

PROTOCOL

First-Strand cDNA synthesis

1. Reaction Components

Component	Volume
Total RNA/mRNA	50 ng-5 µg/5-500 ng
Anchored Oligo(dT)$_{20}$ Primer (0.5 µg /µl)	1 µl
or Random Primer(N9) (0.1 µg/µl)	1 µl
or GSP	2 pmol
2×TS II Reaction Mix	10 µl
TransScript® II RT/RI Enzyme Mix	1 µl
RNase-free Water	to 20 µl

2. Incubation

- For anchored oligo(dT)$_{20}$ primer or GSP, incubate at 50°C for 15 minutes (for qPCR) or incubate at 50°C for 30 minutes (for PCR).
- For random primer, incubate at 25°C for 10 minutes. After that, incubate at 50°C for 15 minutes (for qPCR) or incubate at 50°C for 30 minutes (for PCR).
- For GC-rich or complex secondary structure RNA template, incubate at 55°C for 30 minutes.

3. Incubate at 85°C for 5 seconds to inactivate enzymes.

RT-PCR

The suggested reaction condition is the same as described on page 42.

TransScript® II One-Step gDNA Removal and cDNA Synthesis SuperMix

AH311-02	50 rxns (20 µl per reaction)
AH311-03	100 rxns (20 µl per reaction)

Storage
at -20°C for one year

Description

Unique genomic DNA remover is combined with *TransScript*® II First-Strand cDNA Synthesis SuperMix to achieve simultaneous genomic DNA removal and cDNA synthesis. After cDNA synthesis, gDNA remover and reverse transcriptase are inactivated by heating at 85°C for 5 seconds.

- Simultaneous genomic DNA removal and cDNA synthesis in one tube to minimize RNA contamination. The product obtained from 15 minutes reaction is used for qPCR; the product obtained from 30 minutes reaction is used for PCR.
- cDNA up to 15 kb.

Applications

- cDNA library construction, 3' and 5' RACE
- Multiple copy and low copy gene detection
- GC-rich or complex secondary structure RNA template

Kit Contents

Component	AH311-02	AH311-03
TransScript® II RT/RI Enzyme Mix	50 µl	100 µl
gDNA Remover	50 µl	100 µl
2×TS II Reaction Mix	500 µl	1 ml
Random Primer(N9) (0.1 µg/µl)	50 µl	100 µl
Anchored Oligo(dT)$_{20}$ Primer (0.5 µg/µl)	50 µl	100 µl
RNase-free Water	500 µl	1 ml

PROTOCOL

First-Strand cDNA synthesis

1. Reaction Components

Component	Volume
Total RNA/mRNA	50 ng-5 µg/5-500 ng
Anchored Oligo(dT)$_{20}$ Primer (0.5 µg/µl)	1 µl
or Random Primer(N9) (0.1 µg/µl)	1 µl
or GSP	2 pmol
2×TS II Reaction Mix	10 µl
TransScript® II RT/RI Enzyme Mix	1 µl
gDNA Remover	1 µl
RNase-free Water	to 20 µl

2. Incubation

- For anchored oligo(dT)$_{20}$ primer or GSP, incubate at 50°C for 15 minutes (for qPCR) or incubate at 50°C for 30 minutes (for PCR).
- For random primer, incubate at 25°C for 10 minutes. After that, incubate at 50°C for 15 minutes (for qPCR) or incubate at 50°C for 30 minutes (for PCR).
- For GC-rich or complex secondary structure RNA template, incubate at 55°C for 30 minutes.

3. Incubate at 85°C for 5 seconds to inactivate enzymes.

RT-PCR

The suggested reaction condition is the same as described on page 42.

TransScript® All-in-One First-Strand cDNA Synthesis SuperMix for PCR

AT321-01	50 rxns (20 µl per reaction)

Storage
at -20°C for one year

Description

TransScript® All-in-One First-Strand cDNA Synthesis SuperMix for PCR provides all the necessary components for cDNA synthesis from total RNA or mRNA. The SuperMix is provided at 5× concentration and used at 1× concentration by adding RNA and H_2O. The resulting cDNA is suitable for regular PCR, not for qPCR.

- One-tube format for simple and fast setup and reducing pipetting variability.
- The optimal ratio of oligo$(dT)_{18}$ primer to random primer(N9) for PCR ready cDNA.
- PCR ready cDNA in 30 minutes (unsuitable for qPCR).
- cDNA up to 12 kb.

Application

Multiple copy and low copy gene detection

Kit Contents

Component	AT321-01
5×*TransScript*® All-in-One SuperMix for PCR	200 µl
RNase-free Water	1 ml

PROTOCOL

First-Strand cDNA synthesis

1. Reaction Components

Component	Volume
Total RNA/mRNA	50 ng-5 µg/5-500 ng
5×*TransScript*® All-in-One SuperMix for PCR	4 µl
RNase-free Water	to 20 µl

2. Incubation

- For RNA template with poly(A)$^+$, incubate at 42°C for 30 minutes.
- For RNA template without poly(A)$^+$, incubate at 25°C for 10 minutes, then at 42°C for 30 minutes.

3. Incubate at 85°C for 5 seconds to inactivate enzymes.

RT-PCR

The suggested reaction condition is the same as described on page 42.

TransScript® All-in-One First-Strand cDNA Synthesis SuperMix for qPCR (One-Step gDNA Removal)

AT341-01	50 rxns (20 μl per reaction)
AT341-02	100 rxns (20 μl per reaction)

Storage
at -20°C for one year

Description
The kit provides all the necessary components for cDNA synthesis from total RNA or mRNA. It is provided at 5× concentration and used at 1× concentration by adding gDNA remover, RNA and H_2O. Simultaneous genomic DNA removal and cDNA synthesis are performed. After cDNA synthesis, gDNA remover and reverse transcriptase are inactivated by heating at 85°C for 5 seconds. The resulting cDNA is suitable for qPCR, not for regular PCR.

- Simultaneous genomic DNA removal and cDNA synthesis.
- The optimal ratio of oligo(dT)$_{18}$ primer to random primer(N9) for qPCR ready cDNA.
- qPCR ready cDNA in 15 minutes.
- cDNA up to 250 bp.

Application
Multiple copy and low copy gene detection

Kit Contents

Component	AT341-01	AT341-02
5×*TransScript*® All-in-One SuperMix for qPCR	200 μl	400 μl
gDNA Remover	50 μl	100 μl
5×*TransScript*® All-in-One No-RT Control SuperMix for qPCR	20 μl	40 μl
RNase-free Water	1 ml	2×1 ml

PROTOCOL

First-Strand cDNA synthesis

1. Reaction Components

Component	Volume
Total RNA/mRNA	≤1 μg/≤100 ng
5×*TransScript*® All-in-One SuperMix for qPCR	4 μl
gDNA Remover	1 μl
RNase-free Water	to 20 μl

2. Incubate at 42°C for 15 minutes.
3. Incubate at 85°C for 5 seconds to inactivate enzymes.

qPCR

Reaction Components

Component	Volume	Final Concentration
Template	Variable	as required
Forward Primer (10 μM)	0.4 μl	0.2 μM
Reverse Primer (10 μM)	0.4 μl	0.2 μM
2×*TransStart*® Top/Tip Green qPCR SuperMix	10 μl	1×
Passive Reference Dye (50×) (optional)	0.4 μl	1×
ddH_2O	Variable	-
Total Volume	20 μl	-

Thermal cycling conditions (three-step)

94°C	30 sec	
94°C	5 sec	40-45 cycles
50-60°C	15 sec*	
72°C	10 sec*	

Dissociation Stage

Thermal cycling conditions (two-step)

94°C	30 sec	
94°C	5 sec	40-45 cycles
60°C	30 sec*	

Dissociation Stage

Fluorescent signals can be collected during the annealing or extension stage. For ABI qPCR instrument, we suggest using the following signal collecting time:

* For ABI Prism7700/7900, the time is 30 seconds.
* For ABI Prism7000/7300, the time is 31seconds.
* For ABI Prism7500, the time is 34 seconds.
* For ABI ViiA 7, the time is at least 19 seconds.

Two-step qPCR is more suitable for higher specificity assay.

Three-step qPCR is more suitable for higher sensitivity assay.

TransScript® II All-in-One First-Strand cDNA Synthesis SuperMix for PCR

AH321-01	50 rxns (20 μl per reaction)

Storage
at -20°C for one year

Description

TransScript® II All-in-One First-Strand cDNA Synthesis SuperMix for PCR provides all the necessary components for cDNA synthesis from total RNA or mRNA. The SuperMix is provided at 5× concentration and used at 1× concentration by adding RNA and H_2O. The resulting cDNA is suitable for regular PCR, not for qPCR.

- One-tube format for simple and fast setup and reduced pipetting variability.
- The optimal ratio of oligo$(dT)_{20}$ primer to random primer(N9) for PCR ready cDNA.
- PCR ready cDNA in 30 minutes (unsuitable for qPCR).
- cDNA up to 15 kb.

Applications

- cDNA library construction, 3' and 5' RACE
- Multiple copy and low copy gene detection
- GC-rich or complex secondary structure RNA template

Kit Contents

Component	AH321-01
5×*TransScript*® II All-in-One SuperMix for PCR	200 μl
RNase-free Water	1 ml

PROTOCOL

First-Strand cDNA synthesis

1. Reaction Components

Component	Volume
Total RNA/mRNA	50 ng-5 μg/5-500 ng
5×*TransScript*® II All-in-One SuperMix for PCR	4 μl
RNase-free Water	to 20 μl

2. Incubation

- For RNA template with poly(A)$^+$, incubate at 50°C for 30 minutes.
- For RNA template without poly(A)$^+$, incubate at 25°C for 10 minutes, then at 50°C for 30 minutes.
- For GC-rich or complex secondary structure RNA template, incubate at 55°C for 30 minutes.

3. Incubate at 85°C for 5 seconds to inactivate enzymes.

RT-PCR

The suggested reaction condition is the same as described on page 42.

TransScript® II All-in-One First-Strand cDNA Synthesis SuperMix for qPCR (One-Step gDNA Removal)

AH341-01	50 rxns (20 μl per reaction)

Storage
at -20°C for one year

Description

The kit provides all the necessary components for cDNA synthesis from total RNA or mRNA. It is provided at 5× concentration and used at 1× concentration by adding gDNA remover, RNA and H_2O. Simultaneous genomic DNA removal and cDNA synthesis are performed. After cDNA synthesis, gDNA remover and reverse transcriptase are inactivated by heating at 85°C for 5 seconds. The resulting cDNA is suitable for qPCR, not for regular PCR.

- Simultaneous genomic DNA removal and cDNA synthesis.
- The optimal ratio of Oligo(dT)$_{20}$ Primer to random primer(N9) for qPCR ready cDNA.
- qPCR ready cDNA in 15 minutes.
- cDNA up to 250 bp.

Applications

- Multiple copy and low copy gene detection
- GC-rich or complex secondary structure RNA template

Kit Contents

Component	AH341-01
5×*TransScript*® II All-in-One SuperMix for qPCR	200 μl
gDNA Remover	50 μl
5×*TransScript*® II All-in-One No-RT Control SuperMix for qPCR	20 μl
RNase-free Water	1 ml

PROTOCOL

First-Strand cDNA synthesis

1. Reaction Components

Component	Volume
Total RNA/mRNA	≤1 μg/≤100 ng
5×*TransScript*® II All-in-One SuperMix for qPCR	4 μl
gDNA Remover	1 μl
RNase-free Water	to 20 μl

2. Incubate at 50°C for 15 minutes.
   For GC-rich or complex secondary structure RNA template, incubate at 55°C for 15 minutes.
3. Incubate at 85°C for 5 seconds to inactivate enzymes.

qPCR

The suggested reaction condition is the same as described on page 61.

TransScript® Two-Step RT-PCR SuperMix

AT401-01	50 rxns (20 µl per RT reaction)
	80 rxns (50 µl per PCR)

Storage
at -20°C for one year

Description

TransScript® Two-Step RT-PCR SuperMix performs first-strand cDNA synthesis and PCR in two steps. 5×*TransScript*® All-in-One SuperMix for PCR is used for reverse transcription and 2×*TransTaq*® HiFi PCR SuperMix II is used for PCR.

- Amplification of fragment up to 12 kb.

Application

Multiple copy and low copy gene detection

Kit Contents

Component	AT401-01
5×*TransScript*® All-in-One SuperMix for PCR	200 µl
2×*TransTaq*® HiFi PCR SuperMix II	2×1 ml
RNase-free Water	1 ml

PROTOCOL

First-Strand cDNA synthesis

The suggested reaction condition is the same as described on page 59.

RT-PCR

Reaction Components

Component	Volume	Final Concentration
cDNA	2 µl	as required
Forward Primer (10 µM)	1 µl	0.2 µM
Reverse Primer (10 µM)	1 µl	0.2 µM
2×*TransTaq*® HiFi PCR SuperMix II	25 µl	1×
ddH_2O	to 50 µl	Not applicable

The suggested reaction condition is the same as described on page 42.

TransScript® II Two-Step RT-PCR SuperMix

AH401-01	50 rxns (20 μl per RT reaction)
	80 rxns (50 μl per PCR)

Storage
at -20°C for one year

Description

TransScript® II Two-Step RT-PCR SuperMix performs first-strand cDNA synthesis and PCR in two steps. 5×*TransScript*® II All-in-One SuperMix for PCR is used for reverse transcription and 2×*TransTaq*® HiFi PCR SuperMix II is used for PCR.

- Amplification of fragment up to 15 kb.

Applications

- cDNA library construction, 3' and 5' RACE
- Multiple copy and low copy gene detection
- GC-rich or complex secondary structure RNA template

Kit Contents

Component	AH401-01
5×*TransScript*® II All-in-One SuperMix for PCR	200 μl
2×*TransTaq*® HiFi PCR SuperMix II	2×1 ml
RNase-free Water	1 ml

PROTOCOL

First-Strand cDNA synthesis

The suggested reaction condition is the same as described on page 62.

RT-PCR

Reaction Components

Component	Volume	Final Concentration
cDNA	2 μl	as required
Forward Primer (10 μM)	1 μl	0.2 μM
Reverse Primer (10 μM)	1 μl	0.2 μM
2×*TransTaq*® HiFi PCR SuperMix II	25 μl	1×
ddH_2O	to 50 μl	Not applicable

The suggested reaction condition is the same as described on page 42.

EasyScript® One-Step RT-PCR SuperMix

Mix (+dye)	AE411-02	200 rxns (20 µl per reaction)

Storage
at -20°C for one year

Description

One-Step RT-PCR combines the first-strand cDNA synthesis with PCR in the same tube to simplify reaction setup and reduce the possibility of contamination. Only gene-specific primers can be used for One-Step RT-PCR. *EasyScript*® RT and *TransTaq*® HiFi DNA Polymerase are used in the kit.

- Amplification of fragment up to 4 kb.

Application

Multiple copy gene detection

Kit Contents

Component	AE411-02
EasyScript® One-Step Enzyme Mix	80 µl
2×One-Step Reaction Mix	2×1 ml
RNase-free Water	2×1 ml

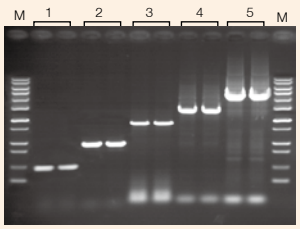
PROTOCOL

Reaction Components

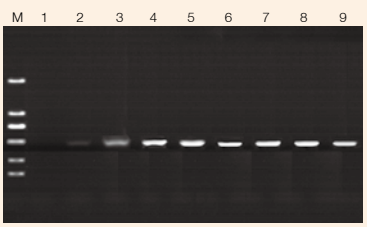
Component	Volume	Final Concentration
RNA Template	1 pg~1 µg	as required
Forward GSP (10 µM)	0.4 µl	0.2 µM
Reverse GSP (10 µM)	0.4 µl	0.2 µM
2×One-Step Reaction Mix	10 µl	1×
EasyScript® One-Step Enzyme Mix	0.4 µl	-
RNase-free Water	to 20 µl	-

Thermal cycling conditions

45°C	15-30 min	
94°C	2-5 min	
94°C	30 sec	35-40 cycles
50-60°C	30 sec	
72°C	1-2 kb/min	
72°C	5-10 min	



RT-PCR with *EasyScript*® One-Step RT-PCR SuperMix
M: 1Kb Plus DNA Ladder
1: β-actin 0.5 kb 2: BACH1 1.0 kb
3: REPA 1.8 kb 4: ACTR 3.0 kb
5: VIN 4.6 kb



RT-PCR with *EasyScript*® One-Step RT-PCR SuperMix to amplify β-actin using human total RNA as templates
M: *Trans*2K® DNA Marker
Lanes 1-9: 0 pg, 0.1 pg, 1 pg, 10 pg, 100 pg, 1,000 pg, 10 ng, 100 ng, 1,000 ng

TransScript® One-Step RT-PCR SuperMix

Mix (+dye)	AT411-02	200 rxns (20 μl per reaction)

Storage
at -20°C for one year

Description

One-Step RT-PCR combines the first-strand cDNA synthesis with PCR in the same tube to simplify reaction setup and reduce the possibility of contamination. Only gene-specific primers can be used for One-Step RT-PCR. *TransScript*® RT and *TransTaq*® HiFi DNA Polymerase are used in the kit.

- Amplification of fragment up to 8 kb.

Application

Multiple copy and low copy gene detection

Kit Contents

Component	AT411-02
TransScript® One-Step Enzyme Mix	80 μl
2×One-Step Reaction Mix	2×1 ml
RNase-free Water	2×1 ml

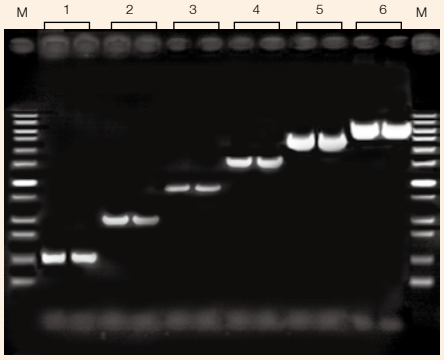
PROTOCOL

Reaction Components

Component	Volume	Final Concentration
RNA Template	1 pg~1 μg	as required
Forward GSP (10 μM)	0.4 μl	0.2 μM
Reverse GSP (10 μM)	0.4 μl	0.2 μM
2×One-Step Reaction Mix	10 μl	1×
TransScript® One-Step Enzyme Mix	0.4 μl	-
RNase-free Water	to 20 μl	-

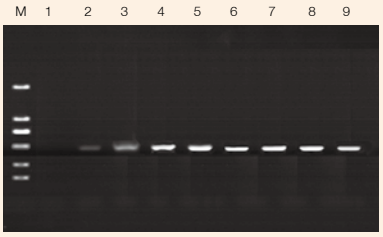
Thermal cycling conditions

45°C	15-30 min	
94°C	2-5 min	
94°C	30 sec	35-40 cycles
50-60°C	30 sec	
72°C	1-2 kb/min	
72°C	5-10 min	



RT-PCR with *TransScript*® One-Step RT-PCR SuperMix

M: 1Kb Plus DNA Ladder
1: β-actin 0.5 kb 2: BACH1 1.0 kb 3: REPA 1.8 kb
4: ACTR 3.0 kb 5: VIN 4.6 kb 6: GNE 5.9 kb



RT-PCR with *TransScript*® One-Step RT-PCR SuperMix to amplify β-actin using human total RNA as templates

M: *Trans2K*® DNA Marker
Lanes 1-9: 0 pg, 0.1 pg, 1 pg, 10 pg, 100 pg, 1,000 pg, 10 ng, 100 ng, 1,000 ng

TransScript® II One-Step RT-PCR SuperMix

Mix (+dye)	AH411-02	200 rxns (20 μl per reaction)

Storage
at -20℃ for one year

Description

One-Step RT-PCR combines the first-strand cDNA synthesis with PCR in the same tube to simplify reaction setup and reduce the possibility of contamination. Only gene-specific primers can be used for One-Step RT-PCR. *TransScript*® II RT and *TransTaq*® HiFi DNA Polymerase are used in the kit.

- Amplification of fragment up to 8 kb.

Applications

- Multiple copy and low copy gene detection
- GC-rich or complex secondary structure RNA template

Kit Contents

Component	AH411-02
TransScript® II One-Step Enzyme Mix	80 μl
2×One-Step Reaction Mix	2×1 ml
RNase-free Water	2×1 ml

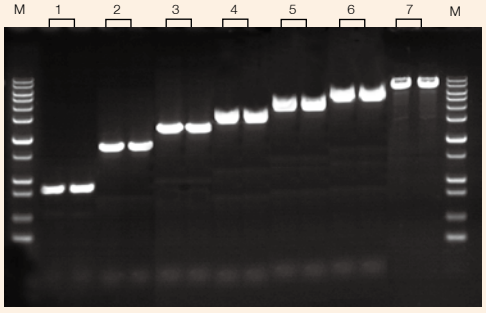
PROTOCOL

Reaction Components

Component	Volume	Final Concentration
RNA Template	1 pg~1 μg	as required
Forward GSP (10 μM)	0.4 μl	0.2 μM
Reverse GSP (10 μM)	0.4 μl	0.2 μM
2×One-Step Reaction Mix	10 μl	1×
TransScript® II One-Step Enzyme Mix	0.4 μl	-
RNase-free Water	to 20 μl	-

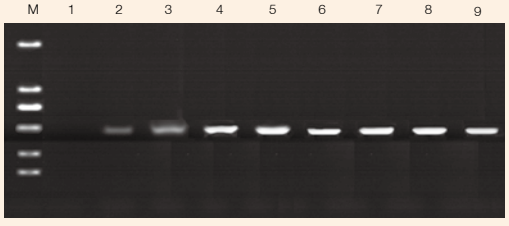
Thermal cycling conditions

50°C	15-30 min	
94°C	2-5 min	
94°C	30 sec	35-40 cycles
50-60°C	30 sec	
72°C	1-2 kb/min	
72°C	5-10 min	



RT-PCR with *TransScript*® II One-Step RT-PCR SuperMix

M: 1Kb Plus DNA Ladder
1: GAPDH 0.9 kb
2: REPA 1.8 kb
3: NCBP 2.5 kb
4: HDP 3.5 kb
5: VIN 4.6 kb
6: Pol 6.8 kb
7: APC 8.5 kb



RT-PCR with *TransScript*® II One-Step RT-PCR SuperMix to amplify β-actin using human total RNA as templates

M: *Trans*2K® DNA Marker
Lanes 1-9: 0 pg, 0.1 pg, 1 pg, 10 pg, 100 pg, 1,000 pg, 10 ng, 100 ng, 1,000 ng

Ribonuclease Inhibitor

AI101-01	2,000 units
AI101-02	5×2,000 units

Concentration
50 units/μl
Storage
at -20°C for one year

Description

Ribonuclease Inhibitor is a recombinant protein purified from *E. coli* strain carrying human placenta ribonuclease inhibitor gene. Ribonuclease Inhibitor specifically inhibits RNase A, RNase B, and RNase C. It is not effective against RNase 1, RNase T1, S1 nuclease, RNase H and aspergillus-originated RNase. It has no inhibition effect on DNA Polymerase, AMV, M-MLV, SP6, T7 and T3 RNA Polymerases.

Unit Definition

One unit is defined as the amount of enzyme required to inhibit 5 ng RNase A by 50%.

Applications

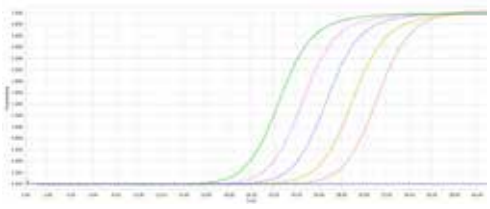
In vitro inhibition of ribonuclease, cDNA synthesis and *in vitro* transcription and translation.

qPCR and qRT-PCR SuperMix

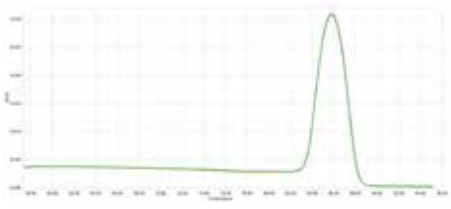
Basic principle of real-time quantitative PCR

Real-time qPCR is a PCR method used to amplify and simultaneously quantify target DNA molecules. Two methods are frequently used for qPCR: double-strand DNA-binding dyes (e.g. SYBR Green I) or fluorescent reporter probes (e.g. TaqMan). In both cases, fluorescence signals are detected during the exponential phase.

Amplification Plots



Dissociation Curve



TransStart® Green qPCR SuperMix

AQ101-01	1 ml
AQ101-02	5×1 ml
AQ101-03	15×1 ml

Contents

- 2×*TransStart*® Green qPCR SuperMix
- Passive Reference Dye (50×)

Storage

at -20°C in dark for one year

Description

TransStart® Green qPCR SuperMix is a ready-to-use qPCR cocktail containing all components, except primer and template. It contains *TransStart*® *Taq* DNA Polymerase, SYBR Green I, dNTPs, PCR enhancer and stabilizer. qPCR SuperMix is provided at 2× concentration and can be used at 1× concentration by adding template, primer, passive reference dye (optional) and ddH_2O.

- *TransStart*® *Taq* DNA Polymerase, hot start with double blocking technique, improves sensitivity, enhances specificity and generates more accurate data.
- Double cation (K^+, NH_4^+) buffer enhances specificity and reduces primer-dimer formation.
- Passive reference dyes are provided for different qPCR instruments.

Passive Reference Dye

- Passive Reference Dye I (50×)
  ABI Prism7000/7300/7700/7900, Eppendorf, ABI Step One, ABI Step One Plus
- Passive Reference Dye II (50×)
  ABI Prism7500, ABI Prism7500 Fast, ABI Q6, ABI Quant Studio 6/7 Flex, ABI ViiA 7, Stratagene Mx3000/Mx3005P, Corbett Rotor Gene 3000

- No Passive Reference Dye
  Roche LightCycler480, Roche Light Cycler96, MJ Research Chromo4, Opticon (II), Takara TP800, Bio-Rad iCycler iQ, iQ5, Bio-Rad CFX96, Bio-Rad C1000 Thermal Cycler, Thermo Pikoreal 96, Corbett Rotor Gene 6000, Corbett Rotor Gene G, Corbett Rotor Gene Q

PROTOCOL

Reaction Components

Component	Volume	Final Concentration
Template	Variable	as required
Forward Primer (10 μM)	0.4 μl	0.2 μM
Reverse Primer (10 μM)	0.4 μl	0.2 μM
2×*TransStart*® Green qPCR SuperMix	10 μl	1×
Passive Reference Dye (50×) (optional)	0.4 μl	1×
ddH_2O	Variable	-
Total Volume	20 μl	-

Thermal cycling conditions (three-step)

94°C	30 sec	
94°C	5 sec	40-45 cycles
50-60°C	15 sec*	
72°C	10 sec*	

Dissociation Stage

Thermal cycling conditions (two-step)

94°C	30 sec	
94°C	5 sec	40-45 cycles
60°C	30 sec*	

Dissociation Stage

Fluorescent signals can be collected during the annealing or extension stage. For ABI qPCR instrument, we suggest using the following signal collecting time:

* For ABI Prism7700/7900, the time is 30 seconds.
* For ABI Prism7000/7300, the time is 31 seconds.
* For ABI Prism7500, the time is 34 seconds.
* For ABI ViiA 7, the time is at least 19 seconds.

Two-step qPCR is more suitable for higher specificity assay.

Three-step qPCR is more suitable for higher sensitivity assay.

TransStart® Green qPCR SuperMix UDG

AQ111-01	1 ml
AQ111-02	5×1 ml
AQ111-03	15×1 ml

Contents

- 2×*TransStart*® Green qPCR SuperMix UDG
- Passive Reference Dye (50×)

Storage

at -20°C in dark for one year

Description

TransStart® Green qPCR SuperMix UDG is a ready-to-use qPCR cocktail containing all components, except primer and template. It contains *TransStart*® *Taq* DNA Polymerase, UDG, SYBR Green I, dNTPs, PCR enhancer and stabilizer. qPCR SuperMix is provided at 2× concentration and can be used at 1× concentration by adding template, primer, passive reference dye (optional) and ddH_2O.

- *TransStart*® *Taq* DNA Polymerase, hot start with double blocking technique, improves sensitivity, enhances specificity and generates more accurate data.
- Double cation (K^+, NH_4^+) buffer enhances specificity and reduces primer-dimer formation.
- Passive reference dyes are provided for different qPCR instruments.
- UDG and dUTP avoid cross contamination.

Passive Reference Dye

- Passive Reference Dye I (50×)
  ABI Prism7000/7300/7700/7900, Eppendorf, ABI Step One, ABI Step One Plus
- Passive Reference Dye II (50×)
  ABI Prism7500, ABI Prism7500 Fast, ABI Q6, ABI Quant Studio 6/7 Flex, ABI ViiA 7, Stratagene Mx3000/Mx3005P, Corbett Rotor Gene 3000
- No Passive Reference Dye
  Roche LightCycler480, Roche Light Cycler96, MJ Research Chromo4, Opticon (II), Takara TP800, Bio-Rad iCycler iQ, iQ5, Bio-Rad CFX96, Bio-Rad C1000 Thermal Cycler, Thermo Pikoreal 96, Corbett Rotor Gene 6000, Corbett Rotor Gene G, Corbett Rotor Gene Q

PROTOCOL

Reaction Components

Component	Volume	Final Concentration
Template	Variable	as required
Forward Primer (10 μM)	0.4 μl	0.2 μM
Reverse Primer (10 μM)	0.4 μl	0.2 μM
2×*TransStart*® Green qPCR SuperMix UDG	10 μl	1×
Passive Reference Dye (50×) (optional)	0.4 μl	1×
ddH_2O	Variable	-
Total Volume	20 μl	-

Thermal cycling conditions (three-step)

50°C	2 min (UDG Incubation)	
94°C	10 min (UDG Inactivation)	
94°C	5 sec	40-45 cycles
50-60°C	15 sec*	
72°C	10 sec*	
Dissociation Stage		

Thermal cycling conditions (two-step)

50°C	2 min (UDG Incubation)	
94°C	10 min (UDG Inactivation)	
94°C	5 sec	40-45 cycles
60°C	30 sec*	
Dissociation Stage		

Fluorescent signals can be collected during the annealing or extension stage. For ABI qPCR instrument, we suggest using the following signal collecting time:

* For ABI Prism7700/7900, the time is 30 seconds.
* For ABI Prism7000/7300, the time is 31 seconds.
* For ABI Prism7500, the time is 34 seconds.
* For ABI ViiA 7, the time is at least 19 seconds.

Two-step qPCR is more suitable for higher specificity assay.

Three-step qPCR is more suitable for higher sensitivity assay.

TransStart® Top Green qPCR SuperMix

AQ131-01	1 ml
AQ131-02	5×1 ml
AQ131-03	15×1 ml
AQ131-04	25×1 ml

Contents

- 2×*TransStart*® Top Green qPCR SuperMix
- Passive Reference Dye (50×)

Storage

at -20°C in dark for one year

Description

TransStart® Top Green qPCR SuperMix is a ready-to-use qPCR cocktail containing all components, except primer and template. It contains *TransStart*® *TopTaq* DNA Polymerase, SYBR Green I, dNTPs, PCR enhancer and stabilizer. qPCR SuperMix is provided at 2× concentration and can be used at 1× concentration by adding template, primer, passive reference dye (optional) and ddH_2O.

- *TransStart*® *TopTaq* DNA Polymerase, hot start with double blocking technique, improves sensitivity, enhances specificity and generates more accurate data.
- Double cation (K^+, NH_4^+) buffer enhances specificity and reduces primer-dimer formation.
- Passive reference dyes are provided for different qPCR instruments.

Passive Reference Dye

- Passive Reference Dye I (50×)
  ABI Prism7000/7300/7700/7900, Eppendorf, ABI Step One, ABI Step One Plus

- Passive Reference Dye II (50×)
  ABI Prism7500, ABI Prism7500 Fast, ABI Q6, ABI Quant Studio 6/7 Flex, ABI ViiA 7, Stratagene Mx3000/Mx3005P, Corbett Rotor Gene 3000
- No Passive Reference Dye
  Roche LightCycler480, Roche Light Cycler96, MJ Research Chromo4, Opticon (II), Takara TP800, Bio-Rad iCycler iQ, iQ5, Bio-Rad CFX96, Bio-Rad C1000 Thermal Cycler, Thermo Pikoreal 96, Corbett Rotor Gene 6000, Corbett Rotor Gene G, Corbett Rotor Gene Q

PROTOCOL

Reaction Components

Component	Volume	Final Concentration
Template	Variable	as required
Forward Primer (10 μM)	0.4 μl	0.2 μM
Reverse Primer (10 μM)	0.4 μl	0.2 μM
2×*TransStart*® Top Green qPCR SuperMix	10 μl	1×
Passive Reference Dye (50×) (optional)	0.4 μl	1×
ddH_2O	Variable	-
Total Volume	20 μl	-

The suggested reaction condition is the same as described on page 72.

TransStart® Tip Green qPCR SuperMix

AQ141-01	1 ml
AQ141-02	5×1 ml
AQ141-03	15×1 ml
AQ141-04	25×1 ml

Contents

- 2×*TransStart*® Tip Green qPCR SuperMix
- Passive Reference Dye (50×)

Storage

at -20°C in dark for one year

Description

TransStart® Tip Green qPCR SuperMix is a ready-to-use qPCR cocktail. It contains a novel *TransStart*® *TipTaq* DNA Polymerase , unique hot start reagents (DNA binding proteins combined with unique chemical), optimized double cation buffer, SYBR Green I, dNTPs, PCR Enhancer and PCR stabilizer. qPCR SuperMix is provided at 2× concentration and can be used at 1× concentration by adding template, primer, passive reference dye (optional) and ddH_2O.

- A combination of chemical blocking technique with *TransStart*® hot start technique to achieve complete blocking. Compared with double blocking *TransStart*® *TopTaq*, this method provides higher sensitivity, higher specificity, better amplification.
- Double cation (K^+, NH_4^+) buffer enhances specificity and reduces primer-dimer formation.
- Passive reference dyes are provided for different qPCR instruments.

Passive Reference Dye

- Passive Reference Dye I (50×)
  ABI Prism7000/7300/7700/7900, Eppendorf, ABI Step One, ABI Step One Plus
- Passive Reference Dye II (50×)
  ABI Prism7500, ABI Prism7500 Fast, ABI Q6, ABI Quant Studio 6/7 Flex, ABI ViiA 7, Stratagene Mx3000/Mx3005P, Corbett Rotor Gene 3000
- No Passive Reference Dye
  Roche LightCycler480, Roche Light Cycler96, MJ Research Chromo4, Opticon (II), Takara TP800, Bio-Rad iCycler iQ, iQ5, Bio-Rad CFX96, Bio-Rad C1000 Thermal Cycler, Thermo Pikoreal 96, Corbett Rotor Gene 6000, Corbett Rotor Gene G, Corbett Rotor Gene Q

PROTOCOL

Reaction Components

Component	Volume	Final Concentration
Template	Variable	as required
Forward Primer (10 μM)	0.4 μl	0.2 μM
Reverse Primer (10 μM)	0.4 μl	0.2 μM
2×*TransStart*® Tip Green qPCR SuperMix	10 μl	1×
Passive Reference Dye (50×) (optional)	0.4 μl	1×
ddH_2O	Variable	-
Total Volume	20 μl	-

The suggested reaction condition is the same as described on page 72.

TransScript® Green Two-Step qRT-PCR SuperMix

AQ201-01	50 rxns (20 μl per RT reaction)
	300 rxns (20 μl per qPCR)

Storage

at -20°C in dark for one year

Description

TransScript® Green Two-Step qRT-PCR SuperMix contains all the necessary reagents for gDNA removal, cDNA synthesis and qPCR.

- gDNA remover and 5×*TransScript*® All-in-One SuperMix for qPCR are provided for simultaneous gDNA removal and cDNA synthesis.
- *TransStart*® Tip Green qPCR SuperMix is provided for qPCR.
- 5×*TransScript*® All-in-One No-RT Control SuperMix for qPCR is provided for experimental control.
- Passive reference dyes are provided for different qPCR instruments.

Application

Multiple copy and low copy gene detection

Passive Reference Dye

- Passive Reference Dye I (50×)
  ABI Prism7000/7300/7700/7900, Eppendorf, ABI Step One, ABI Step One Plus

- Passive Reference Dye II (50×)
  ABI Prism7500, ABI Prism7500 Fast, ABI Q6, ABI Quant Studio 6/7 Flex, ABI ViiA 7, Stratagene Mx3000/Mx3005P, Corbett Rotor Gene 3000
- No Passive Reference Dye
  Roche LightCycler480, Roche Light Cycler96, MJ Research Chromo4, Opticon (II), Takara TP800, Bio-Rad iCycler iQ, iQ5, Bio-Rad CFX96, Bio-Rad C1000 Thermal Cycler, Thermo Pikoreal 96, Corbett Rotor Gene 6000, Corbett Rotor Gene G, Corbett Rotor Gene Q

Kit Contents

Component	AQ201-01
5×*TransScript*® All-in-One SuperMix for qPCR	200 µl
gDNA Remover	50 µl
5×*TransScript*® All-in-One No-RT Control SuperMix for qPCR	20 µl
2×*TransStart*® Tip Green qPCR SuperMix	3×1 ml
Passive Reference Dye (50×)	120 µl
RNase-free Water	1 ml

PROTOCOL

First-Strand cDNA synthesis

1. Reaction Components

Component	Volume
Total RNA/mRNA	≤1 µg / ≤100 ng
5×*TransScript*® All-in-one SuperMix for qPCR	4 µl
gDNA Remover	1 µl
RNase-free Water	to 20 µl

2. Incubate at 42°C for 15 minutes.

3. Incubate at 85°C for 5 seconds to inactivate enzymes.

The suggested reaction components and condition for qPCR are the same as described on page 72.

TransScript® Green miRNA Two-Step qRT-PCR SuperMix

AQ202-01	20 rxns (20 μl per RT reaction)
	500 rxns (20 μl per qPCR)

Storage
at -20℃ in dark for one year

Description

TransScript® Green miRNA Two-Step qRT-PCR SuperMix provides all the necessary components for miRNA detection. High efficient poly(A) tail addition and first-strand cDNA synthesis are performed by *TransScript*® miRNA RT Enzyme Mix (containing tailing enzyme and RT enzyme) and 2×TS miRNA Reaction Mix. *TransStart*® Tip Green qPCR SuperMix is provided for miRNA detection.

- One-Step poly(A) tailing and cDNA synthesis.
- Passive reference dyes are provided for different qPCR instruments.

Application

Multiple copy and low copy gene detection

Passive Reference Dye

- Passive Reference Dye I (50×)
  ABI Prism7000/7300/7700/7900, Eppendorf, ABI Step One, ABI Step One Plus
- Passive Reference Dye II (50×)
  ABI Prism7500, ABI Prism7500 Fast, ABI Q6, ABI Quant Studio 6/7 Flex, ABI ViiA 7, Stratagene Mx3000/Mx3005P, Corbett Rotor Gene 3000
- No Passive Reference Dye
  Roche LightCycler480, Roche Light Cycler96, MJ Research Chromo4, Opticon (II), Takara TP800, Bio-Rad iCycler iQ, iQ5, Bio-Rad CFX96, Bio-Rad C1000 Thermal Cycler, Thermo Pikoreal 96, Corbett Rotor Gene 6000, Corbett Rotor Gene G, Corbett Rotor Gene Q

Kit Contents

Component	AQ202-01
TransScript® miRNA RT Enzyme Mix	20 μl
2×TS miRNA Reaction Mix	200 μl
Universal miRNA qPCR Primer (10 μM)	200 μl
2×*TransStart*® Tip Green qPCR SuperMix	5×1 ml
Passive Reference Dye (50×)	200 μl
RNase-free Water	1 ml

PROTOCOL

Tail addition and First-Strand cDNA synthesis

1. Reaction Components

Component	Volume
Total RNA/miRNA*	x µl
TransScript® miRNA RT Enzyme Mix	1 µl
2×TS miRNA Reaction Mix	10 µl
RNase-free Water	to 20 µl

* Total RNA ≤ 5 µg. Since miRNA cannot be directly quantified by spectrophotometer, we suggest using 1-9 µl for 20 µl reaction.

2. Mix gently, and incubate at 37°C for 1 hour.

3. Incubate at 85°C for 5 seconds to inactivate RT Enzyme Mix.

The suggested reaction components and condition for qPCR are the same as described on page 55-56.

TransScript® II Green Two-Step qRT-PCR SuperMix

AQ301-01	50 rxns (20 µl per RT reaction)
	300 rxns (20 µl per qPCR)

Storage

at -20°C in dark for one year

Description

TransScript® II Green Two-Step qRT-PCR SuperMix contains all the necessary reagents for gDNA removal, cDNA synthesis and qPCR.

- gDNA remover and 5×*TransScript*® II All-in-One SuperMix for qPCR are provided for simultaneous gDNA removal and cDNA synthesis.
- *TransStart*® Tip Green qPCR SuperMix is provided for qPCR.
- 5×*TransScript*® II All-in-One No-RT Control SuperMix for qPCR is provided for experimental control.
- Passive reference dyes are provided for different qPCR instruments.

Applications

- Multiple copy and low copy gene detection
- GC-rich or complex secondary structure RNA template

Passive Reference Dye

- Passive Reference Dye I (50×)
  ABI Prism7000/7300/7700/7900, Eppendorf, ABI Step One, ABI Step One Plus
- Passive Reference Dye II (50×)
  ABI Prism7500, ABI Prism7500 Fast, ABI Q6, ABI Quant Studio 6/7 Flex, ABI ViiA 7, Stratagene Mx3000/Mx3005P, Corbett Rotor Gene 3000
- No Passive Reference Dye
  Roche LightCycler480, Roche Light Cycler96, MJ Research Chromo4, Opticon (II), Takara TP800, Bio-Rad iCycler iQ, iQ5, Bio-Rad CFX96, Bio-Rad C1000 Thermal Cycler, Thermo Pikoreal 96, Corbett Rotor Gene 6000, Corbett Rotor Gene G, Corbett Rotor Gene Q

Kit Contents

Component	AQ301-01
5×*TransScript*® II All-in-One SuperMix for qPCR	200 µl
gDNA Remover	50 µl
5×*TransScript*® II All-in-One No-RT Control SuperMix for qPCR	20 µl
2×*TransStart*® Tip Green qPCR SuperMix	3×1 ml
Passive Reference Dye (50×)	120 µl
RNase-free Water	1 ml

PROTOCOL

First-Strand cDNA synthesis

1. Reaction Components

Component	Volume
Total RNA/mRNA	≤1 µg / ≤100 ng
5×*TransScript*® II All-in-one SuperMix for qPCR	4 µl
gDNA Remover	1 µl
RNase-free Water	to 20 µl

2. Incubation at 50°C for 15 minutes.
   For GC-rich or complex secondary structure RNA template, incubate at 55°C for 15 minutes.
3. Incubate at 85°C for 5 seconds to inactivate enzymes.

The suggested reaction components and condition for qPCR are the same as described on page 72.

TransScript® Green One-Step qRT-PCR SuperMix

AQ211-01	100 rxns (20 µl per reaction)
AQ211-02	400 rxns (20 µl per reaction)

Storage

at -20°C in dark for one year

Description

TransScript® Green One-Step qRT-PCR SuperMix combines the first-strand cDNA synthesis and qPCR in the same tube to simplify reaction setup and reduce the possibility of contamination. Only gene specific primers can be used for this kit. *TransScript*® Green One-Step qRT-PCR SuperMix contains all the necessary reagents for cDNA synthesis and qPCR except total RNA/mRNA template and gene specific primers.

- 5 minutes cDNA synthesis
- cDNA synthesis and qPCR are performed in a single tube using gene specific primers with total RNA or mRNA as templates.
- Passive reference dyes are provided for different qPCR instruments.

Applications

- Multiple copy and low copy gene detection
- Viral RNA and trace RNA detection

Passive Reference Dye

- Passive Reference Dye I (50×)
  ABI Prism7000/7300/7700/7900, Eppendorf, ABI Step One, ABI Step One Plus
- Passive Reference Dye II (50×)
  ABI Prism7500, ABI Prism7500 Fast, ABI Q6, ABI Quant Studio 6/7 Flex, ABI ViiA 7, Stratagene Mx3000/Mx3005P, Corbett Rotor Gene 3000
- No Passive Reference Dye
  Roche LightCycler480, Roche Light Cycler96, MJ Research Chromo4, Opticon (II), Takara TP800, Bio-Rad iCycler iQ, iQ5, Bio-Rad CFX96, Bio-Rad C1000 Thermal Cycler, Thermo Pikoreal 96, Corbett Rotor Gene 6000, Corbett Rotor Gene G, Corbett Rotor Gene Q

Kit Contents

Component	AQ211-01	AQ211-02
TransScript® One-Step RT/RI Enzyme Mix	40 µl	160 µl
2×*TransStart*® Tip Green qPCR SuperMix	1 ml	4x1 ml
Passive Reference Dye (50×)	40 µl	160 µl
RNase-free Water	1 ml	4x1 ml

PROTOCOL

Reaction Components

Component	Volume	Final Concentration
RNA Template	1 pg~1 µg	as required
Forward GSP (10 µM)	0.4 µl	0.2 µM
Reverse GSP (10 µM)	0.4 µl	0.2 µM
2×*TransStart*® Tip Green qPCR SuperMix	10 µl	1×
TransScript® One-Step RT/RI Enzyme Mix	0.4 µl	-
Passive Reference Dye (50×) (optional)	0.4 µl	1×
RNase-free Water	Variable	-
Total volume	20 µl	-

Thermal cycling conditions (three-step)

45°C	5 min	
94°C	30 sec	
94°C	5 sec	40-45 cycles
50-60°C	15 sec*	
72°C	10 sec*	
Dissociation Stage		

Thermal cycling conditions (two-step)

45°C	5 min	
94°C	30 sec	
94°C	5 sec	40-45 cycles
60°C	30 sec*	
Dissociation Stage		

Fluorescent signals can be collected during the annealing or extension stage. For ABI qPCR instrument, we suggest using the following signal collecting time:

* For ABI Prism7700/7900, the time is 30 seconds.
* For ABI Prism7000/7300, the time is 31 seconds.
* For ABI Prism7500, the time is 34 seconds.
* For ABI ViiA 7, the time is at least 19 seconds.

Two-step qPCR is more suitable for higher specificity assay.

Three-step qPCR is more suitable for higher sensitivity assay.

TransScript® II Green One-Step qRT-PCR SuperMix

AQ311-01	100 rxns (20 µl per reaction)
AQ311-02	400 rxns (20 µl per reaction)

Storage
at -20°C in dark for one year

Description

TransScript® II Green One-Step qRT-PCR SuperMix combines the first-strand cDNA synthesis and qPCR in the same tube to simplify reaction setup and reduce the possibility of contamination. Only gene specific primers can be used for this kit. *TransScript*® II Green One-Step qRT-PCR SuperMix contains all the necessary reagents for cDNA synthesis and qPCR except total RNA/mRNA template and gene specific primers.

- 5 minutes cDNA synthesis
- cDNA synthesis and qPCR are performed in a single tube using gene specific primers with total RNA or mRNA as templates.
- Passive reference dyes are provided for different qPCR instruments.

Applications

- Multiple copy and low copy gene detection
- GC-rich or complex secondary structure RNA template
- Viral RNA and trace RNA detection

Passive Reference Dye

- Passive Reference Dye I (50×)
  ABI Prism7000/7300/7700/7900, Eppendorf, ABI Step One, ABI Step One Plus
- Passive Reference Dye II (50×)
  ABI Prism7500, ABI Prism7500 Fast, ABI Q6, ABI Quant Studio 6/7 Flex, ABI ViiA 7, Stratagene Mx3000/Mx3005P, Corbett Rotor Gene 3000
- No Passive Reference Dye
  Roche LightCycler480, Roche Light Cycler96, MJ Research Chromo4, Opticon (II), Takara TP800, Bio-Rad iCycler iQ, iQ5, Bio-Rad CFX96, Bio-Rad C1000 Thermal Cycler, Thermo Pikoreal 96, Corbett Rotor Gene 6000, Corbett Rotor Gene G, Corbett Rotor Gene Q

Kit Contents

Component	AQ311-01	AQ311-02
TransScript® II One-Step RT/RI Enzyme Mix	40 µl	160 µl
2×*TransStart*® Tip Green qPCR SuperMix	1 ml	4×1 ml
Passive Reference Dye (50×)	40 µl	160 µl
RNase-free Water	1 ml	4×1 ml

PROTOCOL

Reaction Components

Component	Volume	Final Concentration
RNA Template	1 pg~1 µg	as required
Forward GSP (10 µM)	0.4 µl	0.2 µM
Reverse GSP (10 µM)	0.4 µl	0.2 µM
2×*TransStart*® Tip Green qPCR SuperMix	10 µl	1×
TransScript® II One-Step RT/RI Enzyme Mix	0.4 µl	-
Passive Reference Dye (50×) (optional)	0.4 µl	1×
RNase-free Water	Variable	-
Total volume	20 µl	-

Thermal cycling conditions (three-step)

50°C	5 min	
94°C	30 sec	
94°C	5 sec	40-45 cycles
50-60°C	15 sec*	
72°C	10 sec*	
Dissociation Stage		

Thermal cycling conditions (two-step)

50°C	5 min	
94°C	30 sec	
94°C	5 sec	40-45 cycles
60°C	30 sec*	
Dissociation Stage		

Fluorescent signals can be collected during the annealing or extension stage. For ABI qPCR instrument, we suggest using the following signal collecting time:

* For ABI Prism7700/7900, the time is 30 seconds.
* For ABI Prism7000/7300, the time is 31 seconds.
* For ABI Prism7500, the time is 34 seconds.
* For ABI ViiA 7, the time is at least 19 seconds.

Two-step qPCR is more suitable for higher specificity assay.

Three-step qPCR is more suitable for higher sensitivity assay.

TransStart® Probe qPCR SuperMix

AQ401-01	1 ml
AQ401-02	5×1 ml
AQ401-03	15×1 ml

Contents

- 2×*TransStart*® Probe qPCR SuperMix
- Passive Reference Dye (50×)

Storage

at -20°C for one year

Description

TransStart® Probe qPCR SuperMix is a ready-to-use qPCR cocktail containing all components, except probe, primer and template. It contains *TransStart*® *Taq* DNA Polymerase, dNTPs, PCR enhancer and stabilizer. qPCR SuperMix is provided at 2× concentration and can be used at 1× concentration by adding template, primer, probe, passive reference dye (optional) and ddH_2O.

- *TransStart*® *Taq* DNA Polymerase, hot start with double blocking technique, improves sensitivity, enhances specificity and generates more accurate data.
- Double cation (K^+, NH_4^+) buffer enhances specificity and reduces primer-dimer formation.
- Passive reference dyes are provided for different qPCR instruments.

Passive Reference Dye

- Passive Reference Dye I (50×)
  ABI Prism7000/7300/7700/7900, Eppendorf, ABI Step One, ABI Step One Plus
- Passive Reference Dye II (50×)
  ABI Prism7500, ABI Prism7500 Fast, ABI Q6, ABI Quant Studio 6/7 Flex, ABI ViiA 7, Stratagene Mx3000/Mx3005P, Corbett Rotor Gene 3000
- No Passive Reference Dye
  Roche LightCycler480, Roche Light Cycler96, MJ Research Chromo4, Opticon (II), Takara TP800, Bio-Rad iCycler iQ, iQ5, Bio-Rad CFX96, Bio-Rad C1000 Thermal Cycler, Thermo Pikoreal 96, Corbett Rotor Gene 6000, Corbett Rotor Gene G, Corbett Rotor Gene Q

PROTOCOL

Reaction Components

Component	Volume	Final Concentration
Template	Variable	as required
Forward Primer (10 μM)	0.4 μl	0.2 μM
Reverse Primer (10 μM)	0.4 μl	0.2 μM
Probe (10 μM)	0.4 μl	0.2 μM
2×*TransStart*® Probe qPCR SuperMix	10 μl	1×
Passive Reference Dye (50×) (optional)	0.4 μl	1×
ddH_2O	Variable	-
Total volume	20 μl	-

Thermal cycling conditions (two-step)

94°C	30 sec	
94°C	5 sec	40-45 cycles
60°C	30 sec*	

For ABI qPCR instrument, we suggest using the following signal collecting time:

* For ABI Prism7700/7900, the time is 30 seconds.
* For ABI Prism7000/7300, the time is 31 seconds.
* For ABI Prism7500, the time is 34 seconds.
* For ABI ViiA 7, the time is at least 19 seconds.

TransScript® Probe One-Step qRT-PCR SuperMix

AQ221-01	100 rxns (20 μl per reaction)
AQ221-02	400 rxns (20 μl per reaction)

Storage

at -20°C for one year

Description

TransScript® Probe One-Step qRT-PCR SuperMix combines the first-strand cDNA synthesis and qPCR in the same tube to simplify reaction setup and reduce the possibility of contamination. Only gene specific primers can be used for this kit. *TransScript*® Probe One-Step qRT-PCR SuperMix contains all the necessary reagents for cDNA synthesis and qPCR except probe, total RNA/mRNA template and gene specific primers.

- 5 minutes cDNA synthesis
- cDNA synthesis and qPCR are performed in a single tube using gene specific primers with total RNA or mRNA as templates.
- Passive reference dyes are provided for different qPCR instruments.

Applications

- Multiple copy and low copy gene detection
- Viral RNA and trace RNA detection

Passive Reference Dye

- Passive Reference Dye I (50×)
  ABI Prism7000/7300/7700/7900, Eppendorf, ABI Step One, ABI Step One Plus
- Passive Reference Dye II (50×)
  ABI Prism7500, ABI Prism7500 Fast, ABI Q6, ABI Quant Studio 6/7 Flex, ABI ViiA 7, Stratagene Mx3000/Mx3005P, Corbett Rotor Gene 3000
- No Passive Reference Dye
  Roche LightCycler480, Roche Light Cycler96, MJ Research Chromo4, Opticon (II), Takara TP800, Bio-Rad iCycler iQ, iQ5, Bio-Rad CFX96, Bio-Rad C1000 Thermal Cycler, Thermo Pikoreal 96, Corbett Rotor Gene 6000, Corbett Rotor Gene G, Corbett Rotor Gene Q

Kit Contents

Component	AQ221-01	AQ221-02
TransScript® One-Step RT/RI Enzyme Mix	40 µl	160 µl
2×*TransStart*® Probe qPCR SuperMix	1 ml	4×1 ml
Passive Reference Dye (50×)	40 µl	160 µl
RNase-free Water	1 ml	4×1 ml

PROTOCOL

Reaction Components

Component	Volume	Final Concentration
RNA Template	Variable	as required
Forward GSP (10 µM)	0.4 µl	0.2 µM
Reverse GSP (10 µM)	0.4 µl	0.2 µM
Probe (10 µM)	0.4 µl	0.2 µM
2×*TransStart*® Probe qPCR SuperMix	10 µl	1×
TransScript® One-Step RT/RI Enzyme Mix	0.4 µl	-
Passive Reference Dye (50×) (optional)	0.4 µl	1×
RNase-free Water	Variable	-
Total volume	20 µl	-

Thermal cycling conditions (two-step)

45°C	5 min	
94°C	30 sec	
94°C	5 sec	40-45 cycles
60°C	30 sec*	

For ABI qPCR instrument, we suggest using the following signal collecting time:

* For ABI Prism7700/7900, the time is 30 seconds.
* For ABI Prism7000/7300, the time is 31 seconds.
* For ABI Prism7500, the time is 34 seconds.
* For ABI ViiA 7, the time is at least 19 seconds.

TransScript® II Probe One-Step qRT-PCR SuperMix

AQ321-01	100 rxns (20 μl per reaction)
AQ321-02	400 rxns (20 μl per reaction)

Storage
at -20°C for one year

Description

TransScript® II Probe One-Step qRT-PCR SuperMix combines the first-strand cDNA synthesis and qPCR in the same tube to simplify reaction setup and reduce the possibility of contamination. Only gene specific primers can be used for this kit. *TransScript*® II Probe One-Step qRT-PCR SuperMix contains all the necessary reagents for cDNA synthesis and qPCR except probe, total RNA/mRNA template and gene specific primers.

- 5 minutes cDNA synthesis
- cDNA synthesis and qPCR are performed in a single tube using gene specific primers with total RNA or mRNA as templates.
- Passive reference dyes are provided for different qPCR instruments.

Applications

- Multiple copy and low copy gene detection
- GC-rich or complex secondary structure RNA template
- Viral RNA and trace RNA detection

Passive Reference Dye

- Passive Reference Dye I (50×)
  ABI Prism7000/7300/7700/7900, Eppendorf, ABI Step One, ABI Step One Plus
- Passive Reference Dye II (50×)
  ABI Prism7500, ABI Prism7500 Fast, ABI Q6, ABI Quant Studio 6/7 Flex, ABI ViiA 7, Stratagene Mx3000/Mx3005P, Corbett Rotor Gene 3000
- No Passive Reference Dye
  Roche LightCycler480, Roche Light Cycler96, MJ Research Chromo4, Opticon (II), Takara TP800, Bio-Rad iCycler iQ, iQ5, Bio-Rad CFX96, Bio-Rad C1000 Thermal Cycler, Thermo Pikoreal 96, Corbett Rotor Gene 6000, Corbett Rotor Gene G, Corbett Rotor Gene Q

Kit Contents

Component	AQ321-01	AQ321-02
TransScript® II One-Step RT/RI Enzyme Mix	40 μl	160 μl
2×*TransStart*® Probe qPCR SuperMix	1 ml	4×1 ml
Passive Reference Dye (50×)	40 μl	160 μl
RNase-free Water	1 ml	4×1 ml

PROTOCOL

Reaction Components

Component	Volume	Final Concentration
RNA Template	Variable	as required
Forward GSP (10 μM)	0.4 μl	0.2 μM
Reverse GSP (10 μM)	0.4 μl	0.2 μM
Probe (10 μM)	0.4 μl	0.2 μM
2×*TransStart*® Probe qPCR SuperMix	10 μl	1×
TransScript® II One-Step RT/RI Enzyme Mix	0.4 μl	-
Passive Reference Dye (50×) (optional)	0.4 μl	1×
RNase-free Water	Variable	-
Total volume	20 μl	-

Thermal cycling conditions (two-step)

50°C 5 min
94°C 30 sec
94°C 5 sec
60°C 30 sec* 40-45 cycles

For ABI qPCR instrument, we suggest using the following signal collecting time:

* For ABI Prism7700/7900, the time is 30 seconds.
* For ABI Prism7000/7300, the time is 31 seconds.
* For ABI Prism7500, the time is 34 seconds.
* For ABI ViiA 7, the time is at least 19 seconds.

High Pure dNTPs

2.5 mM	AD101-01	1 ml
	AD101-02	5×1 ml
10 mM	AD101-11	1 ml
	AD101-12	5×1 ml

Storage
at -20°C for two years

Description
High Pure dNTPs is an equal molar solution of high quality dATP, dCTP, dGTP, and dTTP with purity up to 99%. It is suitable for PCR, qPCR, DNA sequencing and cDNA synthesis.

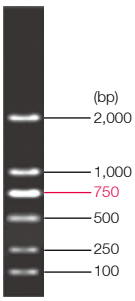
Chapter 2 DNA Molecular Weight Standards

TransGen provides a broad range of double-strand DNA molecular weight markers for conventional electrophoresis. All DNA markers are generated from restriction enzymes digested plasmids. All DNA markers are in ready-to-use format.

DNA Marker Selection Guide

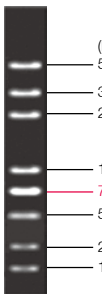
DNA Marker	Agarose	DNA Marker	Agarose
*Trans*2K® DNA Marker	1.5%	1Kb DNA Ladder	1.0%
*Trans*2K® Plus DNA Marker	1.5%	1Kb Plus DNA Ladder	1.0%
*Trans*2K® Plus II DNA Marker	1.5%	100bp DNA Ladder	2.0%
*Trans*5K DNA Marker	1.5%	100bp Plus DNA Ladder	2.0%
*Trans*15K DNA Marker	0.8%	100bp Plus II DNA Ladder	2.0%

*Trans*2K® DNA Marker



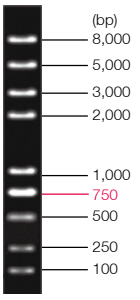
1.5% TAE agarose gel stained with GelStain (loading volume: 5 μl)

*Trans*2K® Plus DNA Marker



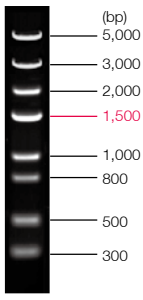
1.5% TAE agarose gel stained with GelStain (loading volume: 5 μl)

*Trans*2K® Plus II DNA Marker



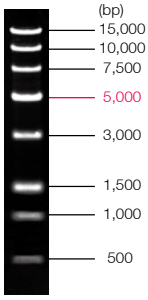
1.5% TAE agarose gel stained with GelStain (loading volume: 5 μl)

*Trans*5K DNA Marker



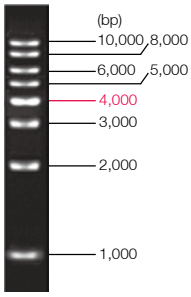
1.5% TAE agarose gel stained with GelStain (loading volume: 5 μl)

*Trans*15K DNA Marker



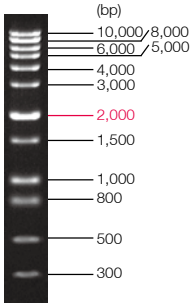
0.8% TAE agarose gel stained with GelStain (loading volume: 5 μl)

1Kb DNA Ladder



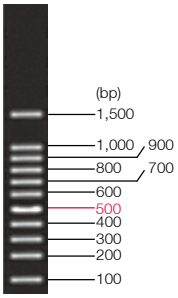
1.0% TAE agarose gel stained with GelStain (loading volume: 5 μl)

1Kb Plus DNA Ladder



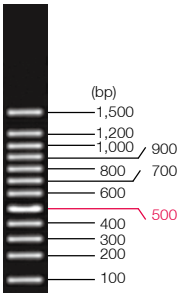
1.0% TAE agarose gel stained with GelStain (loading volume: 5 μl)

100bp DNA Ladder



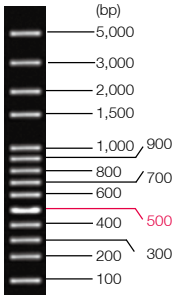
2.0% TAE agarose gel stained with GelStain (loading volume: 5 μl)

100bp Plus DNA Ladder



2.0% TAE agarose gel stained with GelStain (loading volume: 5 μl)

100bp Plus II DNA Ladder



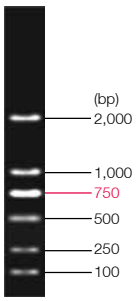
2.0% TAE agarose gel stained with GelStain (loading volume: 5 μl)

*Trans*2K® DNA Marker

BM101-01	500 µl
BM101-02	5×500 µl

Concentration
0.07 mg/ml
Band Size
100 bp, 250 bp, 500 bp, 750 bp (100 ng/5 µl, the double intensity band), 1,000 bp, 2,000 bp.
Storage
at 4°C for six months; at -20°C for two years

*Trans*2K® DNA Marker



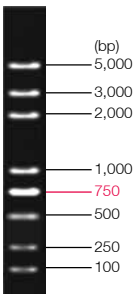
1.5% TAE agarose gel stained with GelStain (loading volume: 5 µl)

*Trans*2K® Plus DNA Marker

BM111-01	500 µl
BM111-02	5×500 µl

Concentration
0.09 mg/ml
Band Size
100 bp, 250 bp, 500 bp, 750 bp (100 ng/5 µl, the double intensity band), 1,000 bp, 2,000 bp, 3,000 bp, 5,000 bp.
Storage
at 4°C for six months; at -20°C for two years

*Trans*2K® Plus DNA Marker



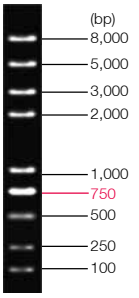
1.5% TAE agarose gel stained with GelStain (loading volume: 5 µl)

*Trans*2K® Plus II DNA Marker

BM121-01	500 µl
BM121-02	5×500 µl

Concentration
0.10 mg/ml
Band Size
100 bp, 250 bp, 500 bp, 750 bp (100 ng/5 µl, the double intensity band), 1,000 bp, 2,000 bp, 3,000 bp, 5,000 bp, 8,000 bp.
Storage
at 4°C for six months; at -20°C for two years

*Trans*2K® Plus II DNA Marker



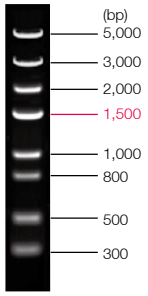
1.5% TAE agarose gel stained with GelStain (loading volume: 5 µl)

*Trans*5K DNA Marker

BM141-01	500 μl
BM141-02	5×500 μl

Concentration
0.095 mg/ml
Band Size
300 bp, 500 bp, 800 bp, 1,000 bp, 1,500 bp (125 ng/5 μl, the double intensity band), 2,000 bp, 3,000 bp, 5,000 bp.
Storage
at 4°C for six months; at -20°C for two years

*Trans*5K DNA Marker



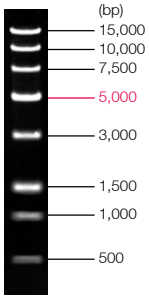
1.5% TAE agarose gel stained with GelStain (loading volume: 5 μl)

*Trans*15K DNA Marker

BM161-01	500 μl
BM161-02	5×500 μl

Concentration
0.09 mg/ml
Band Size
500 bp, 1,000 bp, 1,500 bp, 3,000 bp, 5,000 bp (100 ng/5 μl, the double intensity band), 7,500 bp, 10,000 bp, 15,000 bp.
Storage
at 4°C for six months; at -20°C for two years

*Trans*15K DNA Marker



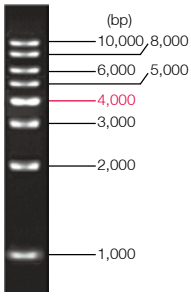
0.8% TAE agarose gel stained with GelStain (loading volume: 5 μl)

1Kb DNA Ladder

BM201-01	500 μl
BM201-02	5×500 μl

Concentration
0.09 mg/ml
Band Size
1,000 bp, 2,000 bp, 3,000 bp, 4,000 bp (100 ng/5 μl, the double intensity band), 5,000 bp, 6,000 bp, 8,000 bp, 10,000 bp.
Storage
at 4°C for six months; at -20°C for two years

1Kb DNA Ladder

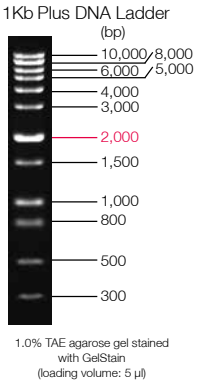


1.0% TAE agarose gel stained with GelStain (loading volume: 5 μl)

1Kb Plus DNA Ladder

BM211-01	500 µl
BM211-02	5×500 µl

Concentration
0.13 mg/ml
Band Size
300 bp, 500 bp, 800 bp, 1,000 bp,
1,500 bp, 2,000 bp (100 ng/5 µl, the double intensity band), 3,000 bp, 4,000 bp,
5,000 bp, 6,000 bp, 8,000 bp, 10,000 bp.
Storage
at 4°C for six months; at -20°C for two years

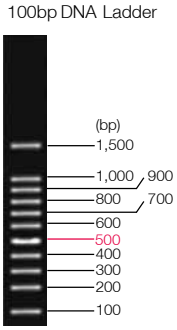


1.0% TAE agarose gel stained with GelStain (loading volume: 5 µl)

100bp DNA Ladder

BM301-01	500 µl
BM301-02	5×500 µl

Concentration
0.12 mg/ml
Band Size
100 bp, 200 bp, 300 bp, 400 bp,
500 bp (100 ng/5 µl, the double intensity band),
600 bp, 700 bp, 800 bp,
900 bp, 1,000 bp, 1,500 bp.
Storage
at 4°C for six months; at -20°C for two years

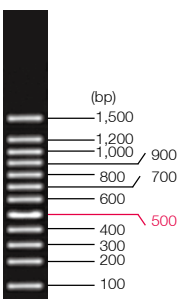


2.0% TAE agarose gel stained with GelStain (loading volume: 5 µl)

100bp Plus DNA Ladder

BM311-01	500 µl
BM311-02	5×500 µl

Concentration
0.13 mg/ml
Band Size
100 bp, 200 bp, 300 bp, 400 bp,
500 bp (100 ng/5 µl, the double intensity band),
600 bp, 700 bp, 800 bp,
900 bp, 1,000 bp, 1,200 bp, 1,500 bp.
Storage
at 4°C for six months; at -20°C for two years



2.0% TAE agarose gel stained with GelStain (loading volume: 5 µl)

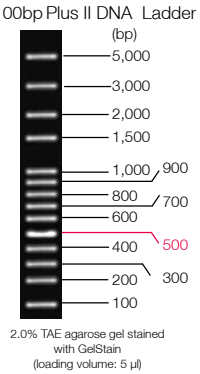
100bp Plus II DNA Ladder

BM321-01	500 μl
BM321-02	5×500 μl

Concentration
0.15 mg/ml

Band Size
100 bp, 200 bp, 300 bp, 400 bp,
500 bp (100 ng/5 μl, the double intensity band),
600 bp, 700 bp, 800 bp,
900 bp, 1,000 bp, 1,500 bp, 2,000 bp,
3,000 bp, 5,000 bp.

Storage
at 4°C for six months; at -20°C for two years



2.0% TAE agarose gel stained with GelStain (loading volume: 5 μl)

GelStain

GS101-01	500 μl
GS101-02	1 ml

Concentration
10,000×

Storage
at 4°C in dark for one year

Description
GelStain is a sensitive, stable and safe staining reagent for DNA/RNA. GelStain uses the same wavelength as ethidium bromide (EB), and it is more sensitive than EB.

Highlights
- No toxicity: GelStain is a specific form of oily macromolecules, which is incapable of entering cells via the cell membrane.
- High sensitivity: GelStain provides high sensitivity, which can detect low amount of DNA even at 10-20 ng.
- Exceptional stability: GelStain can be heated or microwaved.
- Signal to noise ratio: strong fluorescent signal from samples, weak from background.
- Like EB, GelStain can be used before electrophoresis or after electrophoresis. No destaining is needed.
- No optical setting change: standard EB filter and SYBR filter can be used.

Agarose

GS201-01	100 g

Storage
at room temperature for two years

Description
Extremely pure, molecular biology grade Agarose from TransGen is free of DNase, RNase and protease. This product is suitable for routine analysis of nucleic acids by gel electrophoresis and blotting.

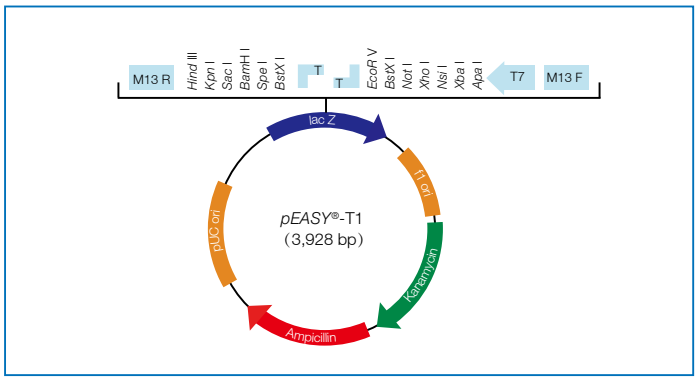
% of Agarose	Resolution (bp)
0.5%	1,000～30,000
0.7%	800～12,000
1.0%	500～10,000
1.2%	400～7,000
1.5%	200～3,000
2.0%	50～2,000

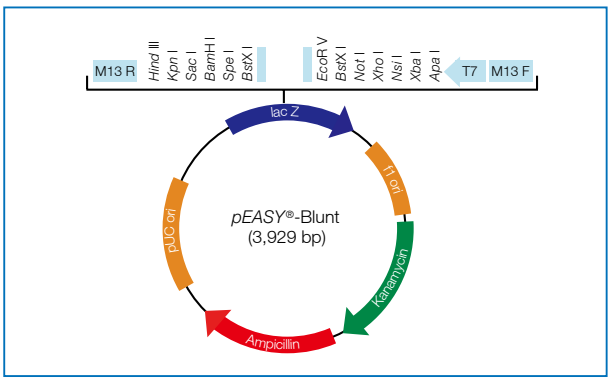
Chapter 3 Cloning and Mutagenesis System

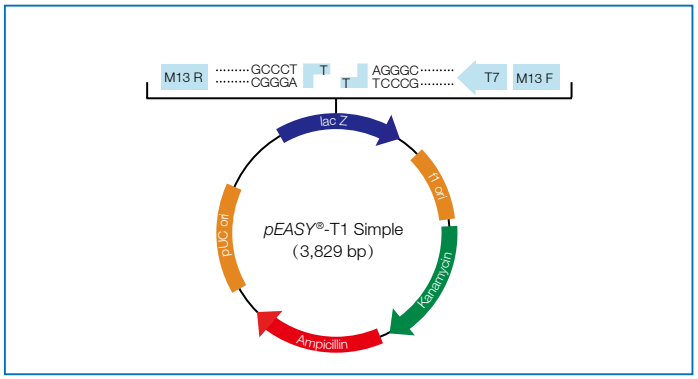
Cloning Vectors

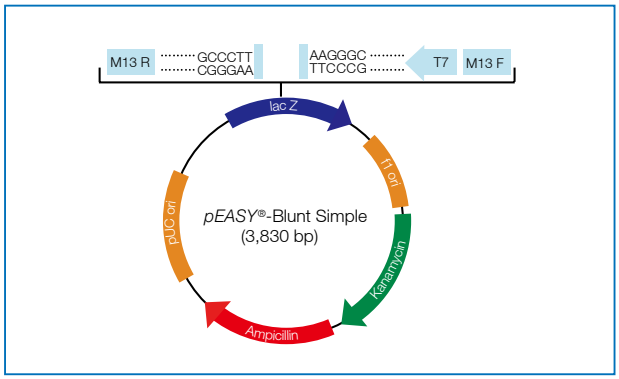
Cloning Competent Cells

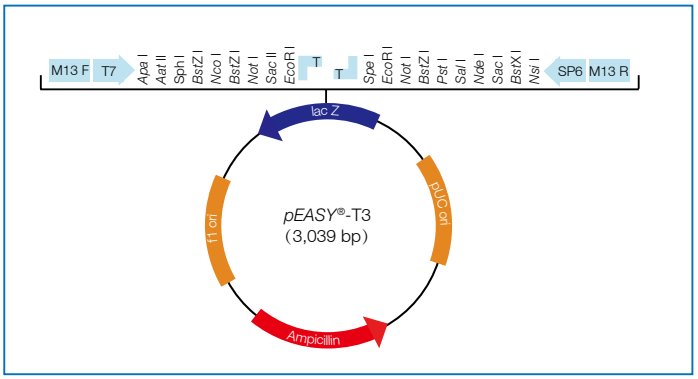
Mutagenesis System

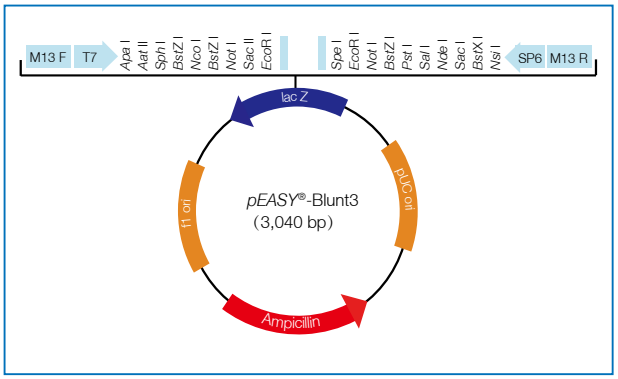

M13 R
Hind III
Kpn I
Sac I
BamH I
Spe I
BstX I
T
T
EcoR V
BstX I
Not I
Xho I
Nsi I
Xba I
Apa I
T7
M13 F
lac Z
f1 ori
pUC ori
pEASY®-T1
(3,928 bp)
Kanamycin
Ampicillin

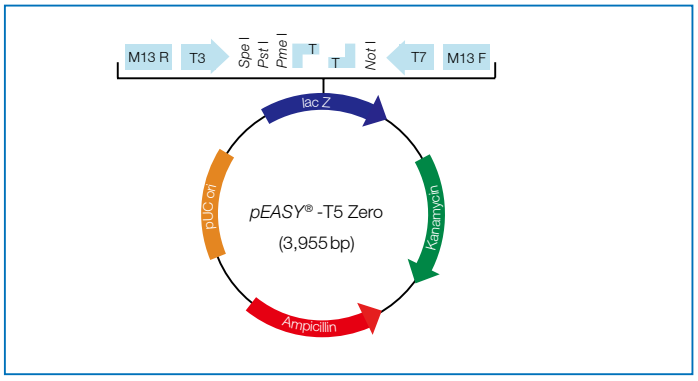

M13 R
Hind III
Kpn I
Sac I
BamH I
Spe I
BstX I
EcoR V
BstX I
Not I
Xho I
Nsi I
Xba I
Apa I
T7
M13 F
lac Z
f1 ori
pUC ori
pEASY®-Blunt
(3,929 bp)
Kanamycin
Ampicillin

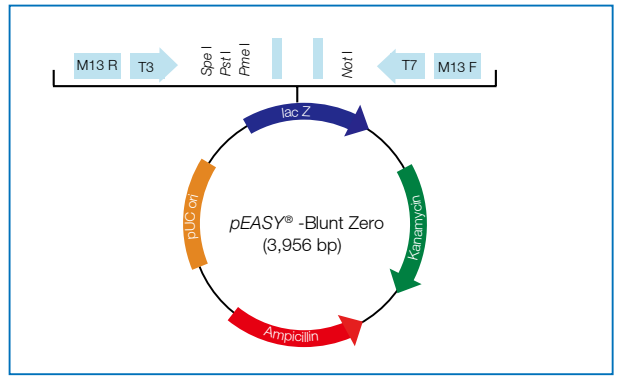

M13 R
GCCCT
CGGGA
T
T
AGGGC
TCCCG
T7
M13 F
lac Z
f1 ori
pUC ori
pEASY®-T1 Simple
(3,829 bp)
Kanamycin
Ampicillin


M13 R
GCCCTT
CGGGAA
AAGGGC
TTCCCG
T7
M13 F
lac Z
f1 ori
pUC ori
pEASY®-Blunt Simple
(3,830 bp)
Kanamycin
Ampicillin


M13 F
T7
Apa I
Aat II
Sph I
BstZ I
Nco I
BstZ I
Not I
Sac II
EcoR I
T
T
Spe I
EcoR I
Not I
BstZ I
Pst I
Sal I
Nde I
Sac I
BstX I
Nsi I
SP6
M13 R
lac Z
f1 ori
pUC ori
pEASY®-T3
(3,039 bp)
Ampicillin


M13 F
T7
Apa I
Aat II
Sph I
BstZ I
Nco I
BstZ I
Not I
Sac II
EcoR I
Spe I
EcoR I
Not I
BstZ I
Pst I
Sal I
Nde I
Sac I
BstX I
Nsi I
SP6
M13 R
lac Z
f1 ori
pUC ori
pEASY®-Blunt3
(3,040 bp)
Ampicillin


M13 R
T3
Spe I
Pst I
Pme I
T
T
Not I
T7
M13 F
lac Z
pUC ori
pEASY® -T5 Zero
(3,955 bp)
Kanamyvin
Ampicillin


M13 R
T3
Spe I
Pst I
Pme I
Not I
T7
M13 F
lac Z
pUC ori
pEASY® -Blunt Zero
(3,956 bp)
Kanamycin
Ampicillin

Advantage of *pEASY*® vectors

- Fast: 5 minutes at room temperature.
- Simple: only add PCR products.
- High efficient: up to 90% clones with correct insert.

Feature and application of *pEASY*® cloning vectors (MCS=multi-cloning site)

Name	Amp$^+$	Kan$^+$	*In vitro* transcription	Sequencing primer	Characteristics	Application
pEASY®-T1	+	+	T7 Promoter	M13 Forward Primer; M13 Reverse Primer; T7 Promoter	Dual resistance, MCS	TA cloning
pEASY®-Blunt	+	+	T7 Promoter	M13 Forward Primer; M13 Reverse Primer; T7 Promoter	Dual resistance, MCS	Blunt cloning
pEASY®-T1 Simple	+	+	T7 Promoter	M13 Forward Primer; SR Primer	Dual resistance, No MCS	TA cloning
pEASY®-Blunt Simple	+	+	T7 Promoter	M13 Forward Primer; SR Primer	Dual resistance, No MCS	Blunt cloning
pEASY®-T3	+	–	T7/SP6 Promoter	M13 Forward Primer; M13 Reverse Primer; T7 Promoter; SP6 Promoter	Dual *Eco*R I, Dual *Not* I restriction enzyme cut sites	TA cloning
pEASY®-Blunt3	+	–	T7/SP6 Promoter	M13 Forward Primer; M13 Reverse Primer; T7 Promoter; SP6 Promoter	Dual *Eco*R I, Dual *Not* I restriction enzyme cut sites	Blunt cloning
pEASY®-T5 Zero	+	+	T3/T7 Promoter	M13 Forward Primer; M13 Reverse Primer	Dual resistance, Zero background	TA cloning
pEASY®-Blunt Zero	+	+	T3/T7 Promoter	M13 Forward Primer; M13 Reverse Primer	Dual resistance, Zero background	Blunt cloning

General notes for cloning using *pEASY*® vectors

- Do not add 5' phosphates to the PCR primers. PCR products with 5' phosphates will not be cloned into *pEASY*® vector.
- Choose the right PCR enzymes for TA cloning or blunt cloning.
- To clone diluted PCR products, increase the amount of PCR products or concentrate the PCR products.
- To clone PCR products with multi-bands, gel purify the products before cloning.
- Cloning reaction time cannot be more than 30 minutes.

pEASY®-T1 Cloning Kit

CT101-01	20 rxns
CT101-02	60 rxns

Storage

*Trans*1-T1 Phage Resistant Chemically Competent Cell at -70°C for six months; others at -20°C for nine months

Description

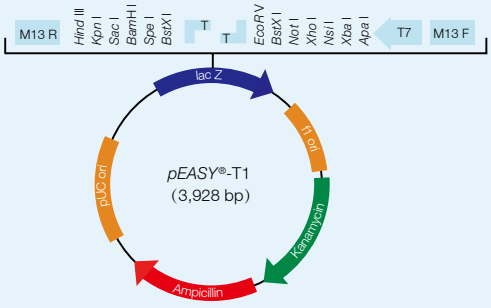
pEASY®-T1 Cloning Kit is designed for cloning and sequencing *Taq*-amplified PCR products.

- 5 minutes fast ligation of *Taq*-amplified PCR products.
- Kanamycin and Ampicillin resistance genes for selection.
- Easy blue/white selection.
- T7 promoter, M13 forward and M13 reverse primers for sequencing.
- T7 promoter for *in vitro* transcription.
- *Trans*1-T1 Phage Resistant Chemically Competent Cell, high transformation efficiency (>10^9 cfu/μg pUC19 DNA) and fast growing.

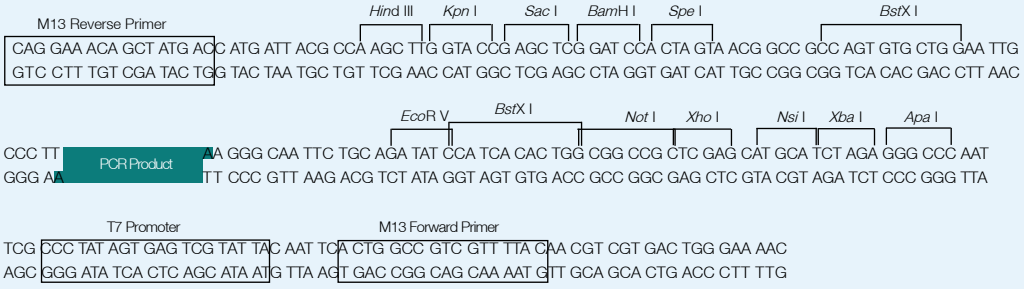
Kit Contents

Component	CT101-01	CT101-02
pEASY®- T1 Cloning Vector (10 ng/μl)	20 μl	3×20 μl
Control Template (5 ng/μl)	5 μl	5 μl
Control Primers (10 μM)	5 μl	5 μl
M13 Forward Primer (10 μM)	50 μl	150 μl
M13 Reverse Primer (10 μM)	50 μl	150 μl
*Trans*1-T1 Phage Resistant Chemically Competent Cell	10×100 μl	30×100 μl

pEASY®-T1 Cloning Vector Map



*LacZ*α fragment: bases 1-544
M13 reverse priming site: bases 205-221
Multiple cloning site: bases 234-354
T7 promoter priming site: bases 361-380
M13 forward priming site: bases 387-403
f1 origin: bases 545-982
Kanamycin resistance ORF: bases 1,316-2,110
Ampicillin resistance ORF: bases 2,128-2,988
pUC origin: bases 3,133-3,806



PROTOCOL

Suggested cloning reaction condition

1. Optimal amount of insert
   Molar ratio of vector to insert = 1:7 (1 kb, ~20 ng; 2 kb, ~40 ng)
2. Optimal volume of vector: 1 µl
3. Optimal reaction volume: 3~5 µl
4. Optimal incubation time
(1) 0.1~1 kb (including 1 kb): 5~10 minutes
(2) 1~2 kb (including 2 kb): 10~15 minutes
(3) 2~3 kb (including 3 kb): 15~20 minutes
(4) ≥3 kb: 20~30 minutes
   Use the maximum incubation time if the insert is gel purified PCR product.
5. Optimal incubation temperature: for most PCR inserts, the optimal temperature is about 25°C; for some PCR inserts, optimal results can be achieved with higher temperature (up to 37°C).

Transformation

1. Add the ligated products to 50 µl of *Trans*1-T1 Phage Resistant Chemically Competent Cell and mix gently (do not mix by pipetting up and down).
2. Incubate on ice for 20~30 minutes.
3. Heat-shock the cells at 42°C for 30 seconds.
4. Immediately place the tube on ice for 2 minutes.
5. Add 250 µl of room temperature SOC or LB medium. Shake the tube at 37°C (200 rpm) for 1 hour.
6. In the meantime, mix 8 µl of 500 mM IPTG with 40 µl of 20 mg/ml X-gal. Spread them evenly onto a selective LB plate. Place the plate at 37°C for 30 minutes.
7. Spread 200 µl or all transformants on the pre-warmed plate. Incubate at 37°C overnight.

Analysis of positive clones

1. Transfer 5~10 white or light blue colonies into 10 µl ddH_2O and vortex.
2. Use 1 µl of the mixture as template for 25 µl PCR using M13 forward and M13 reverse primers.
3. PCR

94°C	10 min	
94°C	30 sec	30 cycles
55°C	30 sec	
72°C	x min*	
72°C	5-10 min	

* (depends on the insert size and PCR enzymes)
the PCR product size from vector self-ligation is 199 bp.

4. Analyze positive clones by restriction enzyme digestion and DNA sequencing.

PCR for control insert (700 bp)

Component	Volume	Final Concentration
Control Template (5 ng/μl)	1 μl	0.1 ng/μl
Control Primers (10 μM)	1 μl	0.2 μM
2×*EasyTaq*® PCR SuperMix	25 μl	1×
ddH_2O	Variable	-
Total volume	50 μl	-

Thermal cycling conditions

94°C	2-5 min	
94°C	30 sec	30 cycles
55°C	30 sec	
72°C	1 min	
72°C	10 min	

Ligate 1 μl of control PCR insert with 1 μl vector. Hundreds of colonies should be produced with cloning efficiency over 90%.

General notes for cloning are the same as described on page 98.

pEASY® -Blunt Cloning Kit

CB101-01	20 rxns
CB101-02	60 rxns

Storage

*Trans*1-T1 Phage Resistant Chemically Competent Cell at -70°C for six months; others at -20°C for nine months

Description

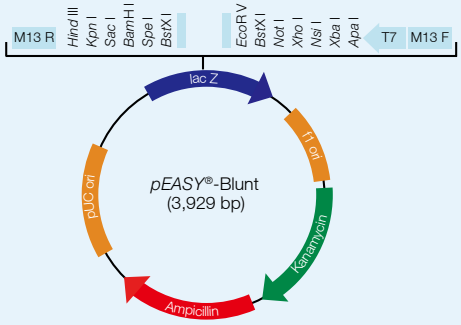
pEASY®-Blunt Cloning Kit is designed for cloning and sequencing *Pfu*-amplified PCR products.

- 5 minutes fast ligation of *Pfu*-amplified PCR products.
- Kanamycin and Ampicillin resistance genes for selection.
- Easy blue/white selection.
- T7 promoter, M13 forward and M13 reverse primers for sequencing.
- T7 promoter for *in vitro* transcription.
- *Trans*1-T1 Phage Resistant Chemically Competent Cell, high transformation efficiency (>10^9 cfu/μg pUC19 DNA) and fast growing.

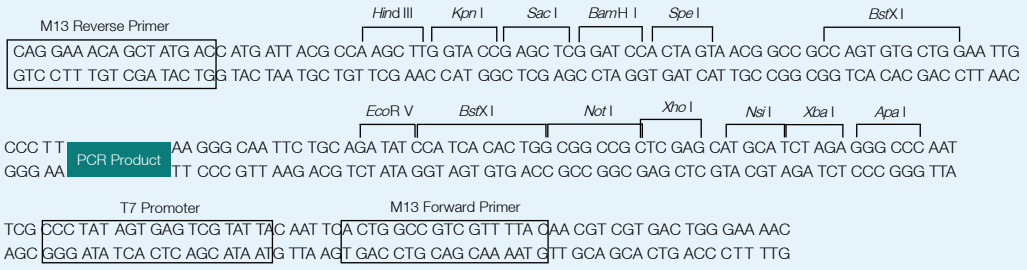
Kit Contents

Component	CB101-01	CB101-02
pEASY® -Blunt Cloning Vector (10 ng/μl)	20 μl	3×20 μl
Control Template (5 ng/μl)	5 μl	5 μl
Control Primers (10 μM)	5 μl	5 μl
M13 Forward Primer (10 μM)	50 μl	150 μl
M13 Reverse Primer (10 μM)	50 μl	150 μl
*Trans*1-T1 Phage Resistant Chemically Competent Cell	10×100 μl	30×100 μl

pEASY®-Blunt Cloning Vector Map



*LacZ*α fragment: bases 1-545
Multiple cloning site: bases 234-355
M13 reverse priming site: bases 205-221
T7 promoter priming site: bases 362-381
M13 forward priming site: bases 388-404
f1 origin: bases 546-983
Kanamycin resistance ORF: bases 1,317-2,111
Ampicillin resistance ORF: bases 2,129-2,989
pUC origin: bases 3,134-3,807



PROTOCOL

Protocols for cloning, transformation and analysis are the same as described on page 100, except the PCR product size from vector self-ligation is 200 bp. General notes for cloning are the same as described on page 98.

pEASY® -T1 Simple Cloning Kit

CT111-01	20 rxns
CT111-02	60 rxns

Storage

*Trans*1-T1 Phage Resistant Chemically Competent Cell at -70°C for six months; others at -20°C for nine months

Description

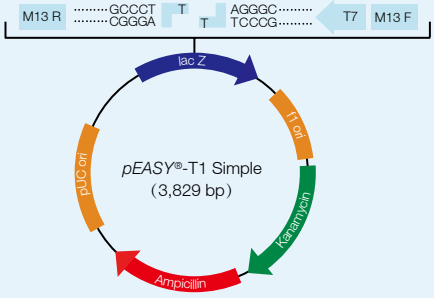
pEASY®-T1 Simple Cloning Vector eliminates the multi-cloning sites of *pEASY*®-T1 Cloning Vector. It is designed for cloning and sequencing *Taq*-amplified PCR products.

- 5 minutes fast ligation of *Taq*-amplified PCR products.
- Kanamycin and Ampicillin resistance genes for selection.
- Easy blue/white selection.
- SR primer and M13 forward primer for sequencing.
- T7 promoter for *in vitro* transcription.
- *Trans*1-T1 Phage Resistant Chemically Competent Cell, high transformation efficiency (>10^9 cfu/μg pUC19 DNA) and fast growing.

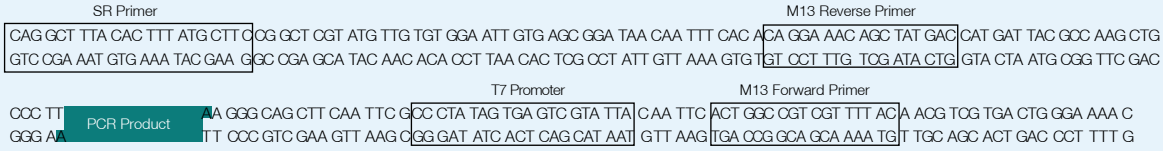
Kit Contents

Component	CT111-01	CT111-02
pEASY®- T1 Simple Cloning Vector (10 ng/μl)	20 μl	3×20 μl
Control Template (5 ng/μl)	5 μl	5 μl
Control Primers (10 μM)	5 μl	5 μl
M13 Forward Primer (10 μM)	50 μl	150 μl
M13 Reverse Primer (10 μM)	50 μl	150 μl
SR Primer (10 μM)	50 μl	150 μl
*Trans*1-T1 Phage Resistant Chemically Competent Cell	10×100 μl	30×100 μl

pEASY®-T1 Simple Cloning Vector Map



LacZα fragment: bases 1-445
M13 reverse priming site: bases 205-221
T7 promoter priming site: bases 262-281
M13 forward priming site: bases 288-304
f1 origin: bases 446-883
Kanamycin resistance ORF: bases 1,217-2,011
Ampicillin resistance ORF: bases 2,029-2,889
pUC origin: bases 3,034-3,707



PROTOCOL

Protocols for cloning, transformation and analysis are the same as described on page 100, except the PCR product size from vector self-ligation is 100 bp. General notes for cloning are the same as described on page 98.

pEASY® -Blunt Simple Cloning Kit

CB111-01	20 rxns
CB111-02	60 rxns

Storage

*Trans*1-T1 Phage Resistant Chemically Competent Cell at -70°C for six months; others at -20°C for nine months

Description

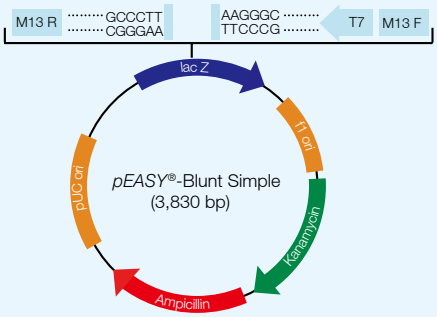
pEASY® -Blunt Simple Cloning Vector eliminates the multi-cloning sites of *pEASY*® -Blunt Cloning Vector. It is designed for cloning and sequencing *Pfu*-amplified PCR products.

- 5 minutes fast ligation of *Pfu*-amplified PCR products.
- Kanamycin and Ampicillin resistance genes for selection.
- Easy blue/white selection.
- SR primer and M13 forward primer for sequencing.
- T7 promoter for *in vitro* transcription.
- *Trans*1-T1 Phage Resistant Chemically Competent Cell, high transformation efficiency (>10^9 cfu/μg pUC19 DNA) and fast growing.

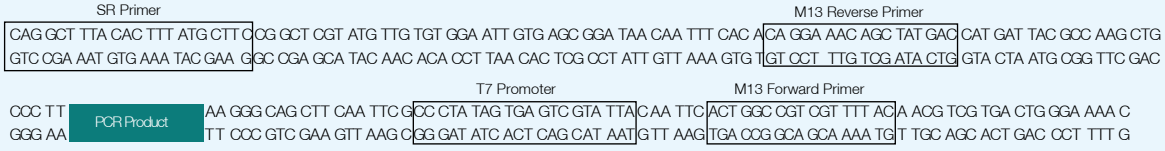
Kit Contents

Component	CB111-01	CB111-02
pEASY® -Blunt Simple Cloning Vector (10 ng/μl)	20 μl	3×20 μl
Control Template (5 ng/μl)	5 μl	5 μl
Control Primers (10 μM)	5 μl	5 μl
M13 Forward Primer (10 μM)	50 μl	150 μl
M13 Reverse Primer (10 μM)	50 μl	150 μl
SR Primer (10 μM)	50 μl	150 μl
*Trans*1-T1 Phage Resistant Chemically Competent Cell	10×100 μl	30×100 μl

pEASY®-Blunt Simple Cloning Vector Map



*LacZ*α fragment: bases 1-446
M13 reverse priming site: bases 205-221
T7 promoter priming site: bases 263-282
M13 forward priming site: bases 289-305
f1 origin: bases 447-884
Kanamycin resistance ORF: bases 1,218-2,012
Ampicillin resistance ORF: bases 2,030-2,890
pUC origin: bases 3,035-3,708



PROTOCOL

Protocols for cloning, transformation and analysis are the same as described on page 100, except the PCR product size from vector self-ligation is 101 bp. General notes for cloning are the same as described on page 98.

pEASY® -T3 Cloning Kit

CT301-01	20 rxns
CT301-02	60 rxns

Storage

*Trans*1-T1 Phage Resistant Chemically Competent Cell at -70°C for six months; others at -20°C for nine months

Description

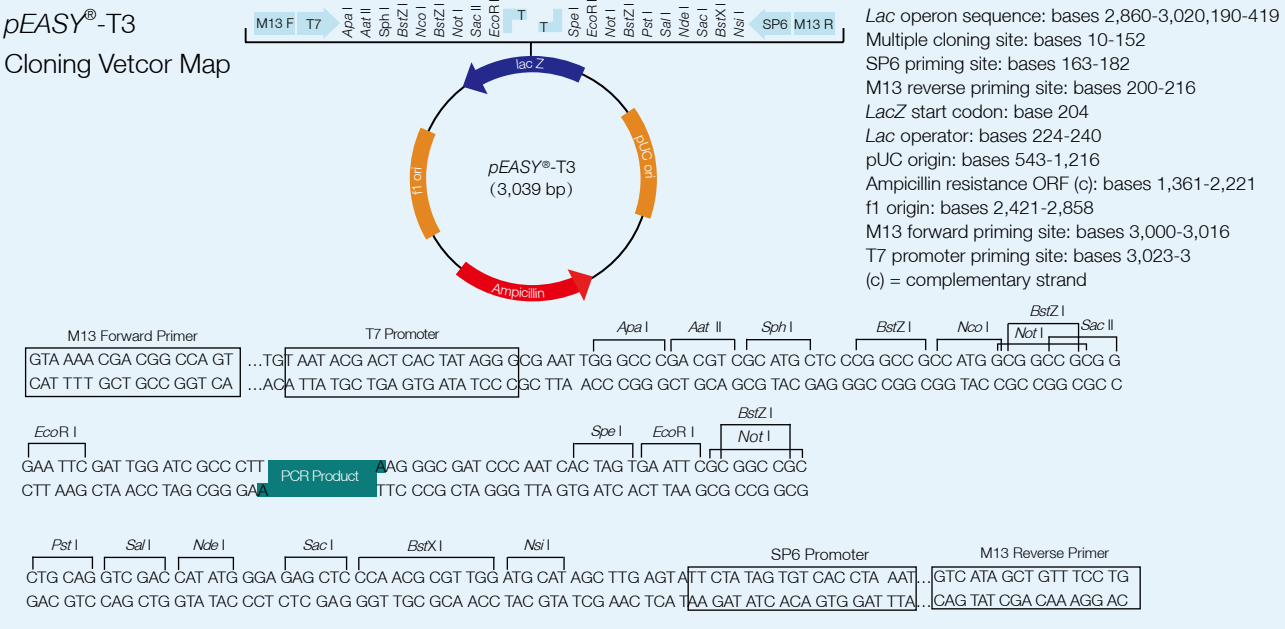
pEASY® -T3 Cloning Vector provides dual *Eco*R I and dual *Not* I restriction sites. It is designed for cloning and sequencing *Taq*-amplified PCR products. The cloned insert can be released from a single enzyme digestion.

- 5 minutes fast ligation of *Taq*-amplified PCR products.
- Ampicillin resistance gene for selection.
- Easy blue/white selection.
- T7 promoter, SP6 promoter, M13 forward and M13 reverse primers for sequencing.
- T7 promoter and SP6 promoter for *in vitro* transcription.
- *Trans*1-T1 Phage Resistant Chemically Competent Cell, high transformation efficiency (>10^9 cfu/μg pUC19 DNA) and fast growing.

Kit Contents

Component	CT301-01	CT301-02
pEASY® -T3 Cloning Vector (10 ng/μl)	20 μl	3×20 μl
Control Template (5 ng/μl)	5 μl	5 μl
Control Primers (10 μM)	5 μl	5 μl
M13 Forward Primer (10 μM)	50 μl	150 μl
M13 Reverse Primer (10 μM)	50 μl	150 μl
*Trans*1-T1 Phage Resistant Chemically Competent Cell	10x100 μl	30x100 μl

pEASY®-T3 Cloning Vetcor Map



Lac operon sequence: bases 2,860-3,020,190-419
Multiple cloning site: bases 10-152
SP6 priming site: bases 163-182
M13 reverse priming site: bases 200-216
LacZ start codon: base 204
Lac operator: bases 224-240
pUC origin: bases 543-1,216
Ampicillin resistance ORF (c): bases 1,361-2,221
f1 origin: bases 2,421-2,858
M13 forward priming site: bases 3,000-3,016
T7 promoter priming site: bases 3,023-3
(c) = complementary strand

PROTOCOL

Protocols for cloning, transformation and analysis are the same as described on page 100, except the PCR product size from vector self-ligation is 253 bp. General notes for cloning are the same as described on page 98.

pEASY® -Blunt3 Cloning Kit

CB301-01	20 rxns
CB301-02	60 rxns

Storage

*Trans*1-T1 Phage Resistant Chemically Competent Cell at -70°C for six months; others at -20°C for nine months

Description

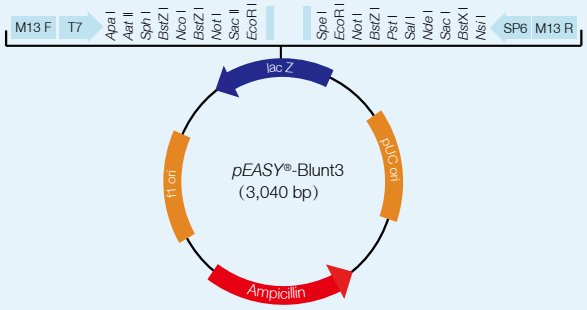
pEASY® -Blunt3 Cloning Vector provides dual *EcoR* I and dual *Not* I enzyme digestion sites. It is designed for cloning and sequencing *Pfu*-amplified PCR products. The cloned insert can be released from a single enzyme digestion.

- 5 minutes fast ligation of *Pfu*-amplified PCR products.
- Ampicillin resistance gene for selection.
- Easy blue/white selection.
- T7 promoter, SP6 promoter, M13 forward and M13 reverse primers for sequencing.
- T7 promoter and SP6 promoter for *in vitro* transcription.
- *Trans*1-T1 Phage Resistant Chemically Competent Cell, high transformation efficiency (>10^9 cfu/μg pUC19 DNA) and fast growing.

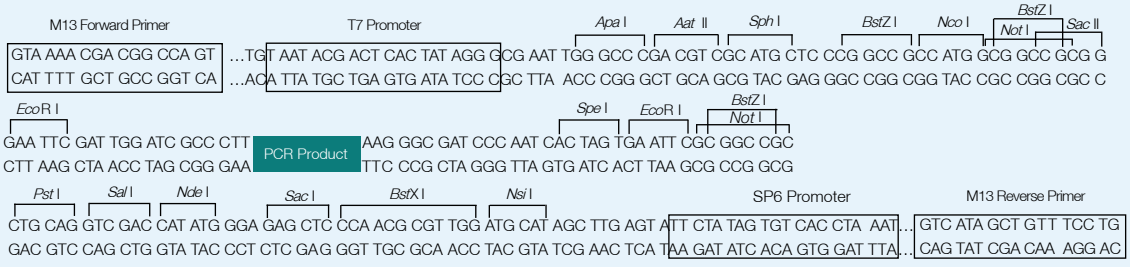
Kit Contents

Component	CB301-01	CB301-02
pEASY® -Blunt3 Cloning Vector (10 ng/μl)	20 μl	3×20 μl
Control Template (5 ng/μl)	5 μl	5 μl
Control Primers (10 μM)	5 μl	5 μl
M13 Forward Primer (10 μM)	50 μl	150 μl
M13 Reverse Primer (10 μM)	50 μl	150 μl
*Trans*1-T1 Phage Resistant Chemically Competent Cell	10x100 μl	30x100 μl

pEASY®-Blunt3 Cloning Vector Map



Lac operon sequence: bases 2,861-3,021,191-420
Multiple cloning site: bases 10-153
SP6 priming site: bases 164-183
M13 reverse priming site: bases 201-217
LacZ start codon: base 205
Lac operator: bases 225-241
pUC origin: bases 544-1,217
Ampicillin resistance ORF (c): bases 1,362-2,222
f1 origin: bases 2,422-2,859
M13 forward priming site: bases 3,001-3,017
T7 promoter priming site: bases 3,024-3
(c) = complementary strand



PROTOCOL

Protocols for cloning, transformation and analysis are the same as described on page 100, except the PCR product size from vector self-ligation is 254 bp. General notes for cloning are the same as described on page 98.

pEASY® -T5 Zero Cloning Kit

CT501-01	20 rxns
CT501-02	60 rxns

Storage

*Trans*1-T1 Phage Resistant Chemically Competent Cell at -70°C for six months; others at -20°C for nine months

Description

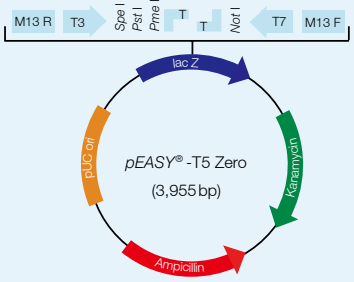
pEASY® -T5 Zero Cloning Vector contains a suicide gene. Ligation of PCR fragment disrupts the expression of the gene. Cells that contain non-recombinant vector are killed upon plating. Therefore, blue/white selection is not required.

- 5 minutes fast ligation of *Taq*-amplified PCR products.
- High cloning efficiency. Positive clones up to 100%.
- No blue/white selection needed.
- Suitable for short and large fragment cloning.
- Kanamycin and Ampicillin resistance genes for selection.
- M13 forward primer and M13 reverse primer for sequencing.
- T3 promoter and T7 promoter for *in vitro* transcription.
- *Trans*1-T1 Phage Resistant Chemically Competent Cell, high transformation efficiency (>10^9 cfu/μg pUC19 DNA) and fast growing.

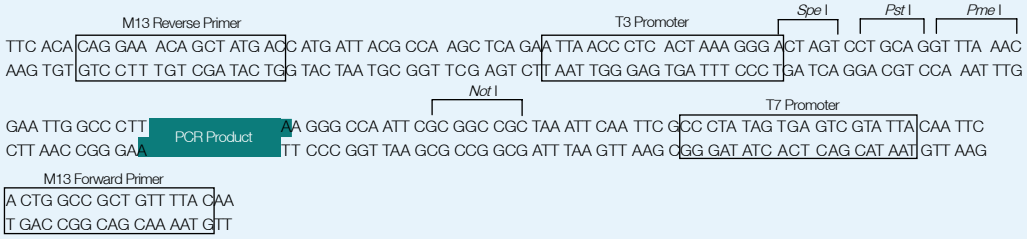
Kit Contents

Component	CT501-01	CT501-02
pEASY® -T5 Zero Cloning Vector (10 ng/μl)	20 μl	3×20 μl
Control Template (5 ng/μl)	5 μl	5 μl
Control Primers (10 μM)	5 μl	5 μl
M13 Forward Primer (10 μM)	50 μl	150 μl
M13 Reverse Primer (10 μM)	50 μl	150 μl
*Trans*1-T1 Phage Resistant Chemically Competent Cell	10×100 μl	30×100 μl

pEASY®-T5 Zero Cloning Vector Map



*LacZ*α fragment: bases 217-809
M13 reverse priming site: bases 207-223
T7 promoter priming site: bases 327-346
M13 Forward priming site: bases 353-369
Kanamycin resistance ORF: bases 1,158-1,952
Ampicillin resistance ORF (c): bases 2,202-3,062
pUC origin: bases 3,160-3,833
(c) = complementary strand



PROTOCOL

Protocols for cloning, transformation and analysis are the same as described on page 100. General notes for cloning are the same as described on page 98.

pEASY® -Blunt Zero Cloning Kit

CB501-01	20 rxns
CB501-02	60 rxns

Storage

*Trans*1-T1 Phage Resistant Chemically Competent Cell at -70°C for six months; others at -20°C for nine months

Description

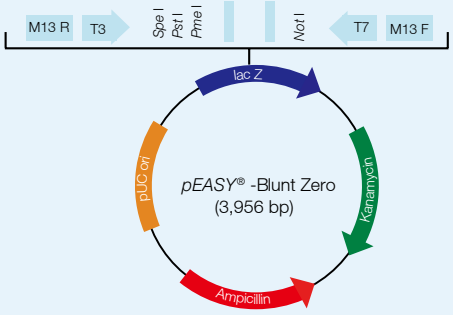
pEASY®-Blunt Zero Cloning Vector contains a suicide gene. Ligation of PCR fragment disrupts the expression of the gene. Cells that contain non-recombinant vector are killed upon plating. Therefore, blue/white selection is not required.

- 5 minutes fast ligation of *Pfu*-amplified PCR products.
- High cloning efficiency. Positive clones up to 100%.
- No blue/white selection needed.
- Suitable for short and large fragment cloning.
- Kanamycin and Ampicillin resistance genes for selection.
- M13 forward primer and M13 reverse primer for sequencing.
- T3 promoter and T7 promoter for *in vitro* transcription.
- *Trans*1-T1 Phage Resistant Chemically Competent Cell, high transformation efficiency (>10^9 cfu/μg pUC19 DNA) and fast growing.

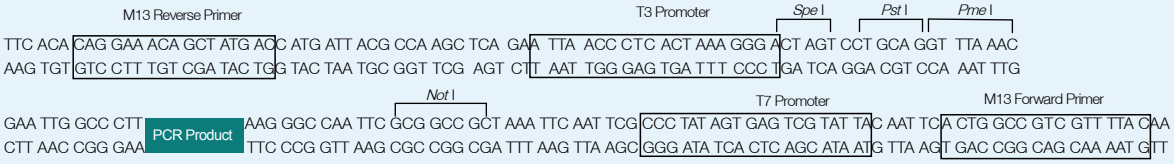
Kit Contents

Component	CB501-01	CB501-02
pEASY® -Blunt Zero Cloning Vector (10 ng/μl)	20 μl	3×20 μl
Control Template (5 ng/μl)	5 μl	5 μl
Control Primers (10 μM)	5 μl	5 μl
M13 Forward Primer (10 μM)	50 μl	150 μl
M13 Reverse Primer (10 μM)	50 μl	150 μl
*Trans*1-T1 Phage Resistant Chemically Competent Cell	10×100 μl	30×100 μl

pEASY®-Blunt Zero Cloning Vector Map



LacZα fragment: bases 217-810
M13 reverse priming site: bases 205-221
T7 promoter priming site: bases 328-347
M13 Forward priming site: bases 354-370
Kanamycin resistance ORF: bases 1,159-1,953
Ampicillin resistance ORF (c): bases 2,203-3,063
pUC origin: bases 3,161-3,834
(c) = complementary strand



PROTOCOL

Protocols for cloning, transformation and analysis are the same as described on page 100. General notes for cloning are the same as described on page 98.

pEASY® -Uni Seamless Cloning and Assembly Kit

CU101-01	10 rxns

Storage

*Trans*1-T1 Phage Resistant Chemically Competent Cell at -70°C for six months; others at -20°C for one year

Description

This kit takes advantage of proprietary assembly mix and homologous recombination. This kit can achieve directional cloning of PCR fragments that share 15-25 bp overlapping sequences into any linearized vector.

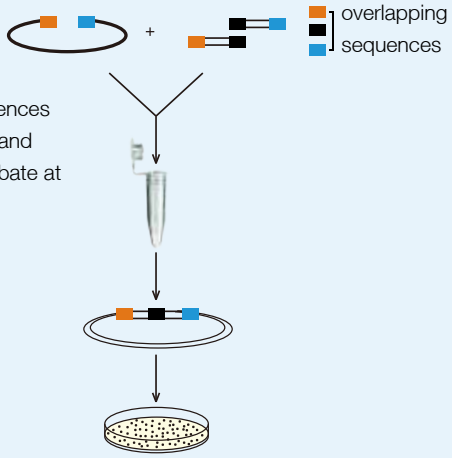
- Fast: 15 minutes.
- Broad: no restriction enzyme digestions. Can be cloned into any sites.
- High efficiency: up to 95% cloning efficiency.
- Seamless: no extra sequences introduced; up to 5 fragments assembly.

Kit Contents

Component	CU101-01
2×Assembly Mix	50 µl
*Trans*1-T1 Phage Resistant Chemically Competent Cell	5×100 µl
Linearized pUC19 Control Vector (10 ng/µl)	3 µl
Control Insert (1 kb, 20 ng/µl)	3 µl

Principle

1. Prepare linearized vector by PCR/Enzyme digestion
2. PCR amplify inserts with 15-25 bp overlapping sequences
3. Mix vector, DNA fragments and Assembly Mix together, incubate at 50°C for 15 minutes
4. Transformation



PROTOCOL

Cloning

Preparation of Vector and Inserts

A: Preparation of Vector

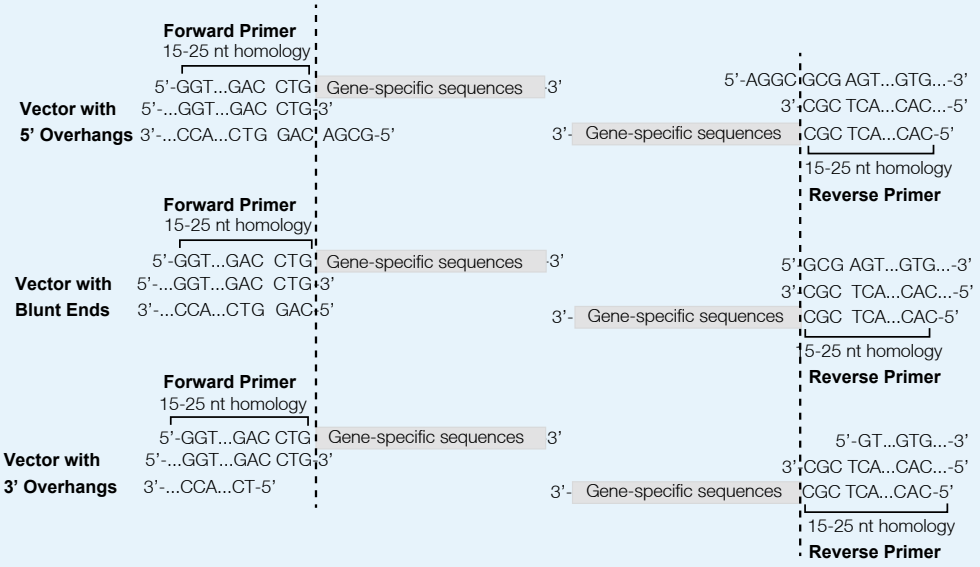
(1) Enzyme digestion: digest plasmid vector with restriction enzyme(s) to generate the linearized vector. Purify the digested vector using Gel Extraction Kit (Cat. No. EG101).

(2) PCR amplification: prepare the linearized vector by high-fidelity DNA polymerase. If a single expected band is generated, use PCR Purification Kit (Cat. No. EP101) to purify the product. Otherwise, use Gel Extraction Kit to recover the product.

In order to increase the positive cloning efficiency, we suggest using DMT enzyme to digest plasmid template before PCR purification or gel extraction. Add DMT enzyme (Cat. No. GD111) after PCR amplification (1 µl of DMT enzyme for a 50 µl PCR system), and incubate at 37°C for 30 minutes.

B: Preparation of Inserts

(1) Forward primer (5'-3'): 15-25 nt homology of linearized vector + 20-25 nt target specific sequence.
Reverse primer (5'-3'): 15-25 nt homology of linearized vector + 20-25 nt target specific sequence.
Example:



(2) Primers for multiple fragments

(3) We suggest using high-fidelity DNA polymerases to generate both the linear vector and fragments.

(4) Reaction conditions

- Use 0.2-0.4 µM (final concentration) primers for PCR.
- Use 60-68°C as annealing temperature.

(5) Purification of target DNA fragments

- To increase the cloning efficiency, if the recombinant vector has the same selection marker as the parental plasmid for PCR fragments, pretreat the PCR fragments with DMT enzyme before purification.
- If product is single band, we recommend using PCR Purification Kit (Cat. No. EP101) to purify your fragments.
- If products are multibands, we recommend using Gel Extraction Kit (Cat. No. EG101) to recover your fragments.

Setting up the cloning reaction

Component	Volume
2×Assembly Mix	5 µl
Linearized vector(5-100 ng)	x µl*
Inserts	y µl*
ddH_2O	to 10 µl

* In a 10 µl system, we recommend using 0.01-0.025 pmols of vector and insert respectively, for optimal cloning efficiency, use 1:2 (vector: insert) molar ratio. pmols= (weight in ng)/(base pairs×0.65 kDa)
For example
100 ng of 2,000 bp insert is equal to 100/(2,000×0.65), which is about 0.08 pmols. 100 ng of 5,000 bp insert is equal to 100/(5,000×0.65), which is about 0.03 pmols. Gently mix and incubate at 50°C for 15 minutes. Place it on ice for a few seconds. The reaction mixture can be directly used for transformation or stored at -20°C.

Transformation

(1) Thaw a vial of *Trans*1-T1 Phage Resistant Chemically Competent Cell on ice.
(2) Transfer 2 µl of reaction mixture into 50 µl of *Trans*1-T1 Phage Resistant Chemically Competent Cell and mix gently by flicking the tube (do not vortex). Incubate on ice for 30 minutes.
(3) Heat-shock at 42°C for 30 seconds, and immediately place on ice for 2 minutes.
(4) Add 450 µl of room temperature SOC/LB medium. Incubate at 37°C for 1 hour at 250 rpm.
(5) Pre-warm LB plate containing the appropriate selection antibiotic at 37°C.
(6) Spread 100 µl of cells on the selection plate and incubate overnight at 37°C.

Analysis of Positive Clones

Analyzing positive clones by PCR

(1) Pick single colony into 10 µl of sterile water. Mix by vortexing or pipetting up and down.
(2) Add 1 µl of mixture into 25 µl of PCR system. Identify the positive clones by appropriate forward and reverse primer.

Analyzing positive clones by restriction enzyme digestion

Pick several single colony and culture them overnight in LB medium containing the appropriate selection antibiotic. Isolate plasmid DNA by *EasyPure*® Plasmid MiniPrep Kit. Analyze the plasmids by restriction enzyme digestion.

Sequencing

Perform sequence analysis using vector universal primers

Cloning reaction for control insert

Component	Volume
2×Assembly Mix	5 µl
Linearized pUC19 Control Vector	1 µl
Control Insert	1 µl
ddH_2O	3 µl

Reaction conditions, transformation and analysis of positive clones are the same as described above.

Cloning Competent Cells

Selection Guide

Name	Cat. No.	Transformation Efficiency	Blue/White Selection/ (*lacZ*ΔM15)	Low Recombination Rate (recA)	High Quality Plasmid DNA Prepared (endA1)	Cloning of Toxic Gene	Phage Resistance
*Trans*10	CD101	10^8 cfu/μg DNA	•	•	•	•	—
*Trans*5α	CD201	10^8 cfu/μg DNA	•	•	•	—	—
*Trans*109	CD301	10^8 cfu/μg DNA	•	••	••	—	—
*Trans*110	CD311	10^6 cfu/μg DNA	•	••	••	—	—
*Trans*1-Blue	CD401	10^8 cfu/μg DNA	•	•	•	—	—
*Trans*2-Blue	CD411	10^9 cfu/μg DNA	•	•	•	—	—
*Trans*1-T1	CD501	10^9 cfu/μg DNA	•	•	•	—	•
DMT	CD511	10^8 cfu/μg DNA	•	•	•	—	•
*Trans*Stbl3	CD521	10^8 cfu/μg DNA	—	•	—	—	—
*Trans*DB3.1	CD531	10^8 cfu/μg DNA	—	•	•	•	—

*Trans*10 Chemically Competent Cell

CD101-01	10×100 μl
CD101-02	20×100 μl

Storage
at -70°C for six months

Characteristics
- High transformation efficiency: >10^8 cfu/μg (pUC19 DNA).
- Str^R.
- Blue/white selection.
- Toxic gene cloning and stable replication of plasmid DNA.

Genotype
F^- *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) φ80*lacZ*ΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara-leu*)7697 *gal*U *gal*K *rps*L (Str^R) *end*A1 *nup*G

*Trans*5α Chemically Competent Cell

CD201-01	10×100 μl
CD201-02	20×100 μl

Storage
at -70°C for six months

Characteristics
- High transformation efficiency: >10^8 cfu/μg (pUC19 DNA).
- Reduced recombination of cloned DNA.
- Blue/white selection.

Genotype
F^-φ80 *lacZ*ΔM15 Δ(*lacZYA-argF*) U169 *end*A1 *rec*A1 *hsd*R17 (r_k^-, m_k^+) *sup*E44λ- *thi*-1 *gyr*A96 *rel*A1 *pho*A

*Trans*109 Chemically Competent Cell

CD301-02	10×100 μl
CD301-03	20×100 μl

Storage
at -70°C for six months

Characteristics
- High transformation efficiency: >10^8 cfu/μg (pUC19 DNA).
- The lowest homologous recombination is favorable for plasmid DNA preparation.
- Routine cloning.
- Blue/white selection.

Genotype
*end*A1 *rec*A1 *gyr*A96 *thi*-1 *hsd*R17 (r_k^-, m_k^+) *rel*A1 *sup*E44 Δ(*lac-pro*AB) [F'*tra*D36 *pro*AB *lacI*^{q}ZΔM15]

*Trans*110 Chemically Competent Cell

CD311-02	10×100 μl

Storage
at -70°C for six months

Characteristics
- Transformation efficiency: >10^6 cfu/μg (pUC19 DNA).
- Unmethylated DNA due to dam$^-$/dcm$^-$.
- Str^R.

Genotype
*rps*L (Str^R) *thr leu thi*-1 *lac*Y *gal*K *gal*T *ara ton*A *tsx dam dcm sup*E44 Δ(*lac-pro*AB) /F' [*tra*D36 *pro*AB *lacI*^{q} *lac*ZΔM15]

*Trans*1-Blue Chemically Competent Cell

CD401-02	10×100 μl
CD401-03	20×100 μl

Storage
at -70°C for six months

Characteristics
- High transformation efficiency: >10^8 cfu/μg (pUC19 DNA).
- Tet^R.
- Blue/white selection.

Genotype
*rec*A1 *end*A1 *gyr*A96 *thi*-1 *hsd*R17 *sup*E44 *rel*A1 *lac* [F' *pro*AB *lacI*^{q}ZΔM15: Tn10 (Tet^R)]

*Trans*2-Blue Chemically Competent Cell

CD411-02	10×100 μl
CD411-03	20×100 μl

Storage
at -70°C for six months

Characteristics
- High transformation efficiency: >10^9 cfu/μg (pUC19 DNA).
- Suitable for larger plasmid transformation.
- Reduced preference for plasmid size, suitable for library construction.
- Tet^R and Cam^R.
- Blue/white selection.

Genotype
Tet^RΔ(*mcr*A)183 Hte[F′ {*pro*AB *lacI*^{q} *lacZ*ΔM15 *Tn*10(Tet^R) Amy Cam^R}] Δ(*mcr*CB-*hsd*SMR-*mrr*)173 *end*A1 *sup*E44 *thi*-1 *rec*A1 *gyr*A96 *rel*A1

*Trans*1-T1 Phage Resistant Chemically Competent Cell

CD501-01	5×100 μl
CD501-02	10×100 μl
CD501-03	20×100 μl

Storage
at -70°C for six months

Characteristics
- High transformation efficiency: >10^9 cfu/μg (pUC19 DNA).
- Fast-growing, colonies are visible in 8~9 hours.
- Resistance to T1 and T5 phage.
- Blue/white selection.

Genotype
F^- φ80(*lacZ*)ΔM15 Δ*lac*X74 *hsd*R(r_K^-, m_K^+) Δ*rec*A1398 *end*A1 *ton*A

DMT Chemically Competent Cell

CD511-01	10×50 μl
CD511-02	20×50 μl

Storage
at -70°C for six months

Characteristics
- High transformation efficiency: >10^8 cfu/μg (pUC19 DNA).
- Resistance to T1 and T5 phage.
- *In vivo* digestion of methylated DNA, suitable for site-directed mutagenesis.

Genotype
F^- φ80 *lacZ*ΔM15 Δ(*lacZ*YA-argF)U169 *rec*A1 *end*A1 *hsd*R17(r_K^-, m_K^+) *pho*A *sup*E44 *thi*-1 *gyr*A96 *rel*A1 *ton*A

*Trans*Stbl3 Chemically Competent Cell

CD521-01	10×100 μl

Storage
at -70°C for six months

Characteristics
- High transformation efficiency: >10^8 cfu/μg (pUC19 DNA).
- Suitable for lentivirus and retrovirus plasmid vectors transformation.
- Str^R
- Reduced the frequency of homologous recombination of long terminal repeats.

Genotype
F^- *mcr*B *mrr hsd*S20(r_B^-, m_B^-) *rec*A13 *sup*E44 *ara*-14 *gal*K2 *lac*Y1 *pro*A2 *rps*L20 (Str^R) *xyl*-5 λ-*leu mtl*-1

*Trans*DB3.1 Chemically Competent Cell

CD531-01	10×100 μl

Storage
at -70°C for six months

Characteristics
- High transformation efficiency: >10^8 cfu/μg (pUC19 DNA).
- Transformation and propagation of plasmids containing the ccdB gene.
- Str^R.

Genotype
F^- *gyr*A462 *end*A1 Δ(*sr*1-*rec*A) *mcr*B *mrr hsd*S20(r_B^-, m_B^-) *sup*E44*ara*-14 *gal*K2 *lac*Y1 *pro*A2 *rps*L20(Sm^R) *xyl*-5 λ- *leu mtl*1

Fast Mutagenesis System

FM111-01	10 rxns
FM111-02	20 rxns

Application

Site-directed mutagenesis for plasmid ≤20 kb

Storage

DMT Chemically Competent Cell at -70°C for six months; others at -20°C for two years

Kit Contents

Component	FM111-01	FM111-02
2×*TransStart*® *FastPfu* PCR SuperMix	250 μl	500 μl
DMT Enzyme (10 units/μl)	10 μl	20 μl
DMT Chemically Competent Cell	10×50 μl	20×50 μl
ddH_2O	1 ml	1 ml
SControl Plasmid (5 ng/μl)	10 μl	20 μl
SControl Primers (10 μM)	10 μl	20 μl

PROTOCOL

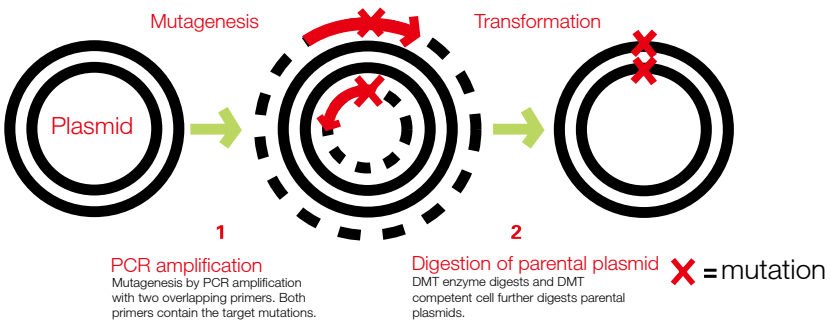
Highlights

- Mutation sites on both primers to improve mutation efficiency.
- Partially overlapping primers for exponential DNA amplification.
- Fast (4 kb/min) and high fidelity (54-fold fidelity as compared to *EasyTaq*® DNA Polymerase) 2x*TransStart*® *FastPfu* PCR SuperMix for DNA amplification.
- Double digestions (*in vitro* and *in vivo*) of parental plasmids to enhance mutation efficiency.

Primer Design

- Both primers (forward and reverse) should be approximately at 25-30 nucleotides in length.
- Primers should have an overlapping region of 15-20 nucleotides for exponential amplification.
- Primers should have an extension region of at least 10 nucleotides.
- The mutation site should be located on both primers.

Principle



Fast MultiSite Mutagenesis System

FM201-01	10 rxns

Storage

DMT Chemically Competent Cell at -70°C for six months; others at -20°C for two years

Description

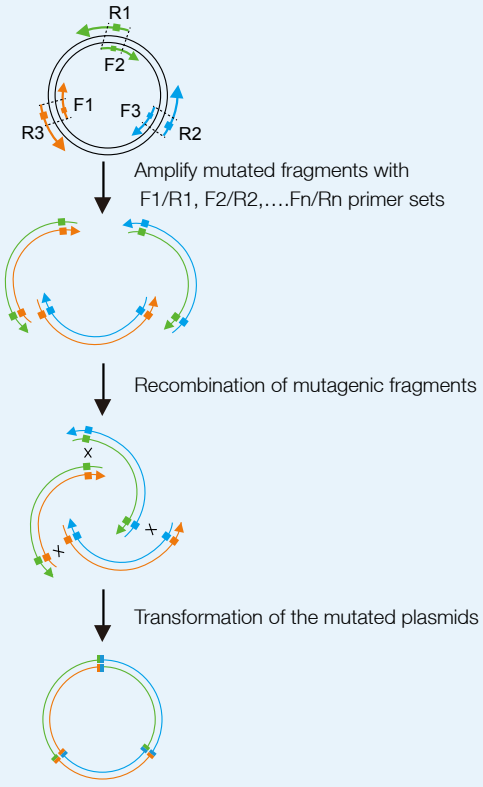
Fast MultiSite Mutagenesis System is used for generating mutated PCR fragments by introducing mutation sites on overlapping regions. High fidelity *TransStart*® *FastPfu* PCR SuperMix is included for amplification. This kit uses proprietary assembly mix and homologous recombination to seamlessly assemble up to six mutagenesis fragments.

- Fast: Amplified with fast & high-fidelity 2×*TransStart*® *FastPfu* PCR SuperMix; only 15 minutes for recombination.
- Flexible: Able to be cloned into any site to realize single-site/ multi-site, continuous/non-continuous mutagenesis.
- Efficient: >90% mutagenesis efficiency.

Kit Contents

Component	FM201-01
2×*TransStart*® *FastPfu* PCR SuperMix	1 ml
DMT Enzyme (10 units/μl)	30 μl
2×Assembly Mix	50 μl
DMT Chemically Competent Cell	10×50 μl
ddH_2O	1 ml

Cloning principle



PROTOCOL

Preparation of multisite mutagenic fragment

(1) Primer Design

- Both primers contain overlapping region at the 5' ends and extension region at the 3' ends, with mutation site on overlapping region, as shown in figure 1.
- Primer length: Both primers (forward and reverse) should be approximately at 25-40 nucleotides in length, excluding the mutation site. Primers should have an overlapping region of 15-25 nucleotides and have an extension region of at least 10 nucleotides.

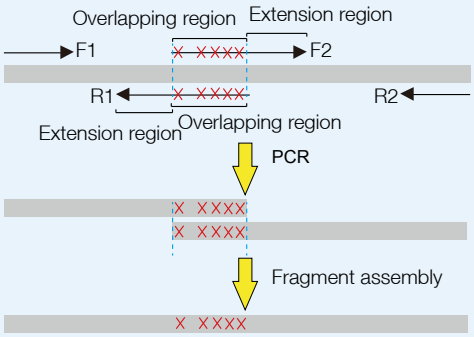


Figure 1

(2) Preparation of mutated fragment

- The mutation sites are located on one pair of primers, as shown in figure 2.
- The mutation sites are located on multiple pairs of primers, with F1/R1, F2/R2,….Fn/Rn for amplification, as shown in figure 3.

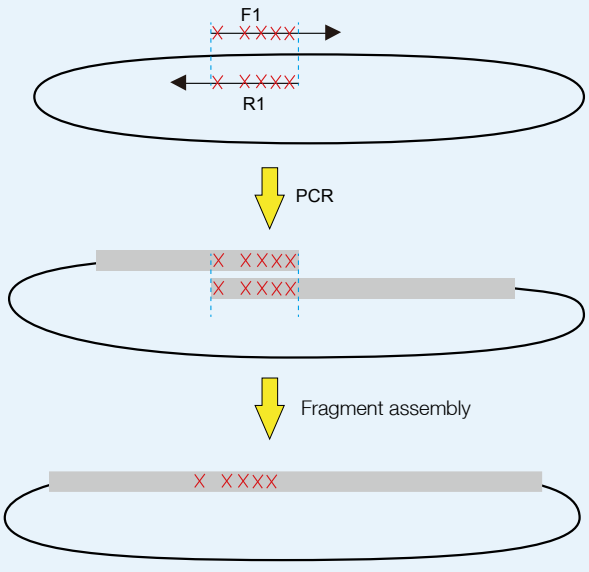


Figure 2

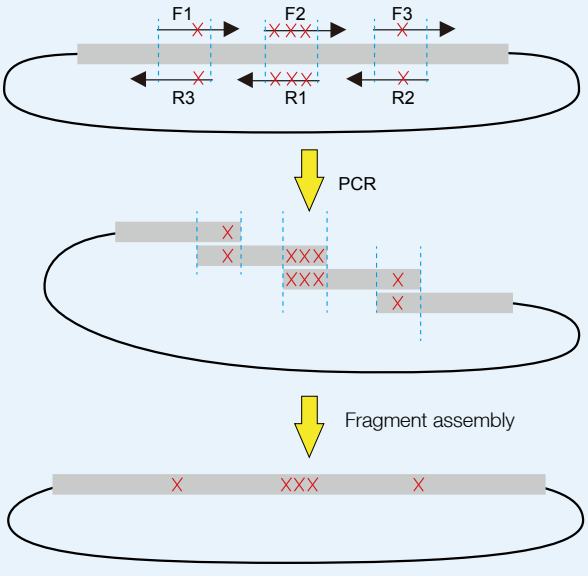


Figure 3

PCR System

Component	Volume	Final Concentration
Plasmid	1-10 ng	as required
Forward Primer (10 μM)	1 μl	0.2 μM
Reverse Primer (10 μM)	1 μl	0.2 μM
2×*TransStart*® *FastPfu* PCR SuperMix	25 μl	1×
ddH_2O	to 50 μl	Not applicable

PCR

95°C	3 min	
95°C	20 sec	25 cycles*2
55°C-65°C*1	20 sec	
72°C	2-4 kb/min	
72°C	5-10 min	

Notes

*1. Annealing temperature depends on primers.

*2. We suggest performing 25 cycles for PCR. For low yield PCR products, we suggest using 30 cycles.

Electrophoresis Analysis

Amplified PCR products can be checked by electrophoresis with 10 μl of PCR product on a 1% agarose gel.

(3) Digestion of PCR Product with DMT

Add 1 μl of DMT enzyme into PCR product, mix thoroughly and incubate at 37°C for 1 hour.

(4) Purification of PCR products

For PCR product with the single expected band, we suggest using PCR Purification Kit to purify PCR products; for PCR product with multibands, we suggest using Quick Gel Extraction Kit to purify PCR products.

Assembly of Mutated Fragments

Component	Volume
2×Assembly Mix	5 μl
Amplified fragment A	x μl*
Amplified fragment B	y μl*
……	……
Amplified fragment N	z μl*
ddH_2O	to 10 μl

*Suggested amount is 20-150 ng

Gently mix and perform reaction at 50°C for 15 minutes. After reaction, transfer the reaction tube on ice for a few seconds.

Transformation

(1) Add 2 μl of assembly products into 50 μl of DMT Chemically Competent Cell (DNA should be added immediately after thawing the cells on ice) and mix by tapping gently. Incubate on ice for 20-30 minutes.

(2) Heat-shock at 42°C for exactly 45 seconds, quickly remove from 42°C water bath and place on ice for 2 minutes.

(3) Add 250 μl of SOC or LB medium (pre-warm to room temperature), and incubate at 37°C for 1 hour with shaking at 200 rpm.

(4) Pre-warm a selective plate at 37°C for 30 minutes.

(5) Spread 100-200 μl of transformants on the plate and incubate at 37°C overnight.

Positive Clone Analysis

Analyze the clones by sequencing.

Chapter 4 Nucleic Acid Purification

Genomic DNA Purification

Plasmid DNA Purification and *E.coli* Medium

DNA Purification

RNA Purification

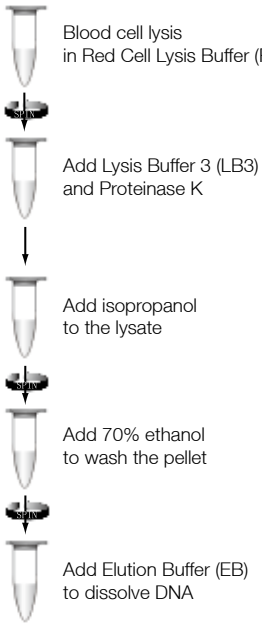
BloodZol

EE131-01	For 50 ml blood
EE131-02	For 200 ml blood

Storage

Proteinase K solution at -20°C for one year; others at room temperature (15-25°C) for one year

Procedures



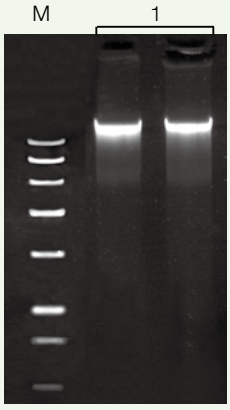
Description

BloodZol provides an easy and fast method to isolate high quality genomic DNA from 0.1-20 ml of fresh or frozen blood. Isolated DNA is free of contaminants and enzyme inhibitors. Red Cell Lysis Buffer is provided to remove non-nucleated red cells and reduce hemoglobin contamination. Genomic DNA is precipitated with isopropanol.

- High quality, free of contaminants and inhibitors.
- Suitable for EDTA, sodium citrate and heparin-anticoagulated fresh and frozen blood.
- No organic solvents.
- Isolated DNA is suitable for PCR, restriction enzyme digestion and Southern blot.

Kit Contents

Component	EE131-01	EE131-02
Red Cell Lysis Buffer (RCL)	125 ml	2×250 ml
Lysis Buffer 3 (LB3)	30 ml	120 ml
Elution Buffer (EB)	25 ml	80 ml
Proteinase K (20 mg/ml)	250 µl	1 ml



M: *Trans*15K DNA Marker
1: mouse whole blood

DNA yield from different samples

Blood	Amount	Yield
Human whole blood	400 µl	~30 µg
Mouse whole blood	400 µl	~20 µg

PlantZol

EE141-01	100 ml

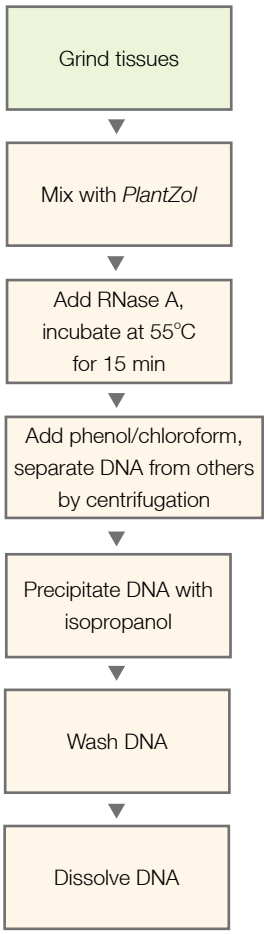
Storage
at room temperature (15-25°C) for one year

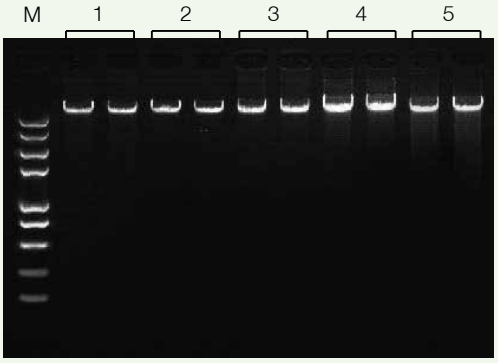
Description

PlantZol provides an easy and fast method to isolate high quality plant genomic DNA. Plant tissue is disrupted by grinding in liquid nitrogen. DNA is released with detergent. DNA is separated from other components by centrifugation and precipitated with isopropanol. *PlantZol* is suitable to isolate DNA from plants rich in polysaccharide and polyphenol.

- Isolated DNA is suitable for PCR, restriction enzyme digestion and Southern blot.

Procedures





M: *Trans*2K® Plus II DNA Marker
1: tobacco leaves
2: soybean leaves
3: wheat leaves
4: rice leaves
5: maize leaves

DNA yield from different fresh plant leaves (100 mg)

Material	Yield
Tobacco leaves	~20 µg
Wheat leaves	~35 µg
Rape leaves	~9 µg
Rice leaves	~29 µg
Soybean leaves	~16 µg
Arabidopsis leaves	~28 µg
Maize leaves	~22 µg
Tomato leaves	~7 µg

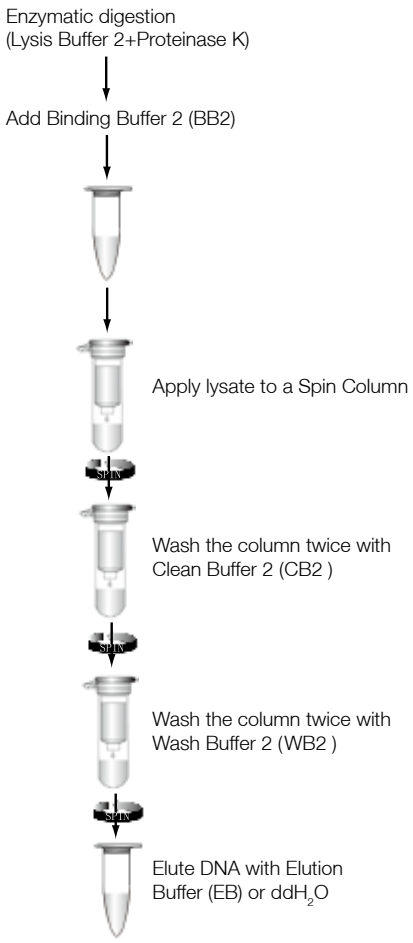
EasyPure® Genomic DNA Kit

RNase A	EE101-01	50 rxns
	EE101-02	200 rxns
RNase A-free	EE101-11	50 rxns
	EE101-12	200 rxns

Storage

RNase A and Proteinase K solutions at -20°C for one year; others at room temperature (15-25°C) for one year

Procedures



Description

EasyPure® Genomic DNA Kit provides a simple and convenient way to isolate high quality genomic DNA from a variety of mammalian cells, tissues, *E.coli* and yeast. Cells and tissues are enzymatically lysed. DNA is bound to silica-based column. The isolated DNA is suitable for PCR, restriction enzyme digestion and Southern blot.

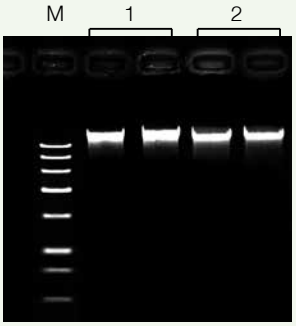
- DNA yield up to 15 µg.
- Complete removal of contaminants and inhibitors.
- Column based purification, no organic extraction or ethanol precipitation.

Kit Contents

Component	EE101-01	EE101-02
	EE101-11	EE101-12
Lysis Buffer 2 (LB2)	6 ml	24 ml
Binding Buffer 2 (BB2)	28 ml	110 ml
Clean Buffer 2 (CB2)	55 ml	2×110 ml
Wash Buffer 2 (WB2)	12 ml	2×22 ml
Elution Buffer (EB)	25 ml	80 ml
RNase A (20 mg/ml)	1 ml (EE101-01)	4×1 ml (EE101-02)
	0 (EE101-11)	0 (EE101-12)
Proteinase K (20 mg/ml)	1 ml	4×1 ml
Genomic Spin Columns with Collection Tubes	50 each	200 each

Sample Requirement

Material	Amount
Mammalian Cells	1-5×10^6 cells
Mammalian Tissues	≤25 mg
Mouse Tail	0.5 cm sections
E.coli Cells	≤2×10^9 cells
Yeast Cells	≤5×10^7 cells



M: *Trans*15K DNA Marker
1: HeLa cell
2: Mouse tissue

DNA yield from different mouse tissues

Tissue	Amount	Yield
Heart	25 mg	~5 μg
Liver	25 mg	~10 μg
Spleen	25 mg	~12 μg
Lung	25 mg	~5 μg
Kidney	25 mg	~10 μg
Muscle	25 mg	~2.5 μg

EasyPure® Plant Genomic DNA Kit

RNase A	EE111-01	50 rxns
	EE111-02	200 rxns
RNase A-free	EE111-11	50 rxns
	EE111-12	200 rxns

Storage

RNase A at -20°C for one year; others at room temperature (15-25°C) for one year

Procedures

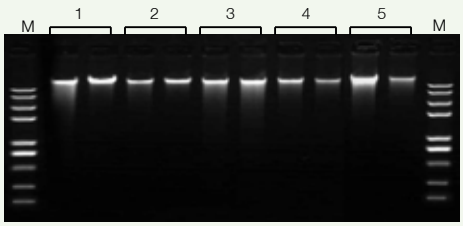
Description

EasyPure® Plant Genomic DNA Kit provides a simple and convenient way to isolate high quality genomic DNA from plant tissues (up to 100 mg). The isolated genomic DNA is suitable for PCR, restriction enzyme digestion and Southern blot.

- DNA yield up to 15 μg.
- Complete removal of pigment, polysaccharides and other impurities.
- Column based purification, no organic extraction or ethanol precipitation.

Kit Contents

Component	EE111-01 / EE111-11	EE111-02 / EE111-12
Resuspension Buffer 1 (RB1)	25 ml	100 ml
Precipitation Buffer 1 (PB1)	6 ml	24 ml
Binding Buffer 1 (BB1)	10 ml	2×20 ml
Wash Buffer 1 (WB1)	12 ml	2×24 ml
Elution Buffer (EB)	25 ml	80 ml
RNase A (10 mg/ml)	1 ml (EE111-01)	4×1 ml (EE111-02)
	0 (EE111-11)	0 (EE111-12)
Genomic Spin Columns with Collection Tubes	50 each	200 each



M: *Trans*15K DNA Marker
1: tobacco leaves
2: wheat leaves
3: sweet potato leaves
4: cayenne leaves
5: soybean leaves

DNA yield from different fresh plant leaves (100 mg)

Material	Yield
Tobacco leaves	~10 μg
Wheat leaves	~7 μg
Sweet potato leaves	~9 μg
Pepper leaves	~6 μg
Rape leaves	~5 μg
Rice leaves	~8 μg
Soybean leaves	~7 μg

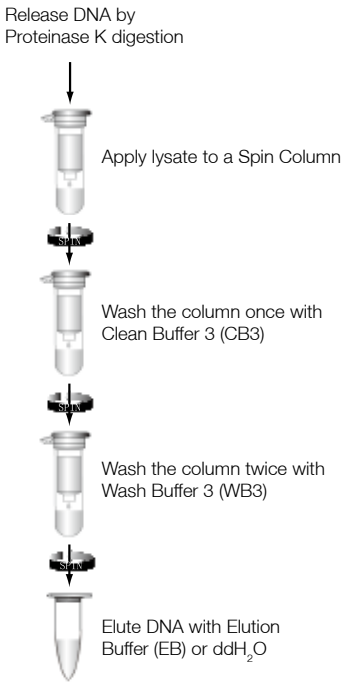
EasyPure® Blood Genomic DNA Kit

RNase A	EE121-01	50 rxns
	EE121-02	200 rxns
RNase A-free	EE121-11	50 rxns
	EE121-12	200 rxns

Storage

RNase A and Proteinase K solutions at -20°C for one year; others at room temperature (15-25°C) for one year

Procedures



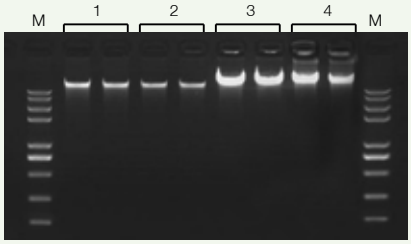
Description

EasyPure® Blood Genomic DNA Kit provides a simple and convenient way to isolate high quality genomic DNA from 5-250 µl of fresh or frozen blood. Whole blood is incubated with binding/lysis buffer to release DNA. DNA is bound to silica-based column. The isolated DNA is suitable for PCR, restriction enzyme digestion and Southern blot.

- Simple and fast, red cell lysis buffer is no longer needed.
- Complete removal of contaminants and inhibitors.
- DNA yield up to 40 µg.
- Column based purification, no organic extraction or ethanol precipitation.
- Suitable for EDTA, sodium citrate and heparin-anticoagulated fresh or frozen blood in a volume of 5 to 250 µl.

Kit Contents

Component	EE121-01 / EE121-11	EE121-02 / EE121-12
Binding Buffer 3 (BB3)	30 ml	110 ml
Clean Buffer 3 (CB3)	6 ml	24 ml
Wash Buffer 3 (WB3)	12 ml	2×22 ml
Elution Buffer (EB)	25 ml	80 ml
RNase A (20 mg/ml)	500 µl (EE121-01)	2×1 ml (EE121-02)
	0 (EE121-11)	0 (EE121-12)
Proteinase K (20 mg/ml)	1 ml	4×1 ml
Genomic Spin Columns with Collection Tubes	50 each	200 each



M: *Trans2K*® Plus II DNA Marker
1: human whole blood
2: mouse whole blood
3: bullfrog whole blood
4: chicken whole blood

DNA yield from different samples

Material	Volume	DNA yield
Human whole blood	100 µl	~6 µg
Mouse whole blood	100 µl	~6 µg
Bullfrog whole blood	20 µl	~20 µg
Chicken whole blood	20 µl	~29 µg

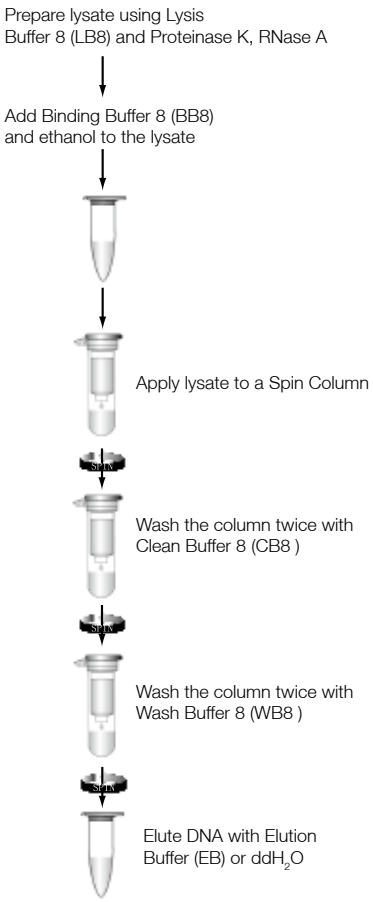
EasyPure® Marine Animal Genomic DNA Kit

RNase A	EE151-01	50 rxns
RNase A-free	EE151-11	50 rxns

Storage

RNase A and Proteinase K solutions at -20°C for one year; others at room temperature (15-25°C) for one year

Procedures



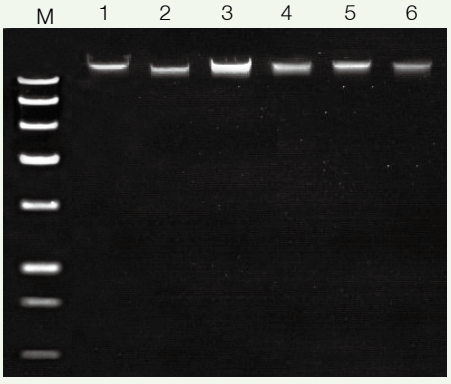
Description

EasyPure® Marine Animal Genomic DNA Kit provides a simple and convenient way to isolate high quality genomic DNA from up to 30 mg marine animals. DNA is bound to silica-based column. The isolated DNA is suitable for PCR, restriction enzyme digestion and Southern blot.

- DNA yield up to 40 μg.
- Complete removal of contaminants and inhibitors.
- Column based purification, no organic extraction or ethanol precipitation.

Kit Contents

Component	EE151-01 / EE151-11
Lysis Buffer 8 (LB8)	12 ml
Binding Buffer 8 (BB8)	9 ml
Clean Buffer 8 (CB8)	12 ml
Wash Buffer 8 (WB8)	12 ml
Elution Buffer (EB)	25 ml
RNase A (10 mg/ml)	1 ml (EE151-01) / 0 (EE151-11)
Proteinase K (20 mg/ml)	1 ml
Genomic Spin Columns with Collection Tubes	50 each



M: *Trans*15K® DNA Marker
Lane 1: white clam
Lane 2: razor clam
Lane 3: oyster
Lane 4: crab
Lane 5: king prawn
Lane 6: scallop

DNA yield from different animal tissues

Material	Amount	DNA yield
Scallop	30 mg	~30 μg
Razor clam	30 mg	~26 μg
Small-sized shrimp	30 mg	~10 μg
Mantis shrimp	30 mg	~16 μg
Crab	30 mg	~2.5 μg
Oyster	30 mg	~38 μg
White clam	30 mg	~50 μg

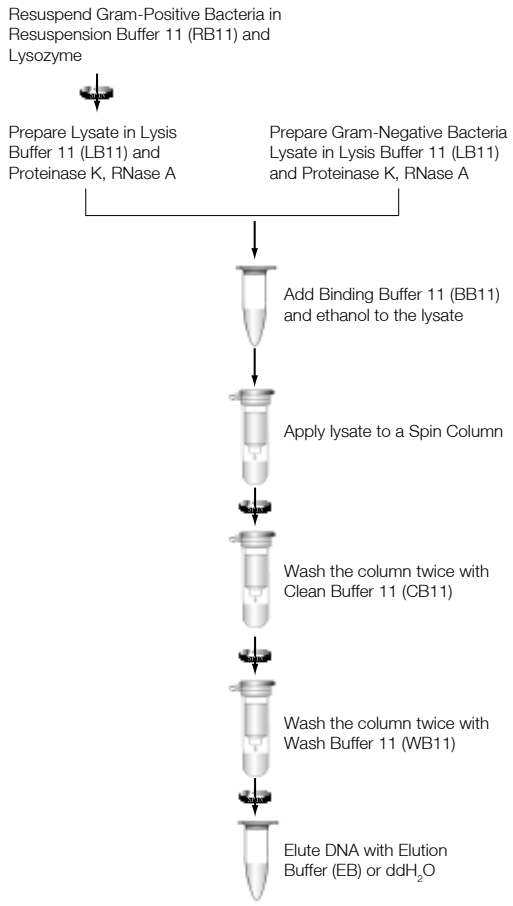
EasyPure® Bacteria Genomic DNA Kit

RNase A	EE161-01	50 rxns
RNase A-free	EE161-11	50 rxns

Storage

RNase A and Proteinase K solutions at -20°C for one year; others at room temperature (15-25°C) for one year

Procedures



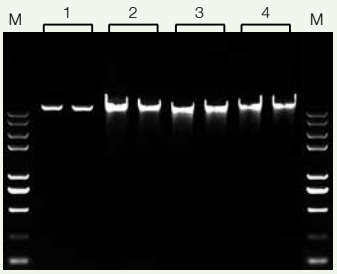
Description

EasyPure® Bacteria Genomic DNA Kit uses lysozyme and moderate lysis buffer to lyse cells. Proteinase K is used for protein digestion and RNase A used for RNA digestion. DNA is specifically bound to silica-based column in hypersaline condition, and DNA is eluted by low salt and high pH solution. This kit is suitable for isolating high quality genomic DNA from Gram-positive and Gram-negative bacteria. The isolated DNA is suitable for PCR, restriction enzyme digestion, and Southern blot.

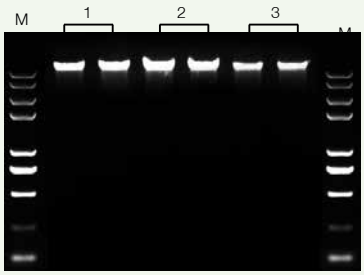
- Fast: the whole process can be completed in 50 minutes
- High yield: DNA yield up to 20 μg

Kit Contents

Component	EE161-01
	EE161-11
Resuspension Buffer 11 (RB11)	12 ml
Lysis Buffer 11 (LB11)	6 ml
Binding Buffer 11 (BB11)	10 ml
Clean Buffer 11 (CB11)	55 ml
Wash Buffer 11 (WB11)	12 ml
Elution Buffer (EB)	25 ml
RNase A (10 mg/ml)	1 ml (EE161-01)
	0 (EE161-11)
Proteinase K (20 mg/ml)	1 ml
Genomic Spin Columns With Collection Tubes	50 each



Extraction from Gram-positive Bacteria
1: *Streptomyces coelicolor*
2: *Staphylococcus aureus*
3: *Lactobacillus acidophilus*
4: *Bacillus subtilis*
M: *Trans*2K® Plus II DNA Marker



Extraction from Gram- negative Bacteria
1: *Escherichia coli*
2: *Citrobacter freundii*
3: *Pseudomonas fluorescens*
M: *Trans*2K® Plus II DNA Marker

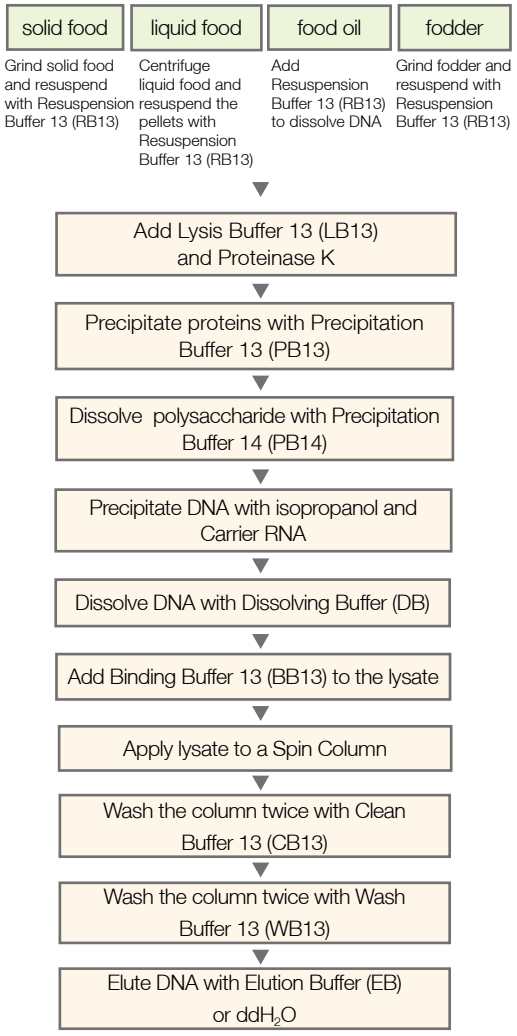
EasyPure® Food and Fodder Security Genomic DNA Kit

EE171-01	50 rxns

Storage

Proteinase K and Carrier RNA solutions at -20°C for one year; others at room temperature (15-25°C) for one year

Procedures



Description

This kit uses modified cetyltrimethylammonium bromide (CTAB) lysis method to lysis cells. DNA is bound to high-adsorption silica-based column and eluted with elution buffer without phenol/chloroform. This kit is designed for total DNA extraction from highly processed food material due to high temperature, or/and extreme pH. It is also suitable to isolate trace amount of animal DNA from fodder. The purified DNA can be used for the detection of genetically modified organisms, animal species in food and fodder.

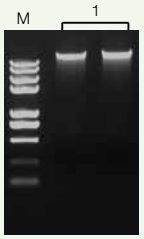
- Strong lysis, fast extraction
- High purity, high efficiency DNA isolation

Kit Contents

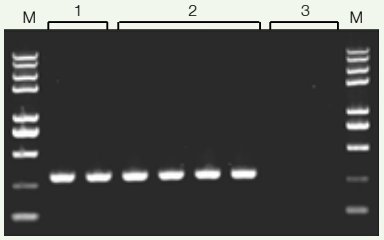
Component	EE171-01
Resuspension Buffer 13 (RB13)	180 ml
Lysis Buffer 13 (LB13)	30 ml
Precipitation Buffer 13 (PB13)	12 ml
Precipitation Buffer 14 (PB14)	18 ml
Dissolving Buffer (DB)	6 ml
Binding Buffer 13 (BB13)	10 ml
Carrier RNA (1 μg/μl)	50 μl
Clean Buffer 13 (CB13)	55 ml
Wash Buffer 13 (WB13)	12 ml
Elution Buffer (EB)	10 ml
Proteinase K (20 mg/ml)	1 ml
Genomic Spin Columns With Collection Tubes	50 each

Sample Requirement

Material	Amount
Seeds and flour	200 mg
Liquid processed food (e.g. soybean sauce, soybean milk)	20 ml
Oil (e.g. soy oil, rapeseed oil)	20 ml
Processed food (e.g. instant noodle, chips, ketchup)	200 mg
Cocoa nuts, chocolate	200 mg
Raw meat (e.g. beef, lamb, pork)	200 mg
Meat-derived processed food	200 mg
Fodder for cattle and sheep	200 mg



1: genomic DNA from soybean sauce
M: *Trans*2K® Plus II DNA Marker



Amplify plant 18S rDNA from isolated soybean sauce genomic DNA
1: positive control (soybean genomic DNA)
2: genomic DNA from soybean sauce
3: negative control
M: *Trans*2K® Plus II DNA Marker

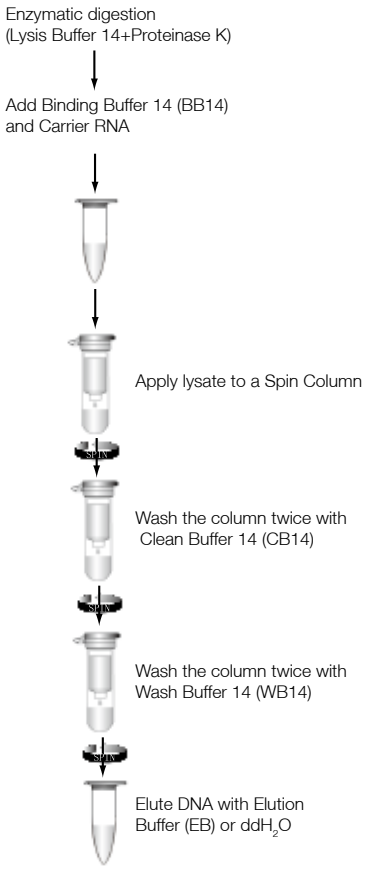
EasyPure® Micro Genomic DNA Kit

EE181-01	50 rxns

Storage

Proteinase K and Carrier RNA solutions at -20°C for one year; others at room temperature (15-25°C) for one year

Procedures



Description

EasyPure® Micro Genomic DNA Kit uses enzyme digestion method to lyse samples. The unique lysis buffer in this kit can efficiently lyse small volume of cells from a variety of materials including blood, dried blood spots, serum/plasma, mouthwash, hair follicles, tissues, microdissected tissues. DNA from the lysate will bind to silica-based column and elute with elution buffer. The isolated DNA is suitable for PCR, restriction enzyme digestion, and other downstream applications.

Kit Contents

Component	EE181-01
Lysis Buffer 14 (LB14)	6 ml
Binding Buffer 14 (BB14)	28 ml
Clean Buffer 14 (CB14)	28 ml
Wash Buffer 14 (WB14)	12 ml
Elution Buffer (EB)	5 ml
Carrier RNA (1 μg/μl)	55 μl
Proteinase K (20 mg/ml)	1 ml
Genomic Spin Columns with Collection Tubes	50 each

Sample Requirement

Material	Amount
Cultured cells	1×10^4-10^6 cells
Tissues	≤10 mg
Microdissected tissues	≤10 mg
Formalin fixed tissues	≤10 mg
E. coli	≤1×10^9 cells
Anti-coagulant blood	1-50 μl
Serum/plasma	50-250 μl
Mouthwash	2-20 ml
Dried blood spots	5 mm^2-100 mm^2
Hair follicles	1-20 pieces

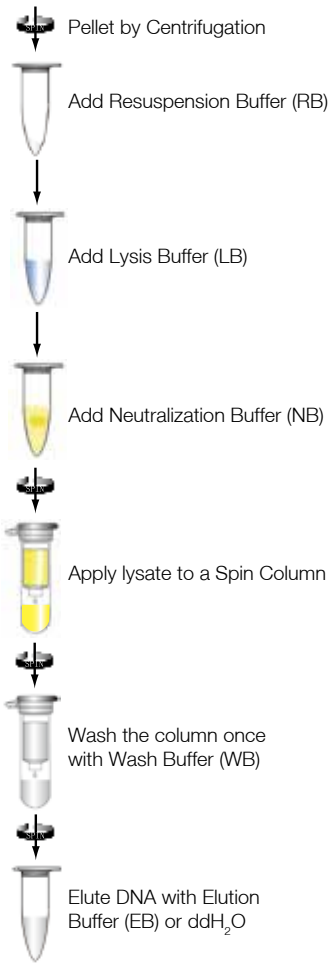
EasyPure® Plasmid MiniPrep Kit

EM101-01	50 rxns
EM101-02	200 rxns

Storage

RNase A at -20°C for one year; others at room temperature (15-25°C) for one year

Procedures



Description

EasyPure® Plasmid MiniPrep Kit uses a modified alkaline lysis method to isolate high-quality plasmid DNA from ≤20 ml (LB) or ≤4 ml (*ArtMedia*® Plasmid Culture) of bacterial culture. Unique formulated lysis buffer and neutralization buffer permit error-free visual identification of complete bacterial cell lysis and neutralization. The purified plasmid DNA is suitable for a variety of molecular biology applications, including restriction enzyme digestion, ligation, transformation and DNA sequencing.

- Simple and fast: the whole procedure can be performed in 20 minutes.
- High yield: DNA yield up to 40 µg.
- Error-free visualization: colored buffers to visualize lysis and neutralization.

Kit Contents

Component	EM101-01	EM101-02
Resuspension Buffer (RB)	15 ml	60 ml
Lysis Buffer (LB, Blue)	15 ml	60 ml
Neutralization Buffer (NB, Yellow)	20 ml	80 ml
Wash Buffer (WB)	10 ml	2×20 ml
Elution Buffer (EB)	5 ml	10 ml
RNase A (10 mg/ml)	150 µl	600 µl
Mini-Plasmid Spin Columns with Collection Tubes	50 each	2×100 each

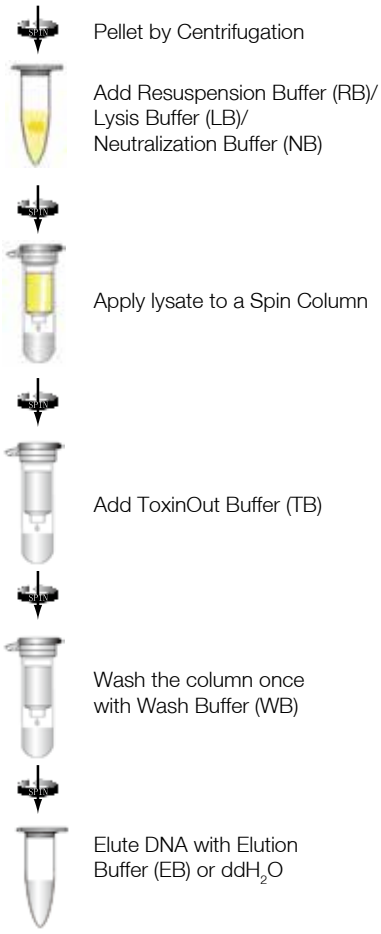
EasyPure® HiPure Plasmid MiniPrep Kit

EM111-01	50 rxns

Storage

RNase A at -20°C for one year; others at room temperature (15-25°C) for one year

Procedures



Description

EasyPure® HiPure Plasmid MiniPrep Kit provides an efficient way to isolate high yield (up to 40 µg) and high quality plasmid DNA from ≤20 ml (LB) or ≤4 ml (*ArtMedia*® Plasmid Culture) of bacterial culture. Unique formulated lysis buffer and neutralization buffer permit error-free visual identification of complete bacterial cell lysis and neutralization. Endotoxin is removed by a simple incubation on column with a novel buffer. The purified plasmid DNA is suitable for a variety of molecular biology applications, including restriction enzyme digestion, ligation, transformation, DNA sequencing, and transfection.

- Fast: the whole procedure can be performed in 20 minutes.
- Simple: endotoxin is removed on column.
- High yield: DNA yield up to 40 µg.
- Error-free visualization: colored buffers to visualize lysis and neutralization.

Kit Contents

Component	EM111-01
Resuspension Buffer (RB)	15 ml
Lysis Buffer (LB, Blue)	15 ml
Neutralization Buffer (NB, Yellow)	20 ml
ToxinOut Buffer (TB)	15 ml
Wash Buffer (WB)	10 ml
Elution Buffer (EB)	5 ml
RNase A (10 mg/ml)	150 µl
Mini-Plasmid Spin Columns with Collection Tubes	50 each

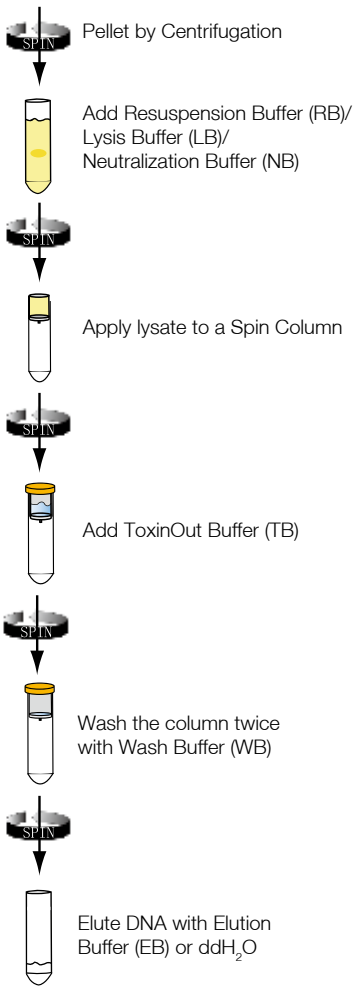
EasyPure® HiPure Plasmid MaxiPrep Kit

EM121-01	10 rxns

Storage

RNase A at -20°C for one year; others at room temperature (15-25°C) for one year

Procedures



Description

EasyPure® HiPure Plasmid MaxiPrep Kit uses a modified alkaline lysis method to isolate high quality plasmid DNA from ≤ 500 ml (LB) or ≤100 ml (*ArtMedia*® Plasmid Culture) of bacterial culture. Unique formulated lysis buffer and neutralization buffer permit error-free visual identification of complete bacterial cell lysis and neutralization. Endotoxin is removed by a simple incubation on column with a novel buffer. The purified DNA is suitable for a variety of molecular biology applications including restriction enzyme digestion, ligation, transformation, DNA sequencing, and transfection.

- Fast: the whole procedure can be performed in one hour.
- Simple: endotoxin is removed on column.
- High yield: DNA yield up to 1 mg.
- Error-free visualization: colored buffers to visualize lysis and neutralization.

Kit Contents

Component	EM121-01
Resuspension Buffer (RB)	120 ml
Lysis Buffer (LB, Blue)	120 ml
Neutralization Buffer (NB, Yellow)	160 ml
ToxinOut Buffer (TB)	60 ml
Wash Buffer (WB)	25 ml
Elution Buffer (EB)	30 ml
RNase A (10 mg/ml)	1.2 ml
Maxi-Plasmid Spin Columns with Collection Tubes	10 each

ArtMedia® Plasmid Culture

EM201-01	95 ml+5 ml

Storage
at 2-8°C for six months

Description

ArtMedia® Plasmid Culture is an enriched bacteria growth medium, which is suitable for growing various *E.coli* strains. It improves bacterial growth rate, increases cell density and obtains high yields of plasmid DNA. Under the same culture condition, *ArtMedia*® Plasmid Culture produces 3-7 folds as much of plasmid DNA as compared with traditional LB medium.

Kit Contents

Component	EM201-01
AM1	95 ml
AM2	5 ml

Suitable bacterial strains

*Trans*1-T1, *Trans*5α, *Trans*10, *Trans*109, *Trans*110, *Trans*1-Blue, *Trans*2-Blue, etc.

DNA yield

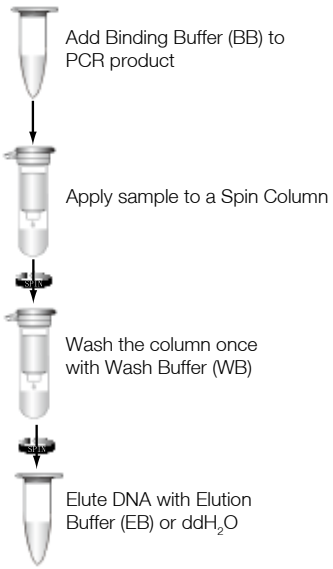
Medium	Volume	DNA yield
LB	1 ml	~2 μg
ArtMedia® Plasmid Culture	0.5 ml	~7 μg
ArtMedia® Plasmid Culture	1 ml	~14 μg
ArtMedia® Plasmid Culture	2 ml	~28 μg

EasyPure® PCR Purification Kit

EP101-01	50 rxns
EP101-02	200 rxns

Storage
at room temperature (15-25°C) for one year

Procedures



Description

EasyPure® PCR Purification Kit provides a simple and fast method to purify PCR product and enzyme-digested DNA. DNA is specifically bound to silica-based column. This kit can effectively remove impurities, including proteins, organic compounds, inorganic salt ion and primers. The purified DNA is suitable for restriction enzyme digestion, ligation, transformation and sequencing.

- Effective removal of primers, dNTPs, enzymes and inorganic salt ion.
- 95%-100% recoveries for PCR fragments of 100 bp to 10 kb.
- 5 minutes procedure.
- Purified DNA ideal for using in all molecular biology experiments, including restriction enzyme digestion, ligation and sequencing.

Kit Contents

Component	EP101-01	EP101-02
Binding Buffer (BB)	30 ml	120 ml
Wash Buffer (WB)	10 ml	2×20 ml
Elution Buffer (EB)	5 ml	10 ml
PCR Spin Columns with Collection Tubes	50 each	2×100 each

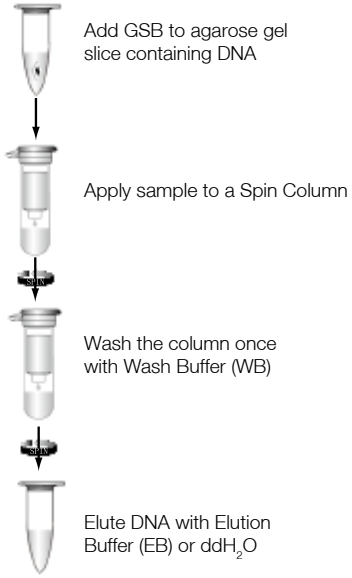
Lane 1: before purification
Lane 2: after purification
50 µl 1Kb Plus DNA Ladder

EasyPure® Quick Gel Extraction Kit

EG101-01	50 rxns
EG101-02	200 rxns

Storage
at room temperature (15-25°C) for one year

Procedures



Description

EasyPure® Quick Gel Extraction Kit is designed for rapid purification and recovery of DNA from TAE or TBE agarose gel. DNA is specifically bound to a silica-based column. The purified DNA is suitable for a variety of molecular biology applications, including restriction enzyme digestion, ligation, cloning, and DNA sequencing.

- DNA fragments size of 100 bp to 10 kb.
- Colored GSB solution (yellow) to monitor gel dissolving efficiency.
- Less than 20 minutes procedures.

Kit Contents

Component	EG101-01	EG101-02
Gel Solubilization Buffer (GSB,Yellow)	30 ml	120 ml
Wash Buffer (WB)	10 ml	2×20 ml
Elution Buffer (EB)	5 ml	10 ml
Gel Spin Columns with Collection Tubes	50 each	2×100 each

100, 500, 1,000, 2,000 bp of *Trans*2K® DNA Marker was purified from agarose gels

TransZol

ET101-01	100 ml

Storage
at 4°C in dark for one year

Description

TransZol is a ready-to-use reagent for the isolation of total RNA from cells and tissues. *TransZol* combines phenol and guanidine thiocyanate in a mono-phase solution to inhibit RNase. After lysis and centrifugation, RNA remains in the aqueous phase and others in the interphase or organic phase. RNA is precipitated by addition of isopropanol.

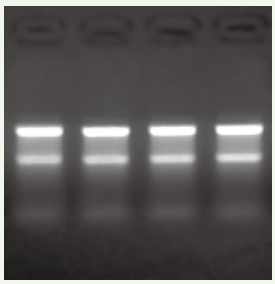
- Isolate RNA from a variety of species: animal, plant, yeast, bacteria and virus.
- The whole procedure can be completed in one hour.
- Simultaneous isolation of RNA, DNA and protein from the same sample.
- Pink solution for easy visualizing different phases.
- Unique dissolving solution for long-term RNA storage.

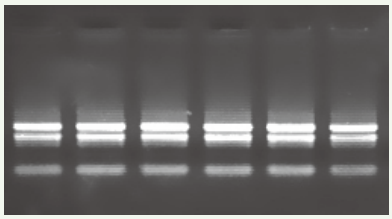
Procedures

Sample preparation
▼
Mix sample with *TransZol* (about 5 min)
▼
Separation (about 20 min)
▼
RNA precipitation (about 20 min)
▼
Wash and dissolve (about 10 min)

Kit Contents

Component	ET101-01
TransZol	100 ml
RNA Dissolving Solution	15 ml


total RNA from mouse liver

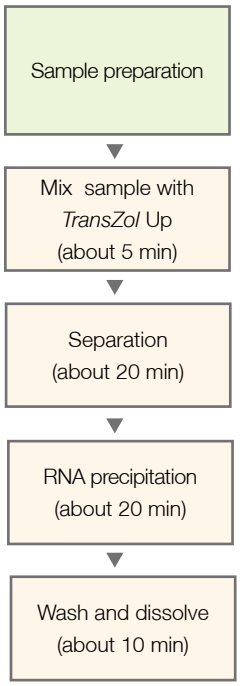

total RNA from tobacco leaves

TransZol Up

ET111-01	100 ml

Storage
at 4°C in dark for one year

Procedures



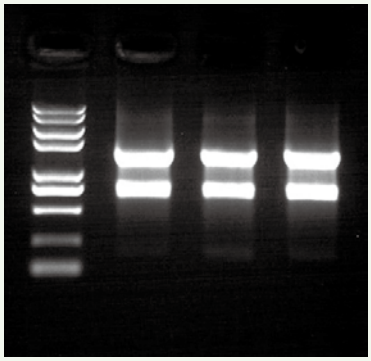
Description

TransZol Up is a ready-to-use reagent for the isolation of total RNA from cells and tissues. Unique lysis buffer is used to disrupt cells. After centrifugation, the solution is separated into an upper colorless aqueous phase containing RNA and a lower pink organic phase. RNA is precipitated and recovered with isopropanol. Proteins can be recovered from organic phase with isopropanol. Compared with other total RNA extraction reagents, *TransZol* Up provides a powerful lysis buffer to extract RNA from a variety of species.

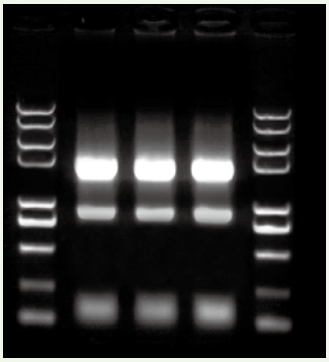
- Suitable for isolating RNA from a variety of species including animal, plant and bacteria.
- Superior lysis capability and higher RNA yield.
- The whole procedure can be completed in one hour.
- Pink solution for easy visualizing different phases.
- Unique dissolving solution for long-term RNA storage.

Kit Contents

Component	ET111-01
TransZol Up	100 ml
RNA Dissolving Solution	15 ml



TransZol Up isolates RNA from rat liver



TransZol Up isolates RNA from HeLa cells

RNA yield from different samples

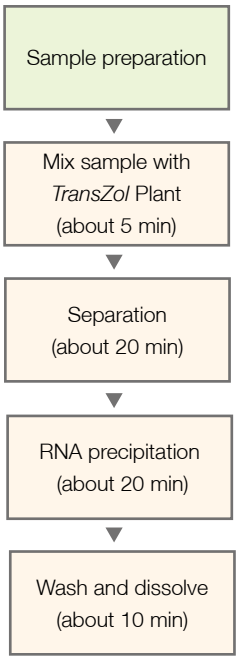
Material	Amount	RNA yield
Tobacco	100 mg	~10 μg
Human blood	200 μl	~2 μg
HeLa cell	2×10^6 cells	~10 μg
Mouse liver	100 mg	~16 μg
Rat liver	100 mg	~28 μg

TransZol Plant

ET121-01	100 ml

Storage
at room temperature (15-25°C) for one year

Procedures



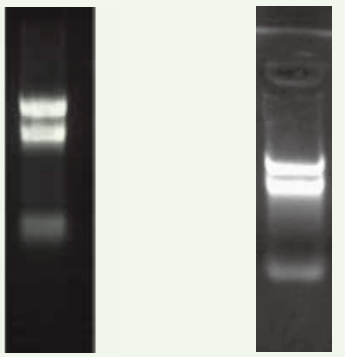
Description

TransZol Plant is a ready-to-use reagent for the isolation of total RNA from polysaccharide-rich and/or polyphenol plant tissues, such as champignon, banana fruit, mango fruit, potato, carrot, sansevieria. It uses a modified CTAB method to lyse samples and phenol/chloroform to remove proteins and others impurities. It is also suitable for the isolation of total RNA from animal tissues like fat, connective tissues etc.

- Superior lysis capability and higher RNA yield.
- The whole procedure can be completed in one hour.
- Pink solution for easy visualizing different phases.
- Unique dissolving solution for long-term RNA storage.

Kit Contents

Component	ET121-01
TP I Buffer	100 ml
TP II Buffer	100 ml
RNA Dissolving Solution	15 ml



Potato tuber Chlorophytum comosum

RNA yield from different samples

Material	Amount	RNA yield
Papaya	100 mg	~7 μg
Banana	100 mg	~8.5 μg
Apple	100 mg	~4 μg
Chinese yam	100 mg	~9 μg
Pear	100 mg	~1.5 μg
Chlorophytum comosum leaves	100 mg	~7.5 μg
Potato tuber	100 mg	~7 μg
Pine needle	100 mg	~2.5 μg

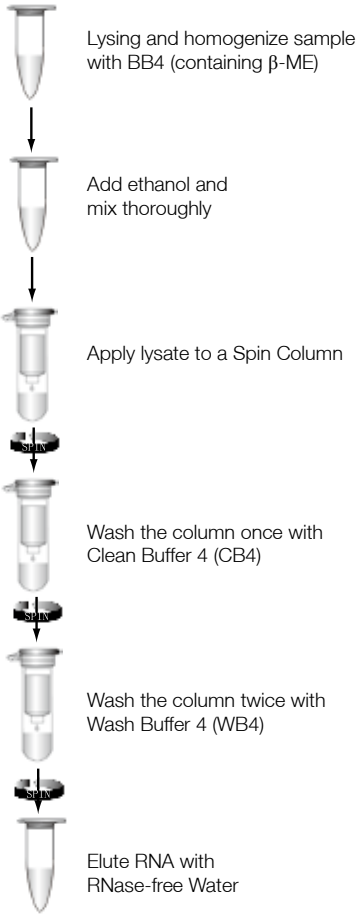
EasyPure® RNA Kit

ER101-01	50 rxns

Storage

Proteinase K and DNase I solutions at -20°C for one year; others at room temperature (15-25°C) for one year

Procedures



Description

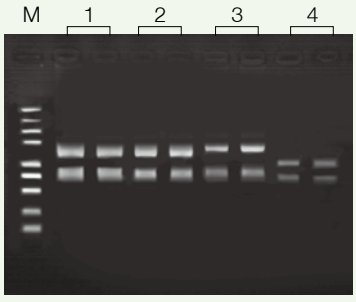
EasyPure® RNA Kit provides a simple and fast column based method to isolate total RNA from animal cells, animal tissues, bacteria and yeast. Cells and tissues are enzymatically lysed. DNA is digested with DNase I. RNA is bound to silica membrane. After washing, high quality RNA is eluted from the column. RNA is free of protein contamination, and is suitable for RT-PCR, qRT-PCR and Northern blot.

Kit Contents

Component	ER101-01
Binding Buffer 4 (BB4)	40 ml
Clean Buffer 4 (CB4)	60 ml
Wash Buffer 4 (WB4)	12 ml
Proteinase K (20 mg/ml)	1 ml
DNase I (3 units/μl)	1500 units
DNase I Reaction Buffer	4×1 ml
RNase-free Water	10 ml
RNase-free Tube (1.5 ml)	50 each
RNA Spin Columns with Collection Tubes	50 each

Sample Requirement

Material	Amount	volume of BB4/β-ME
Animal cell	≤5×10^6	0.3-0.6 ml
Animal tissue	≤20 mg	0.3-0.6 ml
Bacterial cell	≤1×10^9	0.35 ml



M: *Trans*2K® Plus II DNA Marker
1: mouse brain tissue
2: mouse kidney tissue
3: HeLa cell
4: *E.coli* cell

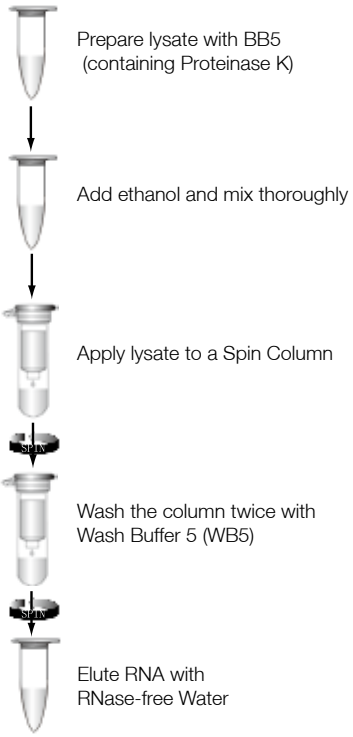
EasyPure® Viral DNA/RNA Kit

ER201-01	50 rxns

Storage

Carrier RNA and Proteinase K solutions at -20°C for one year; others at room temperature (15-25°C) for one year

Procedures



Description

EasyPure® Viral DNA/RNA Kit provides a simple and fast column based method to isolate viral DNA/RNA from up to 200 µl of plasma, serum, body fluid and mammalian cell supernatant. Samples are lysed with unique lysis buffer and DNA/RNA is enriched by carrier RNA. DNA/RNA is bound to silica membrane. After washing, high quality DNA/RNA is eluted from the column. DNA/RNA is free of protein contamination, and is suitable for PCR, RT-PCR, qPCR and qRT-PCR.

Kit Contents

Component	ER201-01
Binding Buffer 5 (BB5)	15 ml
Wash Buffer 5 (WB5)	12 ml
Proteinase K (20 mg/ml)	1 ml
Carrier RNA (1 µg/µl)	310 µl
RNase-free Water	10 ml
RNase-free Tube (1.5 ml)	50 each
RNA Spin Columns with Collection Tubes	50 each

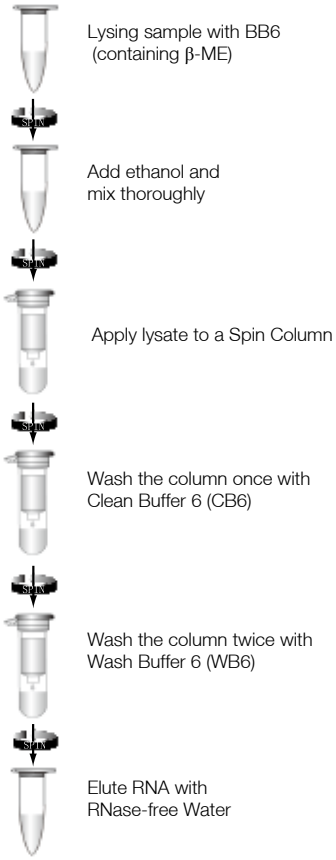
EasyPure® Plant RNA Kit

ER301-01	50 rxns

Storage

DNase I at -20°C for one year; others at room temperature (15-25°C) for one year

Procedures



Description

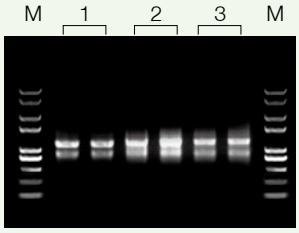
EasyPure® Plant RNA Kit provides a simple and fast column based method to isolate RNA from plant tissue. Samples are lysed with detergent to inactivate RNase. DNA is digested with DNase I. RNA is bound to silica membrane. After washing, high quality RNA is eluted from the column. RNA is free of protein contamination, and is suitable for RT-PCR, qRT-PCR, Microarray analysis and Northern blot.

Kit Contents

Component	ER301-01
Binding Buffer 6 (BB6)	60 ml
Wash Buffer 6 (WB6)	12 ml
Clean Buffer 6 (CB6)	60 ml
DNase I (3 units/μl)	1500 units
DNase I Reaction Buffer	4×1 ml
RNase-free Water	10 ml
RNase-free Tube (1.5 ml)	50 each
RNA Spin Columns with Collection Tubes	50 each

Sample Requirement

Material	Volume of BB6/β-ME
≤100 mg	0.5 ml
100-200 mg	1 ml



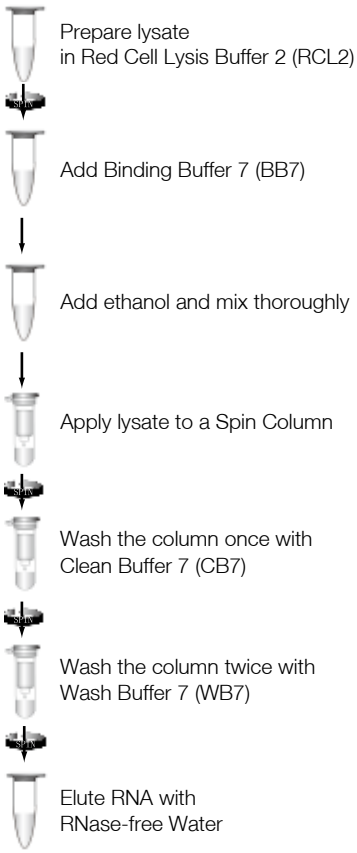
M: *Trans*2K® Plus II DNA Marker
1: corn leaves
2: wheat leaves
3: soybean leaves

EasyPure® Blood RNA Kit

ER401-01	50 rxns

Storage
DNase I at -20°C for one year; others at room temperature (15-25°C) for one year

Procedures



Description

EasyPure® Blood RNA Kit provides a simple and fast column based method to isolate total RNA from 50 µl-1.5 ml of fresh or anticoagulated blood. Blood is lysed and DNA is digested with DNase I. RNA is bound to silica membrane. After washing, high quality RNA is eluted. Purified RNA is suitable for RT-PCR, qRT-PCR and Northern blot.

Kit Contents

Component	ER401-01
Red Cell Lysis Buffer 2 (RCL2)	125 ml
Binding Buffer 7 (BB7)	40 ml
Clean Buffer 7 (CB7)	60 ml
Wash Buffer 7 (WB7)	12 ml
DNase I (3 units/µl)	1500 units
DNase I Reaction Buffer	4×1 ml
RNase-free Water	10 ml
RNA Spin Columns with Collection Tubes	50 each
RNase-free Tube (1.5 ml)	50 each

Sample Requirement

Fresh or anticoagulated blood can be kept at 4°C for one week. Do not freeze blood sample. Blood sample should be extracted as soon as possible and mixed well before use.

Amount of Blood	Volume of BB7
<500 µl	300 µl
500 µl-1.5 ml	600 µl

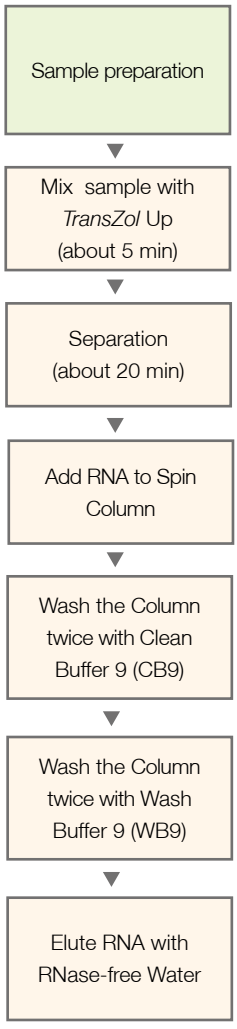
TransZol Up Plus RNA Kit

ER501-01	100 rxns

Storage

TransZol Up at 4°C in dark for one year, others at room temperature (15°C-25°C) for one year

Procedures



Description

TransZol Up Plus RNA Kit is a ready-to-use reagent for the isolation of total RNA from cells and tissues. After lysis and centrifugation, the solution separates into an upper colorless aqueous phase (containing RNA), intermediate phase and a lower pink organic phase. RNA is specifically bound to silica-based spin column. It is a new modified version of *TransZol* Up. Compared with other total RNA extraction methods, *TransZol* Up Plus RNA Kit provides powerful lysis and easy column based purification.

- Wide application: suitable for isolating RNA from a variety of species including human, animal, plant and bacteria.
- Powerful lysis capability: complete lysis, higher RNA yield and higher purity.
- Rapid extraction: the whole procedure can be completed in one hour.
- Visible operation: pink solution for easy visualizing different phases.

Kit Contents

Component	ER501-01
TransZol Up	100 ml
Clean Buffer 9 (CB9)	110 ml
Wash Buffer 9 (WB9)	24 ml
RNase-free Water	40 ml
RNase-free Tube (1.5 ml)	100 each
RNA Spin Columns with Collection Tubes	100 each

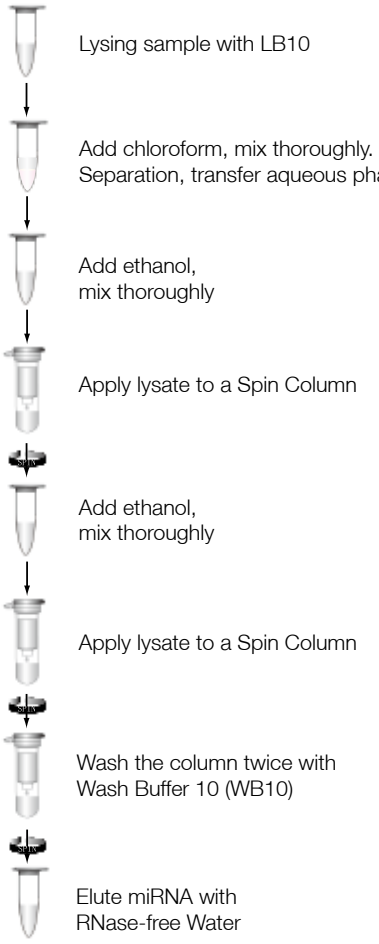
EasyPure® miRNA Kit

ER601-01	50 rxns

Storage

LB10 at 4°C in dark for one year; others at room temperature (15-25°C) for one year

Procedures



Description

EasyPure® miRNA Kit provides a simple and fast column based method to isolate small RNA (≤200 nt) from cells, tissues, fresh blood and virus. Samples are lysed with lysis buffer. The addition of chloroform to the sample separates the solution into an upper colorless aqueous phase containing RNA, an interphase and a lower organic phase. High molecular RNA (28S rRNA, 18S rRNA, mRNA) is bound to a silica membrane. Small RNA in the flow-through can be bound to a miRNA spin column.

Kit Contents

Component	ER601-01
Lysis Buffer 10 (LB10)	55 ml
Wash Buffer 10 (WB10)	12 ml
RNA Spin Columns with Collection Tubes	50 each
miRNA Spin Columns with Collection Tubes	50 each
RNase-free Tube (1.5 ml)	50 each
RNase-free Water	10 ml

Sample Requirement

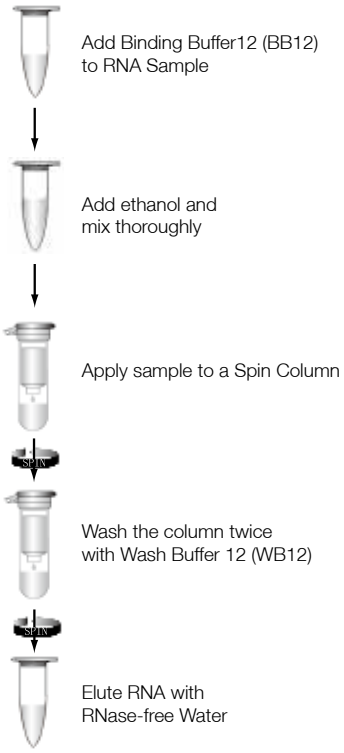
Material	Amount
Tissue	50-100 mg
Cell	1×10^7 cells
Fresh Blood	50-200 µl

EasyPure® RNA Purification Kit

ER701-01	25 rxns

Storage
at room temperature (15°C- 25°C) for one year

Procedures



Description

EasyPure® RNA Purification Kit uses silica-based spin column for specific RNA binding. The kit can be used for RNA purification from DNase I-treated total RNA, *in vitro* transcription product, RNA-labelled product, synthetic RNA. This kit permits effective removal of proteins, organic chemicals, inorganic salt ion and other impurities. Purified RNA is suitable for RT-PCR, qRT-PCR, Northern blot and other applications.

Kit Contents

Component	ER701-01
Binding Buffer 12 (BB12)	10 ml
Wash Buffer 12 (WB12)	8 ml
RNase-free Water	1.5 ml
RNase-free Tube (1.5 ml)	25 each
RNA Spin Columns with Collection Tubes	25 each

RNAhold®

EH101-01	100 ml

Storage

at room temperature for one year

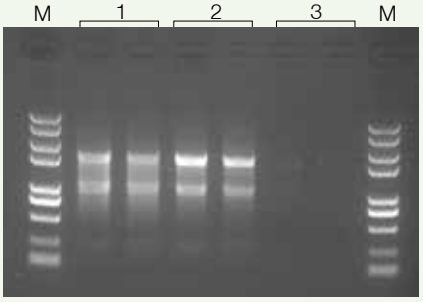
Note

Tissues stored in *RNAhold*® solution can freeze and thaw at least 20 times without significantly affecting the yield or the integrity of the recoverable RNA.

Description

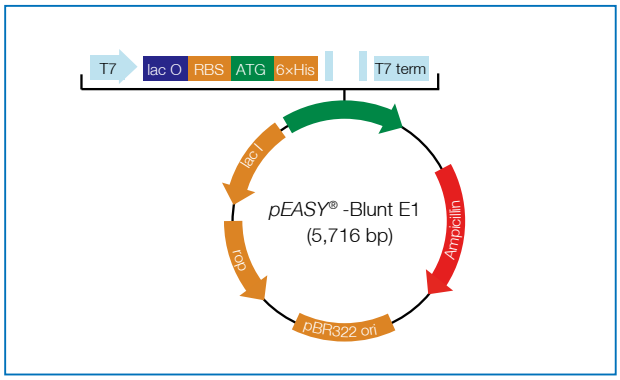
RNAhold® is an aqueous, nontoxic tissue preservation solution. It can inactivate RNase and keep RNA intact by permeating cells and tissues. Cells and tissues can be stored at this solution for one week at room temperature without RNA degradation. It can be used for RNA preservation with bacteria, cells and most fresh animal tissues.

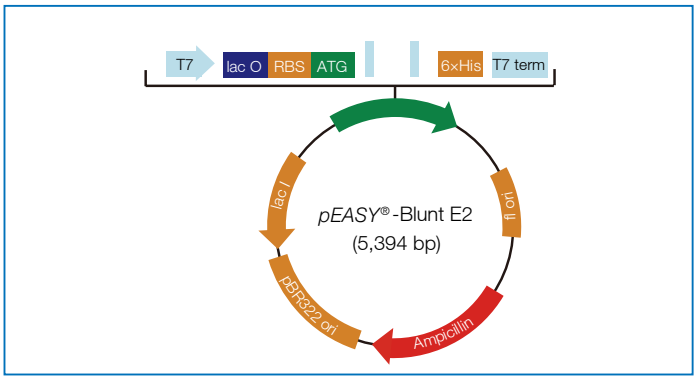
- Immediate RNase inactivation.
- Sample can be stored at room temperature for 1 week, 2-8°C for 1 month, -20°C or -80°C for long term storage.
- Ideal for field sample collection.

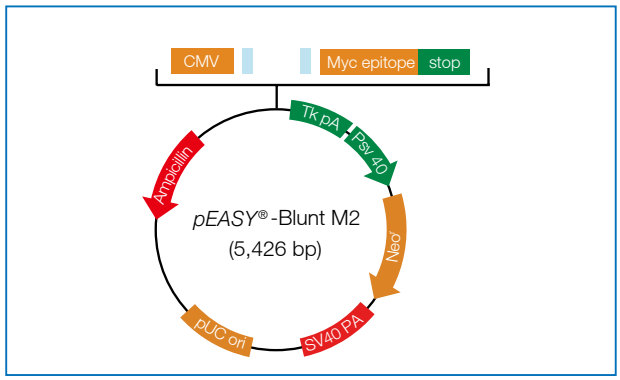


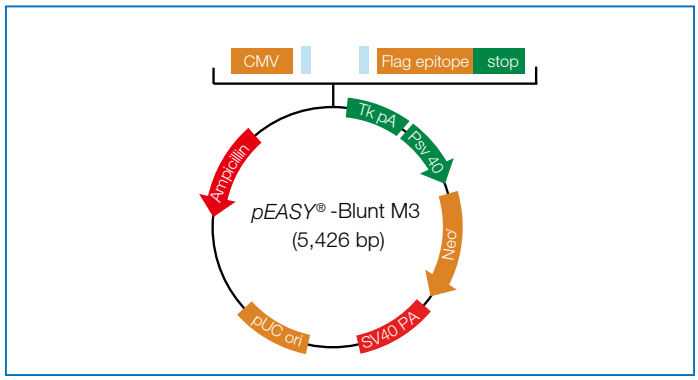
M: *Trans*2K® Plus II DNA Marker
1: HeLa cells stored at 37°C for 1 day with *RNAhold*®
2: HeLa cells stored at room temperature for 1 week with *RNAhold*®
3: HeLa cells stored at room temperature for 1 week without *RNAhold*®

Chapter 5 Gene Expression









Feature and application of *pEASY*® expression vectors

Name	Amp$^+$	Promoter	Sequencing primer	Characteristics	Application
pEASY®-Blunt E1	+	T7*lac*	T7 Promoter Primer; T7 Terminator Primer	N-terminal 6×His tag	Prokaryotic Expression
pEASY®-Blunt E2	+	T7*lac*	T7 Promoter Primer; T7 Terminator Primer	C-terminal 6×His tag	Prokaryotic Expression
pEASY®-Blunt M2	+	Enhanced CMV	CMV Forward Primer; TK PolyA Reverse Primer	C-terminal Myc tag; Neomycin resistance	Mammalian Expression
pEASY®-Blunt M3	+	Enhanced CMV	CMV Forward Primer; TK PolyA Reverse Primer	C-terminal Flag tag; Neomycin resistance	Mammalian Expression

pEASY®-Blunt E1 Expression Kit

CE111-01	10 rxns

Storage

*Trans*1-T1 Phage Resistant Chemically Competent Cell at -70°C for six months; others at -20°C for nine months

Description

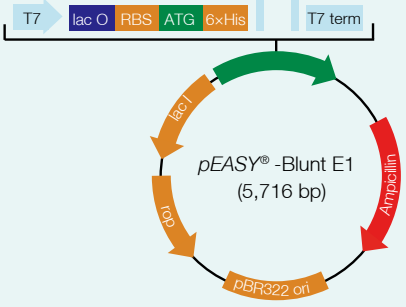
pEASY®-Blunt E1 Expression Vector is constructed from pET vector, it utilizes a highly efficient, five-minute blunt cloning strategy to clone PCR product into high-efficient expression vector. The size of control insert is 750 bp, and expressed target protein is about 27 kDa.

- 5 minutes fast ligation of *Pfu*-amplified PCR products.
- Ampicillin resistance.
- T7 promoter primer and T7 terminator primer for sequencing.
- Bacteriophage T7*lac* promoter for high level expression.
- N-terminal 6xHis tag for easy purification.
- *Trans*1-T1 Phage Resistant Chemically Competent Cell, high transformation efficiency (>10^9 cfu/μg pUC19 DNA) and fast growing.
- E1 Expression Plasmid included as negative control.

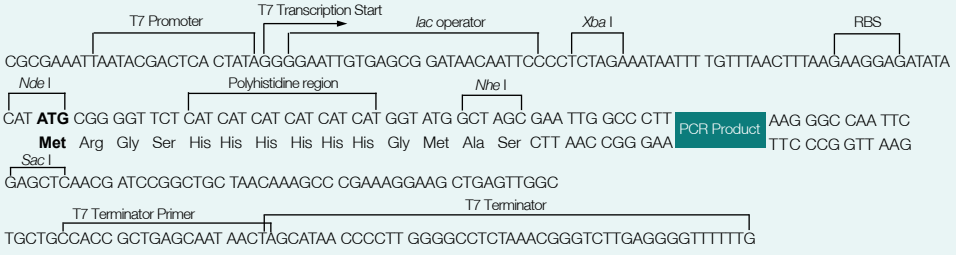
Kit Contents

Component	CE111-01
pEASY®-Blunt E1 Expression Vector (15 ng/μl)	10 μl
EControl Template (5 ng/μl)	10 μl
EControl Forward Primer (10 μM)	10 μl
EControl Reverse Primer (10 μM)	10 μl
E1 Expression Plasmid (Negative Control) (15 ng/μl)	10 μl
T7 Promoter Primer (10 μM)	50 μl
T7 Terminator Primer (10 μM)	50 μl
*Trans*1-T1 Phage Resistant Chemically Competent Cell	5×100 μl

pEASY®-Blunt E1 Prokaryotic Expression Vector Map



T7 promoter: bases 209-225
T7 transcription start: base 226
Lac operator(lacO): bases 228-252
RBS: bases 282-288
His·Tag coding sequence: bases 309-326
T7 terminator: bases 436-482
Ampicillin resistance ORF: bases 907-1,767
pBR322 origin: bases 1,922-2,541
ROP ORF: bases 2,953-3,144
*Lac*I ORF: bases 4,459-5,547



PROTOCOL

Cloning reaction

(1) Primer requirement: primer cannot be phosphorylated
(2) PCR Enzyme: high fidelity *Pfu* DNA polymerase
(3) Reaction conditions: for higher cloning efficiency, we recommend 5-10 minutes post PCR 72°C extension. After PCR, use agarose gel electrophoresis to verify the quality and quantity of PCR product.

Suggested cloning reaction conditions

1. Optimal amount of insert
   Molar ratio of vector and insert = 1:7 (1 kb, ~20 ng; 2 kb, ~40 ng)
2. Optimal volume of vector: 1 µl
3. Optimal reaction volume: 3~5 µl
4. Optimal incubation time
(1) 0.1~1 kb (including 1 kb): 5~10 minutes
(2) 1~2 kb (including 2 kb): 10~15 minutes
(3) 2~3 kb (including 3 kb): 15~20 minutes
(4) ≥ 3 kb: 20~30 minutes
   Use the maximum incubation time if the insert is gel purified PCR product.
5. Optimal incubation temperature: for most PCR inserts, the optimal temperature is about 25°C; for some PCR inserts, optimal results can be achieved with higher temperature (up to 37°C).

Transformation

1. Add the ligated products to 50 µl of *Trans*1-T1 Phage Resistant Chemically Competent Cell and mix gently (do not mix by pipetting up and down).
2. Incubate on ice for 20-30 minutes.
3. Heat-shock the cells at 42°C for 30 seconds.
4. Immediately place the tube on ice for 2 minutes.
5. Add 250 µl of room temperature SOC or LB medium. Shake the tube at 37°C (200 rpm) for 1 hour.
6. Pre-warm a selective LB plate at 37°C for 30 minutes.
7. Spread 200 µl or all transformants on the pre-warmed plate. Incubate at 37°C overnight.

Analysis of positive clones

1. Transfer 5~10 colonies into 10 µl ddH_2O.
2. Use 1 µl of the mixture as template for 25 µl PCR using T7 promoter primer and gene reverse primer, or gene forward primer and T7 terminator primer.
3. PCR

94°C	10 min	
94°C	30 sec	30 cycles
55°C	30 sec	
72°C	X min*	
72°C	5-10 min	

*(depends on the insert size and PCR enzymes)

4. Analyze positive clones by restriction enzyme digestion and DNA sequencing.

Target gene expression

1. Competent cell
   BL21(DE3) competent cell series are suitable for prokaryotic protein expression.
2. Protein expression
   Method 1
- Pick single colony and transfer into 5 ml of LB/Amp$^+$ medium and shake at 37°C (250 rpm) until OD_{600} close to 0.5.
- Add IPTG to a final concentration of 0.5-1 mM and shake at 37°C for 3-5 hours.
- Remove a 500 µl aliquot during different time course and centrifuge at the maximum speed.

   Method 2
- Pick single colony and transfer into 5 ml of *ArtMedia*® Protein Expression/Amp$^+$ medium, incubate at 37°C overnight.

3. Check expression
   Aspirate the supernatant and use the pellets for SDS-PAGE.

PCR for control insert (750 bp)

Component	Volume	Final Concentration
EControl Template (5 ng/µl)	1 µl	0.1 ng/µl
EControl Forward Primer (10 µM)	1 µl	0.2 µM
EControl Reverse Primer (10 µM)	1 µl	0.2 µM
2×*TransStart*® *FastPfu* PCR SuperMix	25 µl	1×
ddH_2O	Variable	-
Total Volume	50 µl	-

Thermal cycling conditions

94°C	2-5 min	
94°C	20 sec	30 cycles
55°C	20 sec	
72°C	30 sec	
72°C	10 min	

Ligate 1 µl of control PCR insert with 1 µl vector. Hundreds of colonies should be produced with cloning efficiency over 90%.

pEASY®-Blunt E2 Expression Kit

CE211-01	10 rxns

Storage

*Trans*1-T1 Phage Resistant Chemically Competent Cell at -70°C for six months; others at -20°C for nine months

Description

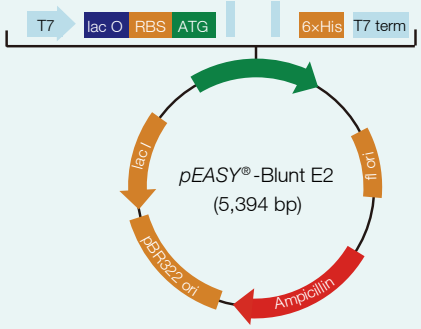
pEASY®-Blunt E2 Expression Vector is constructed from pET vector. It utilizes a highly efficient, five-minute blunt cloning strategy to clone PCR product into high-efficient expression vector. The size of control insert is 750 bp, and expressed target protein is about 27 kDa.

- 5 minutes fast ligation of *Pfu*-amplified PCR products.
- Ampicillin resistance.
- T7 promoter primer and T7 terminator primer for sequencing.
- Bacteriophage T7*lac* promoter for high level expression.
- C-terminal 6×His tag for easy purification.
- *Trans*1-T1 Phage Resistant Chemically Competent Cell, high transformation efficiency (>10^9 cfu/μg pUC19 DNA) and fast growing.
- E2 Expression Plasmid included as negative control.

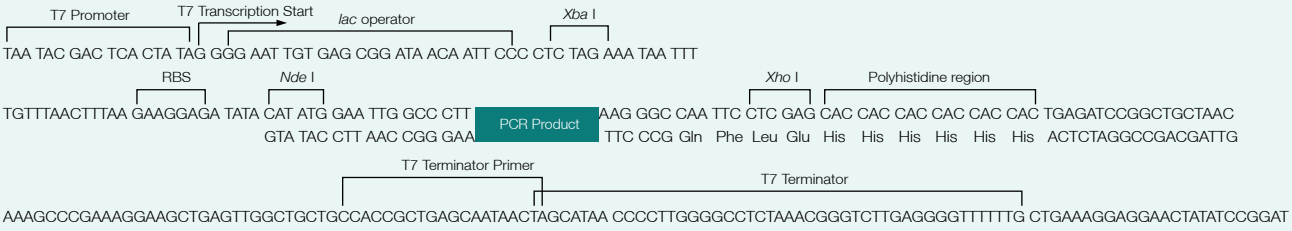
Kit Contents

Component	CE211-01
pEASY®-Blunt E2 Expression Vector (15 ng/μl)	10 μl
EControl Template (5 ng/μl)	10 μl
EControl Forward Primer (10 μM)	10 μl
EControl Reverse Primer (10 μM)	10 μl
E2 Expression Plasmid (Negative Control) (15 ng/μl)	10 μl
T7 Promoter Primer (10 μM)	50 μl
T7 Terminator Primer (10 μM)	50 μl
*Trans*1-T1 Phage Resistant Chemically Competent Cell	5×100 μl

pEASY®-Blunt E2 Prokaryotic Expression Vector Map



T7 promoter: bases 5,117-5,133
T7 transcription start: base 5,134
Lac operator(lacO): bases 5,136-5,160
RBS: bases 5,190-5,196
His·Tag coding sequence: bases 5,238-5,255
T7 terminator: bases 5,323-5,369
ROP ORF: bases 2,648-2,839
*Lac*I ORF: bases 3,651-4,739
pBR origin: bases 1,614-2,233
Ampicillin resistance ORF: bases 599-1,459
f1 origin: bases 13-450



PROTOCOL

Protocols for cloning, transformation, analysis and expression are the same as described on page 152-153.

ArtMedia® Protein Expression

CP101-01	95 ml+5 ml

Storage
at 2-8°C for six months

Description

ArtMedia® Protein Expression is designed for higher protein yield with much less hands-on time. Protein is induced automatically without time-consuming OD monitoring and IPTG induction steps. Simply inoculate prepared *ArtMedia*® with colonies, grow the culture overnight and harvest cells for protein purification.

Kit Contents

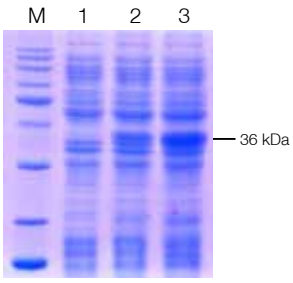
Component	CP101-01
AM3	95 ml
AM4	5 ml

Suitable expression vectors

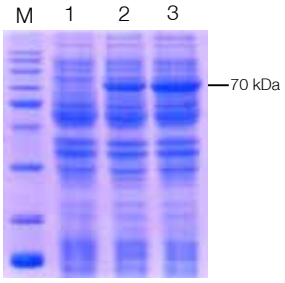
Lactose operons expression vectors: *pEASY*®-Blunt E1, *pEASY*®-Blunt E2, pET, pGEX, pMAL

Strains

BL21 competent cell series.



Target protein: 36 kDa
Vector: pGEX-5X-3 (*tac* promoter)
Strain: BL21
M: *ProteinRuler*® II
Lane 1: LB medium only
Lane 2: LB, OD_{600}=0.5,
induced with 1 mM IPTG, 37°C for 12 hours
Lane 3: 37°C for 12 hours with *ArtMedia*®
Protein Expression



Target protein: 70 kDa
Vector: *pEASY*®-Blunt E1 (T7*lac* promoter)
Strain: *Trans*etta(DE3)
M: *ProteinRuler*® II
Lane 1: LB medium only
Lane 2: LB,OD_{600}=0.5,
induced with 1 mM IPTG, 37°C for 12 hours
Lane 3: 37°C for 12 hours with *ArtMedia*®
Protein Expression

Notes

- AM4 may have a slight precipitate, which will not affect performance. If precipitate is observed, warm the bottle in a 37°C water bath to dissolve the precipitate.
- Add the whole volume of AM4 to AM3 for complete medium. Store the complete medium at 4°C up to 1 month.

Expression Competent Cells

Selection Guide

Name	Cat. No.	Transformation Efficiency	Application
BL21(DE3)	CD601	10^7 cfu/μg DNA	High expression of non-toxic protein
BL21(DE3) pLysS	CD701	10^7 cfu/μg DNA	High expression of toxic protein and non-toxic protein, low background
*Trans*etta(DE3)	CD801	10^7 cfu/μg DNA	Contains tRNAs corresponding to 6 rare codons, application to eukaryotic gene expression
*Trans*B(DE3)	CD811	10^7 cfu/μg DNA	Conducive to the formation of the correctly folded protein with disulfide, enhance the solubility of the protein
BL21	CD901	10^7 cfu/μg DNA	High expression of toxic protein

BL21(DE3) Chemically Competent Cell

CD601-02	10×100 μl
CD601-03	20×100 μl

Storage
at -70°C for six months

Characteristics
- Transformation efficiency: >10^7 cfu/μg (pUC19 DNA).
- DE3 strains contains the λDE3 lysogen that carries the gene for T7 RNA polymerase.
- Suitable for T7 and T7*lac* such as pET, *pEASY*®.
- Suitable for high expression of non-toxic protein.
- Control plasmid I (Amp$^+$) is used for detection of expression function of cell. The protein size is about 25 kDa.

Genotype
F$^-$ *ompT hsdS*$_B$($r_B^-m_B^-$) *gal dcm*(DE3)

BL21(DE3) pLysS Chemically Competent Cell

CD701-02	10×100 μl
CD701-03	20×100 μl

Storage
at -70°C for six months

Characteristics
- Transformation efficiency: >10^7 cfu/μg (pUC19 DNA).
- Cam^R.
- Contains pLysS plasmid that expresses the T7 lysozyme gene to reduce the background of the target gene's expression without disturbing IPTG functioning.
- Suitable for non-toxic and toxic protein expression.
- Control plasmid I (Amp$^+$) is used for detection of expression function of cell. The protein size is about 25 kDa.

Genotype
F$^-$*ompT hsdS*$_B$($r_B^-m_B^-$) *dcm*(DE3) *gal*λ pLysS(Cam^R)

*Trans*etta(DE3) Chemically Competent Cell

CD801-02	10×100 μl
CD801-03	20×100 μl

Storage
at -70°C for six months

Characteristics
- Transformation efficiency: >10^7 cfu/μg (pUC19 DNA).
- Cam^R.
- tRNAs for 6 rare codons AUA, AGG, AGA, CUA, CCC, GGA. Enhance the expression level of proteins in the prokaryotic system.
- Control plasmid I (Amp^+) is used for detection of expression function of cell. The protein size is about 25 kDa.

Genotype
F^- *ompT hsd*S_B (r_B^- m_B^-) *gal dcm* (DE3) pRARE (argU,argW, ileX, glyT, leuW, proL) (Cam^R)

*Trans*B(DE3) Chemically Competent Cell

CD811-02	10×100 μl

Storage
at -70°C for six months

Characteristics
- Transformation efficiency: >10^7 cfu/μg (pUC19 DNA).
- Kan^R and Tet^R.
- Thioredoxin reductase (trxB) and glutathione reductase (gor) mutation greatly facilitates cytoplasmic disulfide bond formation.
- Control plasmid I (Amp^+) is used for detection of expression function of cell. The protein size is about 25 kDa.

Genotype
F^- *ompT hsd*S_B (r_B^- m_B^-) *gal dcm lac*Y1 *ahp*C (DE3) *gor*522::Tn10 *trx*B (Kan^R,Tet^R)

BL21 Chemically Competent Cell

CD901-02	10×100 μl
CD901-03	20×100 μl

Storage
at -70°C for six months

Characteristics
- Transformation efficiency: >10^7 cfu/μg (pUC19 DNA).
- Tet^R.
- Tight expression control ideal for toxic protein expression.
- Control plasmid II (Amp^+) is used for detection of expression function of cell. The protein size is about 26 kDa.

Genotype
E. coli B F^- *dcm ompT hsdS* (r_B^- m_B^-) *gal ara*B::T7RNAP-*tet*A

pEASY®-Blunt M2 Expression Kit

CM211-01	10 rxns

Storage

*Trans*1-T1 Phage Resistant Chemically Competent Cell at -70°C for six months; others at -20°C for nine months

Description

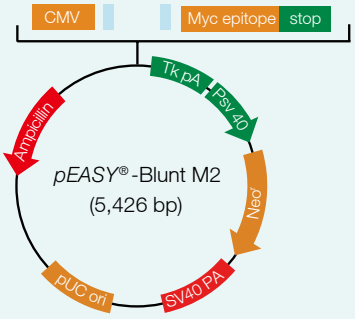
pEASY®-Blunt M2 Expression Vector utilizes a highly efficient, five-minute blunt cloning strategy to clone PCR product into high-efficient expression vector under regulation of enhanced CMV promoter. The size of control insert is 750 bp, and expressed target protein is about 27 kDa.

- 5 minutes fast ligation of *Pfu*-amplified products.
- Enhanced CMV promoter for higher protein yield.
- CMV forward primer and TK polyA reverse primer for sequencing.
- C-terminal Myc tag for protein detection.
- Neomycin resistance gene for stable cell line selection.
- *Trans*1-T1 Phage Resistant Chemically Competent Cell, high transformation efficiency (>10^9 cfu/μg pUC19 DNA) and fast growing.
- M2 Expression Plasmid included as negative control.

Kit Contents

Component	CM211-01
pEASY®-Blunt M2 Expression Vector (15 ng/μl)	10 μl
MControl Template (5 ng/μl)	10 μl
MControl Forward Primer (10 μM)	10 μl
MControl Reverse Primer (10 μM)	10 μl
M2 Expression Plasmid (Negative Control) (15 ng/μl)	10 μl
CMV Forward Primer (10 μM)	50 μl
TK PolyA Reverse Primer (10 μM)	50 μl
*Trans*1-T1 Phage Resistant Chemically Competent Cell	5×100 μl

pEASY®-Blunt M2 Mammalian Expression Vector Map



CMV promoter: bases 4,740-5,327
CMV forward primer binding site: bases 5,277-5,297
Cloning site: bases 6-7
TK PolyA reverse primer binding site: bases 73-91
TK polyadenylation signal: bases 66-337
f1 replication origin: bases 373-801
SV40 early promoter: bases 828-1,136
Neomycin resistance gene: bases 1,211-2,005
SV40 polyadenylation signal: bases 2,181-2,311
pUC origin: bases 2,694-3,367
Ampicillin (bla) resistance gene(c): bases 3,512-4,372
bla promoter(c): bases 4,373-4,471
Myc epitope: bases 19-48
(c) = complementary strand

CMV Forward Primer Binding Site

TGA CGC AAA TGG GCG GTA GGC GTG TAC GGT GGG AGG TCT ATA TAA GCA GAG CTC GTT
ACT GCG TTT ACC CGC CAT CCG CAC ATG CCA CCC TCC AGA TAT ATT CGT CTC GAG CAA

TAG TGA ACC GTC AGA TCG CCT GGA GAC GCC ATC CAC GCT GTT TTG ACC TCC ATA GAA GAC ACC GGG ACC GAT CCA GCC TCC GGA CTC TAG AGG ATC
ATC ACT TGG CAG TCT AGC GGA CCT CTG CGG TAG GTG CGA CAA AAC TGG AGG TAT CTT CTG TGG CCC TGG CTA GGT CGG AGG CCT GAG ATC TCC TAG

Myc epitope

GCC CTT AAG GGC GAT CCG GAA CAA AAA CTC ATC TCA GAA GAG GAT CTG
CGG GAA TTC CCG CTA GGC CTT GTT TTT GAG TAG AGT CTT CTC CTA GAC

PCR Product

TK PolyA

TK PolyA Reverse Primer Binding Site

TAG TAA TGA GTT TAA ACG GGG GAG GCT AAC TGA AAC ACG GAA GGA GAC AAT ACC GGA AGG AAC CCG CG.....TAG C
ATC ATT ACT CAA ATT TGC CCC CTC CGA TTG ACT TTG TGC CTT CCT CTG TTA TGG CCT TCC TTC CTT GG.....ATC G

PROTOCOL

Cloning reaction

1. PCR primer design

(1) Do not add 5´ phosphates to PCR primers.

(2) Forward primer with Kozak consensus sequence: (G/A)NNATGN.

2. Using high fidelity *Pfu* DNA polymerase

3. Add PCR products and vector in a tube, mix gently and incubate at room temperature (20-37°C) for 5 minutes.

Suggested cloning reaction conditions

1. Optimal amount of insert

Molar ratio of vector to insert = 1:7 (1 kb, ~20 ng; 2 kb, 40 ng)

2. Optimal volume of vector: 1 µl

3. Optimal reaction volume: about 3~5 µl

4. Optimal incubation time

(1) 0.1~1 kb (including 1 kb): 5~10 minutes

(2) 1~2 kb (including 2 kb): 10~15 minutes

(3) 2~3 kb (including 3 kb): 15~20 minutes

(4) ≥3 kb: 20~30 minutes

Use the maximum incubation time if the insert is gel purified PCR product.

5. Optimal incubation temperature: for most PCR inserts, the optimal temperature is about 25°C; for some PCR inserts, optimal results can be achieved with higher temperature (up to 37°C).

Transformation

1. Add the ligated products to 50 µl of *Trans*1-T1 Phage Resistant Chemically Competent Cell and mix gently (do not mix by pipetting up and down).

2. Incubate on ice for 20-30 minutes.

3. Heat-shock the cells at 42°C for 30 seconds.

4. Immediately place the tube on ice for 2 minutes.

5. Add 250 µl of room temperature SOC or LB medium. Shake the tube at 37°C (200 rpm) for 1 hour.

6. Pre-warm a selective LB plate at 37°C for 30 minutes.

7. Spread 200 µl or all transformants on the pre-warmed plate. Incubate at 37°C overnight.

Analysis of positive clones

1. Transfer 5-10 colonies into 10 µl ddH_2O.

2. Use 1 µl of the mixture as template for 25 µl PCR using CMV forward primer and gene reverse primer, or gene forward primer and TK polyA reverse primer.

3. PCR

94°C	10 min	
94°C	30 sec	30 cycles
55°C	30 sec	
72°C	X min*	
72°C	5-10 min	

*(depends on the insert size and PCR enzymes)

4. Analyze positive clones by restriction enzyme digestion and DNA sequencing.

Transfection

See *TransLipid*® HL Transfection Reagent for the detailed protocol.

Detection of target protein

Anti-Myc antibody for the detection of proteins with Myc tag.

Selection of stable cell lines

G418 to select stable cell lines.

PCR for control insert (750 bp)

Component	Volume	Final Concentration
MControl Template (5 ng/μl)	1 μl	0.1 ng/μl
MControl Forward Primer (10 μM)	1 μl	0.2 μM
MControl Reverse Primer (10 μM)	1 μl	0.2 μM
2×*TransStart*® *FastPfu* PCR SuperMix	25 μl	1×
ddH_2O	Variable	-
Total Volume	50 μl	-

Thermal cycling conditions

94°C	2-5 min	
94°C	20 sec	30 cycles
55°C	20 sec	
72°C	30 sec	
72°C	10 min	

Ligate 1 μl of control PCR insert with 1 μl vector. Hundreds of colonies should be produced with cloning efficiency over 90%.

pEASY® -Blunt M3 Expression Kit

CM311-01	10 rxns

Storage

*Trans*1-T1 Phage Resistant Chemically Competent Cell at -70°C for six months; others at -20°C for nine months

Description

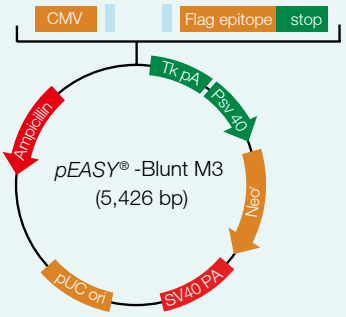
pEASY®-Blunt M3 Expression Vector utilizes a highly efficient, five-minute blunt cloning strategy to clone PCR product into high-efficient expression vector under regulation of enhanced CMV promoter. The size of control insert is 750 bp, and expressed target protein is about 27 kDa.

- 5 minutes fast ligation of *Pfu*-amplified products.
- Enhanced CMV promoter for higher protein yield.
- CMV forward primer and TK polyA reverse primer for sequencing.
- C-terminal Flag-tag for protein detection.
- Neomycin resistance gene for stable cell line selection.
- *Trans*1-T1 Phage Resistant Chemically Competent Cell, high transformation efficiency (>10^9 cfu/μg pUC19 DNA) and fast growing.
- M3 Expression Plasmid included as negative control.

Kit Contents

Component	CM311-01
pEASY®-Blunt M3 Expression Vector (15 ng/μl)	10 μl
MControl Template (5 ng/μl)	10 μl
MControl Forward Primer (10 μM)	10 μl
MControl Reverse Primer (10 μM)	10 μl
M3 Expression Plasmid (Negative Control) (15 ng/μl)	10 μl
CMV Forward Primer (10 μM)	50 μl
TK PolyA Reverse Primer (10 μM)	50 μl
*Trans*1-T1 Phage Resistant Chemically Competent Cell	5x100 μl

pEASY®-Blunt M3 Mammalian Expression Vector Map



CMV promoter: bases 4,734-5,321
CMV forward primer binding site: bases 5,271-5,291
Cloning site: bases 6-7
TK PolyA reverse primer binding site: bases 67-85
TK polyadenylation signal: bases 60-331
f1 replication origin: bases 367-795
SV40 early promoter: bases 822-1,130
Neomycin resistance gene: bases 1,205-1,999
SV40 polyadenylation signal: bases 2,175-2,305
pUC origin: bases 2,688-3,361
Ampicillin (bla) resistance gene(c): bases 3,506-4,366
bla promoter(c): bases 4,367-4,465
Flag epitope: bases 19-42
(c) = complementary strand

CMV Forward Primer Binding Site

```
TGA CGC AAA TGG GCG GTA GGC GTG TAC GGT GGG AGG TCT ATA TAA GCA GAG CTC GTT
ACT GCG TTT ACC CGC CAT CCG CAC ATG CCA CCC TCC AGA TAT ATT CGT CTC GAG CAA

TAG TGA ACC GTC AGA TCG CCT GGA GAC GCC ATC CAC GCT GTT TTG ACC TCC ATA GAA GAC ACC GGG ACC GAT
ATC ACT TGG CAG TCT AGC GGA CCT CTG CGG TAG GTG CGA CAA AAC TGG AGG TAT CTT CTG TGG CCC TGG CTA

                                                                    Flag epitope
CCA GCC TCC GGA CTC TAG AGG ATC GCC CTT PCR Product AAG GGC GAT CCG GAT TAC AAG GAC GAT GAC GAT AAG GAA TTC
GGT CGG AGG CCT GAG ATC TCC TAG CGG GAA             TTC CCG CTA GGC CTA ATG TTT CTG CTA CTG CTA TTT CTT AAG

                TK PolyA   TK PolyA Reverse Primer Binding Site
TAG TAA TGA GTT TAA ACG GGG GAG GCT AAC TGA AAC ACG GAA GGA GAC AAT ACC GGA AGG AAC CCG CG.....TAG C
ATC ATT ACT CAA ATT TGC CCC CTC CGA TTG ACT TTG TGC CTT CCT CTG TTA TGG CCT TCC TTC CTT GG.....ATC G
```

PROTOCOL

Protocols for cloning and transformation are the same as described on page 159.

Analysis of positive clones

1. Transfer 5-10 colonies into 10 µl ddH_2O.
2. Use 1 µl of the mixture as template for 25 µl PCR using CMV forward primer and gene reverse primer, or gene forward primer and TK polyA reverse primer.
3. PCR

94°C	2-5 min	
94°C	30 sec	30 cycles
55°C	30 sec	
72°C	X min*	
72°C	5-10 min	

*(depends on the insert size and PCR enzymes)

4. Analyze positive clones by restriction enzyme digestion and DNA sequencing.

Transfection

See *TransLipid*® HL Transfection Reagent for the detailed protocol.

Detection of target protein

Anti-Flag antibody for the detection of proteins with Flag tag.

Selection of stable cell lines

G418 to select stable cell lines.

Chapter 6 Protein Extraction, Purification and Detection

Protein Extraction

Protein Purification

Unstained Protein Marker

Prestained Protein Marker

Western Protein Marker

Related Products

ProteinExt™ Mammalian Total Protein Extraction Kit

DE101-01	100 ml

Storage
at -20°C for one year

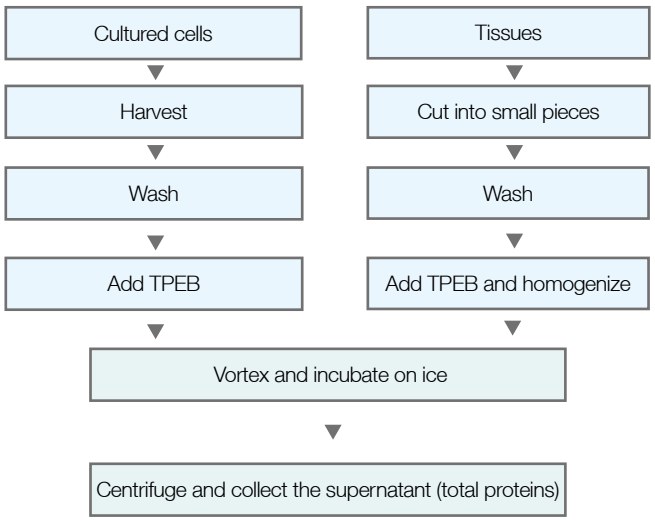
Description

ProteinExt™ Mammalian Total Protein Extraction Kit provides a fast and efficient method to extract total proteins (cytoplasmic, membrane and nuclear proteins) from mammalian cells and tissues without ultracentrifugation. The extracted proteins are suitable for SDS-PAGE, Western blot, ELISA, and other functional assays.

Kit Content

Component	DE101-01
Total Protein Extraction Buffer (TPEB)	100 ml

Procedures



ProteinExt™ Mammalian Nuclear and Cytoplasmic Protein Extraction Kit

DE201-01	50 rxns

Storage
at 2-8°C for one year

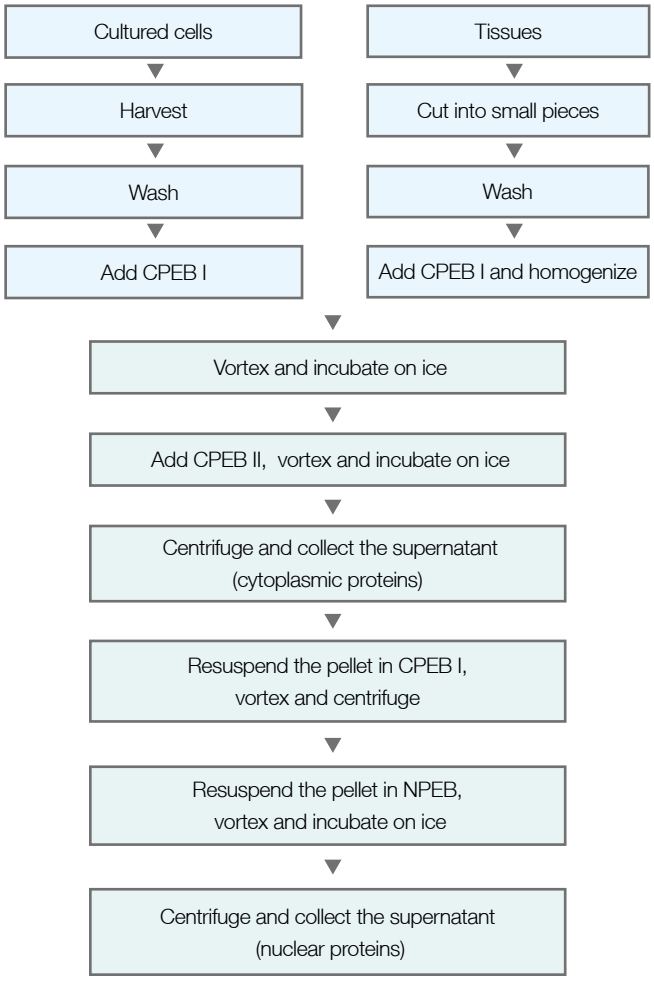
Description

ProteinExt™ Mammalian Nuclear and Cytoplasmic Protein Extraction Kit provides a fast and efficient method to extract nuclear and cytoplasmic proteins from mammalian cells and tissues. Native proteins can be obtained within 80 minutes without ultracentrifugation. The extracted proteins are suitable for a variety of downstream applications, including SDS-PAGE, Western blot, ELISA, enzyme-activity assays, immunoprecipitation and transcription factor activity analysis.

Kit Contents

Component	DE201-01
Cytoplasmic Protein Extraction Buffer I (CPEB I)	75 ml
Cytoplasmic Protein Extraction Buffer II (CPEB II)	3 ml
Nuclear Protein Extraction Buffer (NPEB)	25 ml

Procedures



ProteinExt™ Mammalian Membrane Protein Extraction Kit

DE301-01 50 rxns

Storage
at 2-8°C for one year

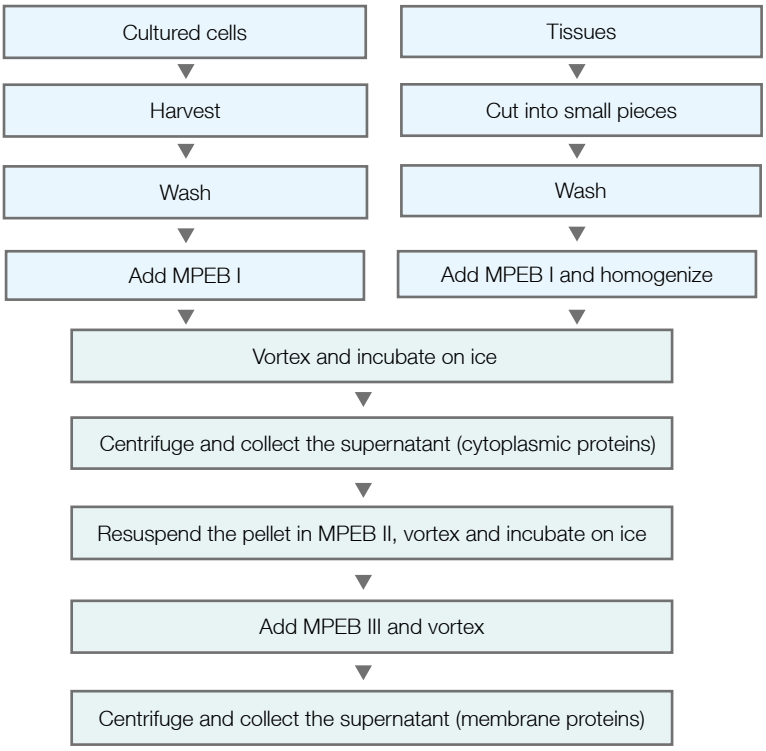
Description

ProteinExt™ Mammalian Membrane Extraction Kit provides a fast and efficient method to extract membrane proteins from mammalian cells and tissues. Native proteins can be obtained within 70 minutes without ultracentrifugation. Membrane proteins with at least 1-2 transmembrane domains are typically extracted with an efficiency of up to 90%. Cross-contamination of cytosolic proteins into the membrane fraction is usually less than 10%. The extracted proteins are suitable for a variety of downstream applications, including SDS-PAGE, Western blot, ELISA, and enzyme-activity assays.

Kit Contents

Component	DE301-01
Membrane Protein Extraction Buffer I (MPEB I)	50 ml
Membrane Protein Extraction Buffer II (MPEB II)	7.5 ml
Membrane Protein Extraction Buffer III (MPEB III)	15 ml

Procedures



ProteinExt™ Mammalian Mitochondria Isolation Kit for Cultured Cells

DE401-01	50 rxns

Storage
MSB at -20°C for one year, others at 2-8°C for one year

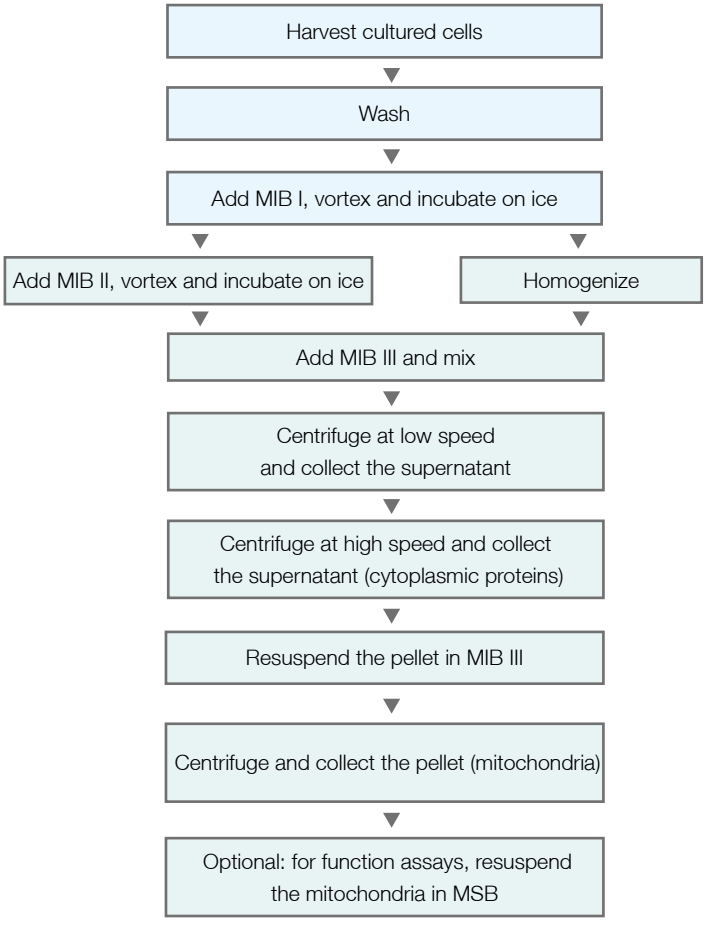
Description

ProteinExt™ Mammalian Mitochondria Isolation Kit for Cultured Cells provides a fast and efficient method to isolate mitochondria from cultured mammalian cells. This kit provides two options for the separation of mitochondria from cytosolic components: a reagent-based method or homogenization-based method. Reagent-based method uses a mild procedure to process single or multiple samples. The isolated mitochondria is suitable for a variety of downstream applications, including protein analysis, apoptosis, signal transduction and metabolic assays.

Kit Contents

Component	DE401-01
Mitochondria Isolation Buffer I (MIB I)	50 ml
Mitochondria Isolation Buffer II (MIB II)	500 µl
Mitochondria Isolation Buffer III (MIB III)	65 ml
Mitochondria Storage Buffer (MSB)	4 ml

Procedures



ProteinExt™ Mammalian Mitochondria Isolation Kit for Tissue

DE501-01 50 rxns

Storage

MSB at -20°C for one year, others at 2-8°C for one year

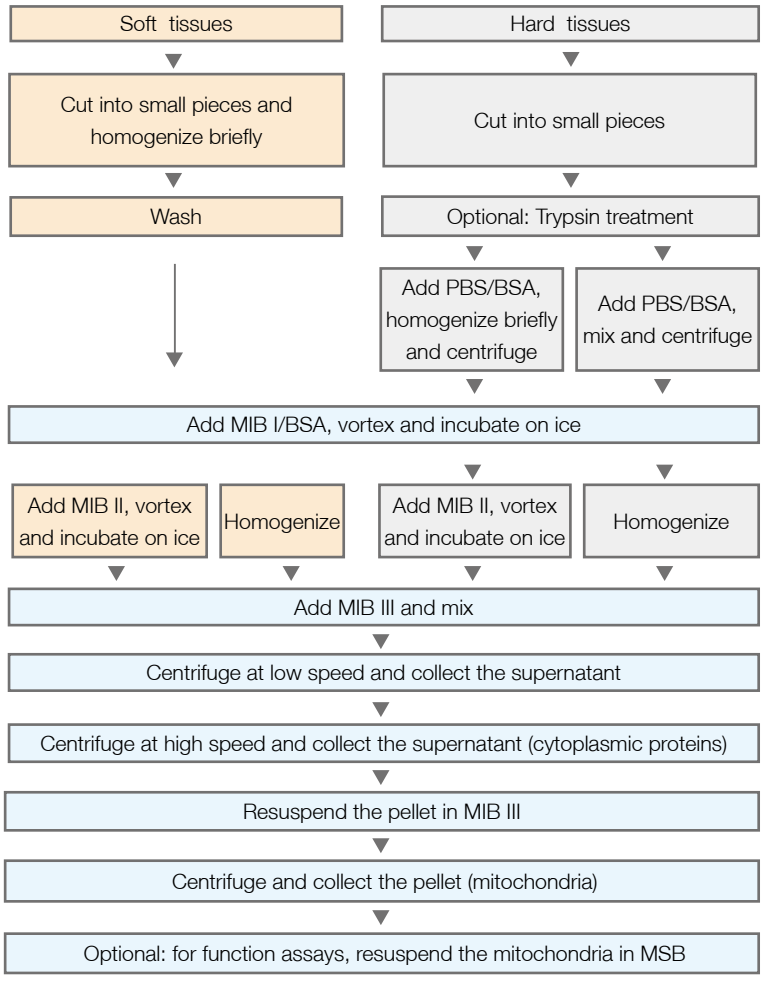
Description

ProteinExt™ Mammalian Mitochondria Isolation Kit for Tissue provides a fast and efficient isolation of mitochondria from tissues with simple procedure. This kit provides two options for the separation of mitochondria from cytosolic components: a reagent-based method or homogenization-based method. Reagent-based method uses a mild procedure to process single or multiple samples. The isolated mitochondria is suitable for a variety of downstream applications, including protein analysis, apoptosis, signal transduction and metabolic assays.

Kit Contents

Component	DE501-01
Mitochondria Isolation Buffer I (MIB I)	50 ml
Mitochondria Isolation Buffer II (MIB II)	500 µl
Mitochondria Isolation Buffer III (MIB III)	65 ml
Mitochondria Storage Buffer (MSB)	4 ml
Bovine Serum Albumin (BSA)	500 mg

Procedures



ProteinIso® Ni-NTA Resin

DP101-01	5 ml
DP101-02	25 ml

Storage
at 2~8°C (20% ethanol) for two years

Description

ProteinIso® Ni-NTA Resin allows rapid affinity purification of His-tagged proteins. The His-tagged proteins bind to Ni^{2+} cations, which are immobilized on the Ni-NTA resin by 4 metal-chelating sites. After wash, the target proteins are recovered by gradient elution. The resin can be used for both native and denatured protein purification.

Resin Specifications

Resin	Cross-linked 6% agarose
Ligand	NTA
Shape	sphere
Pore size	45~165 μm
Binding capacity	10~20 mg/ml wet gel
Recommended flow rate	<300 cm/h
Highest resistance of atmospheric pressure	0.3 Mpa
pH stability	3-13

PROTOCOL

Notes

- Samples should be centrifuged and filtrated with 0.45 μm filter before loading.
- **Equilibration Buffer for soluble protein**
  300 mM NaCl, 50 mM sodium phosphate buffer, 10 mM imidazole, 10 mM Tris-HCl pH 8.0
- **Equilibration Buffer for inclusion body**
  6 M GuHCl, 100 mM sodium phosphate buffer, 10 mM Tris-HCl pH 8.0; or 8 M urea, 100 mM sodium phosphate buffer, 10 mM Tris-HCl pH 8.0

Procedures

1. Prepare Ni-NTA purification column

(1) Thoroughly resuspend the Ni-NTA resin to achieve a homogeneous suspension of the resin in the 20% ethanol storage buffer.

(2) Immediately transfer the resin into a purification column. Ensure that the bottom of the column is plugged with a stopper. Close the valve of the column. Allow the resin to settle.

(3) Equilibrate the column with 5~10 bed volume of equilibration buffer.

2. Prepare samples

To avoid blocking column, samples should be centrifuged and filtrated with 0.45 μm filter before loading.

3. Load samples and wash

Load samples and wash with 5~10 bed volume of equilibration buffer and collect the flow-through in one tube.

4. Elute

Elute target proteins with imidazole or low pH buffer.

5. Regeneration of Ni-NTA resin

(1) Wash the column/resin with 2 bed volume of 6 M GuHCl, 0.2 M acetic acid

(2) 5 bed volume of deionized water

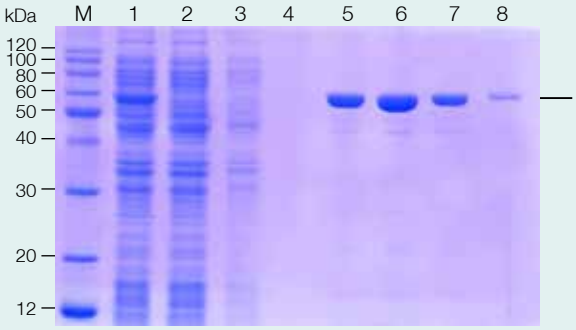
(3) 3 bed volume of 2% SDS

(4) 1 bed volume of 25% ethanol

(5) 1 bed volume of 50% ethanol

(6) 1 bed volume of 75% ethanol

(7) 5 bed volume of 100% ethanol
(8) 1 bed volume of 75% ethanol
(9) 1 bed volume of 50% ethanol
(10) 1 bed volume of 25% ethanol
(11) 1 bed volume of deionized water
(12) 5 bed volume of 100 mM EDTA, pH 8.0
(13) 10 bed volume of deionized water
(14) 5 bed volume of 100 mM $NiSO_4$
(15) Store column/resin in 20% ethanol



M: *ProteinRuler*® II
Lane 1: Sample
Lane 2: Flow-through
Lane 3: Wash
Lane 4: Elution (imidazole 40 mM)
Lane 5: Elution (imidazole 80 mM)
Lane 6: Elution (imidazole 120 mM)
Lane 7: Elution (imidazole 160 mM)
Lane 8: Elution (imidazole 200 mM)

ProteinIso® Ni-IDA Resin

DP111-01	5 ml
DP111-02	25 ml

Storage
at 2~8°C (20% ethanol) for two years

Description

ProteinIso® Ni-IDA Resin allows rapid affinity purification of His-tagged proteins. The His-tagged proteins bind to Ni^{2+} cations, which are immobilized on the Ni-IDA resin by 3 metal-chelating sites. After wash, the target proteins are recovered by gradient elution. The resin can be used for both native and denatured protein purification.

Resin Specifications

Resin	Cross-linked 6% agarose
Ligand	IDA
Shape	sphere
Pore size	90 μm
Binding capacity	20~40 mg/ml wet gel
Recommended flow rate	<300 cm/h
Highest resistance of atmospheric pressure	0.3 Mpa
pH stability	2~14

PROTOCOL

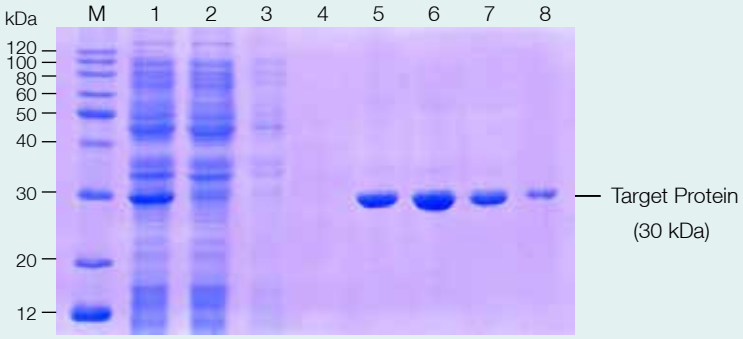
Notes

- Samples should be centrifuged and filtrated with 0.45 μm filter before loading.
- **Equilibration Buffer for soluble protein**
  300 mM NaCl, 50 mM sodium phosphate buffer, 10 mM imidazole, 10 mM Tris-HCl pH 8.0
- **Equilibration Buffer for inclusion body**
  6 M GuHCl, 100 mM sodium phosphate buffer, 10 mM Tris-HCl pH 8.0; or 8 M urea, 100 mM sodium phosphate buffer, 10 mM Tris-HCl pH 8.0

Procedures

1. Prepare Ni-IDA purification column
(1) Thoroughly resuspend the Ni-IDA resin to achieve a homogeneous suspension of the resin in the 20% ethanol storage buffer.
(2) Immediately transfer the resin into a purification column. Ensure that the bottom of the column is plugged with a stopper. Close the valve of the column. Allow the resin to settle.
(3) Equilibrate the column with 5~10 bed volume of equilibration buffer.
2. Prepare samples
To avoid blocking column, samples should be centrifuged and filtrated with 0.45 μm filter before loading.
3. Load samples and wash
Load samples and wash with 5~10 bed volume of equilibration buffer and collect the flow-through in one tube.
4. Elute
Elute target proteins with imidazole or low pH buffer.
5. Regeneration of Ni-IDA resin
(1) Wash the column/resin with 2 bed volume of 6 M GuHCl, 0.2 M acetic acid
(2) 5 bed volume of deionized water
(3) 3 bed volume of 2% SDS
(4) 1 bed volume of 25% ethanol
(5) 1 bed volume of 50% ethanol
(6) 1 bed volume of 75% ethanol
(7) 5 bed volume of 100% ethanol

(8) 1 bed volume of 75% ethanol
(9) 1 bed volume of 50% ethanol
(10) 1 bed volume of 25% ethanol
(11) 1 bed volume of deionized water
(12) 5 bed volume of 100 mM EDTA, pH 8.0
(13) 10 bed volume of deionized water
(14) 5 bed volume of 100 mM $NiSO_4$
(15) Store column/resin in 20% ethanol



M: *ProteinRuler*® II
Lane 1: Sample
Lane 2: Flow-through
Lane 3: Wash
Lane 4: Elution (imidazole 40 mM)
Lane 5: Elution (imidazole 80 mM)
Lane 6: Elution (imidazole 120 mM)
Lane 7: Elution (imidazole 160 mM)
Lane 8: Elution (imidazole 200 mM)

ProteinIso® GST Resin

DP201-01	10 ml

Storage
at 2-8°C (20% ethanol) for two years

Description
ProteinIso® GST Resin allows rapid affinity purification of GST-tagged proteins. GST fusion proteins expressed in *E.coli*, insect cells and mammalian cells can be purified with *ProteinIso*® GST Resin. The GST Resin is only suitable for soluble protein purification.

Resin Specifications

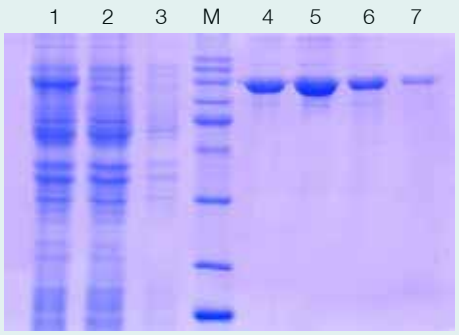
Resin	Cross-linked 4% agarose
Ligand	glutathione
Shape	sphere
Pore size	90 μm
Support density	8 mg GSH/ml wet gel
Binding capacity	10~12 mg/ml wet gel (MW 42 kDa) 3.5 mg/ml wet gel (rat liver)
Maximum flow rate (25°C)	450 cm/h
Recommended flow rate	<150 cm/h
Highest resistance of atmospheric pressure	0.3 Mpa
pH stability	3~10

PROTOCOL

Notes
- Samples should be centrifuged and filtrated with 0.45 μm filter before loading.
- To avoid cross-contamination, do not use the same medium to purify different proteins.
- **Equilibration Buffer**
  140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.3
- **Elution Buffer**
  50 mM Tris-HCl pH 8.0, 10 mM reduced glutathione.

Procedures
1. Prepare GST purification column
(1) Thoroughly resuspend the GST resin to achieve a homogeneous suspension of the resin in the 20% ethanol storage buffer.
(2) Immediately transfer the resin into a purification column. Ensure that the bottom of the column is plugged with a stopper. Close the valve of the column. Allow the resin to settle.
(3) Equilibrate the column with 5~10 bed volume of equilibration buffer.
2. Prepare samples
To avoid blocking column, samples should be centrifuged and filtrated with 0.45 μm filter before loading.
3. Load samples and wash
Load samples and wash with 5~10 bed volume of equilibration buffer and collect the flow-through in one tube.
4. Elute
Elute target protein with elution buffer.
5. Regeneration of GST resin
(1) Wash the column/resin with 2 bed volume of 6 M GuHCl, 0.2 M acetic acid and then 5 bed volume of deionized water or PBS buffer.
Or
(2) 3-4 bed volume of 70% ethanol or 30% isopropanol and then 3-5 bed volume of deionized water.
Or

(3) 2 bed volume of 10-100 mM NaOH and then 10 bed volume of deionized water.
(4) Store column/resin in 20% ethanol.



Lane 1: Sample
Lane 2: Flow-through
Lane 3: Wash
M: *ProteinRuler*® II
Lane 4-7: Elution (10 mM GSH)

ProteinIso® Protein A Resin

DP301-01	5 ml

Storage
at 2-8°C (20% ethanol) for two years

Description
ProteinIso® Protein A Resin is an affinity chromatography resin with high binding capacity for IgG. The recombinant protein A ligand is coupled to highly cross-linked agarose. *ProteinIso*® Protein A Resin is suitable for purification of monoclonal antibody, polyclonal antibody and immunology complex, such as IP, Co-IP.

Resin Specifications

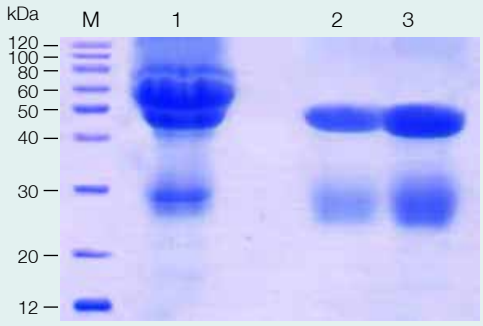
Resin	Cross-linked 4% agarose
Ligand	r-Protein A
Shape	sphere
Pore size	90 μm (45~165)
Support density	6 mg Protein A/ml wet gel
Binding capacity	40~50 mg h-IgG /ml wet gel
Maximum flow rate (25°C)	300 cm/h
Recommended flow rate	<150 cm/h
Highest resistance of atmospheric pressure	0.3 Mpa
pH stability	3~10

PROTOCOL

Notes
- Samples should be centrifuged and filtrated with 0.45 μm filter before loading.
- **Equilibration Buffer**
  20 mM PB, 150 mM KCl pH 7.0
- **Elution Buffer**
  20 mM citric acid pH 3.0-4.0;
  or 100 mM glycine pH 3.0;
  or 20 mM sodium acetate pH 3.0-4.0.
- **Neutralization Buffer**
  1 M Tris-HCl pH 9.0.

Procedures
1. Prepare protein A purification column
(1) Thoroughly resuspend the protein A resin to achieve a homogeneous suspension of the resin in the 20% ethanol storage buffer.
(2) Immediately transfer the resin into a purification column. Ensure that the bottom of the column is plugged with a stopper. Close the valve of the column. Allow the resin to settle.
(3) Equilibrate the column with 5~10 bed volume of equilibration buffer.
2. Prepare samples
To avoid blocking column, samples should be centrifuged and filtrated with 0.45 μm filter before loading.
3. Load samples and wash
Load samples and wash with 5~10 bed volume of equilibration buffer and collect the flow-through in one tube.
4. Elute
Elute antibodies with elution buffer.
Collect the elution containing the target immunoglobulin and immediately neutralized to pH>7.0 with neutralization buffer.
The elution conditions are closely related with binding strength and stability of antibody. When necessary, optimize the elution buffer.
5. Regeneration of Protein A Resin
(1) Wash the column/resin with 3~5 bed volume of 0.1 M citric acid or 0.1 M citric acid /20% ethanol and then 5 bed volume of PBS buffer (pH=7.0).
Or

(2) 3~5 bed volume of 0.05 M NaOH/1 M NaCl or 6 M GuHCl, and then 5 bed volume of deionized water.
(3) Store column/resin in 20% ethanol.



M: *ProteinRuler*® II
Lane 1: 0.33 µl Horse Serum
Lane 2: 100 µl Horse Serum with 10 µl Protein A
Lane 3: 100 µl Horse Serum with 20 µl Protein A

ProteinIso® Protein G Resin

DP401-01	5 ml

Storage
at 2-8°C (20% ethanol) for two years

Description
ProteinIso® Protein G Resin is an affinity chromatography resin with high binding capacity for IgG. The recombinant protein G ligand is coupled to highly cross-linked agarose. *ProteinIso*® Protein G Resin is suitable for purification of monoclonal antibody, polyclonal antibody and immunology complex, such as IP, Co-IP.

Resin Specifications

Resin	Cross-linked 4% agarose
Ligand	r-Protein G
Shape	sphere
Pore size	90 μm (45~165)
Support density	3 mg Protein G/ml wet gel
Binding capacity	20~25 mg h-IgG/ ml wet gel
Maximum flow rate (25°C)	300 cm/h
Recommended flow rate	<150 cm/h
Highest resistance of atmospheric pressure	0.3 Mpa
pH stability	3~10

PROTOCOL

Notes
- Samples should be centrifuged and filtrated with 0.45 μm filter before loading.
- **Equilibration Buffer**
  20 mM PB,150 mM KCl pH 7.0
- **Elution Buffer**
  20 mM citric acid pH 3.0-4.0;
  or 100 mM glycine pH 3.0;
  or 20 mM sodium acetate pH 3.0-4.0.
- **Neutralization Buffer**
  1 M Tris-HCl pH 9.0.

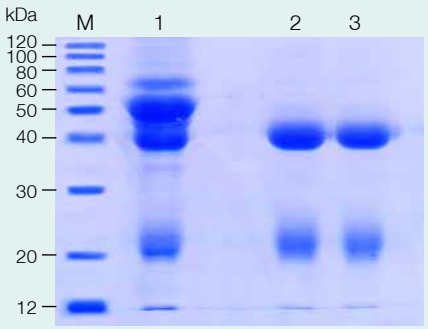
Procedures
1. Prepare protein G purification column
(1) Thoroughly resuspend the protein G resin to achieve a homogeneous suspension of the resin in the 20% ethanol storage buffer.
(2) Immediately transfer the resin into a purification column. Ensure that the bottom of the column is plugged with a stopper. Close the valve of the column. Allow the resin to settle.
(3) Equilibrate the column with 5~10 bed volume of equilibration buffer.
2. Prepare samples
To avoid blocking column, samples should be centrifuged and filtrated with 0.45 μm filter before loading.
3. Load samples and wash
Load samples and wash with 5~10 bed volume of equilibration buffer and collect the flow-through in one tube.
4. Elute
Elute antibodies with elution buffer.
Collect the elution containing the target immunoglobulin and immediately neutralize to pH>7.0 with neutralization buffer.
The elution conditions are closely related with binding strength and stability of antibody. When necessary, optimize the elution buffer.

5. Regeneration of Protein G Resin

(1) Wash the column/resin with 3~5 bed volume of 0.1 M citric acid or 0.1 M citric acid /20% ethanol and then 5 bed volume of PBS buffer (pH=7.0).
Or

(2) 3~5 bed volume of 0.05 M NaOH/1 M NaCl or 6 M GuHCl, and then 5 bed volume of deionized water.

(3) Store column/resin in 20% ethanol.



M: *ProteinRuler*® II

Lane 1: 0.33 µl Horse Serum

Lane 2: 100 µl Horse Serum with 10 µl Protein G

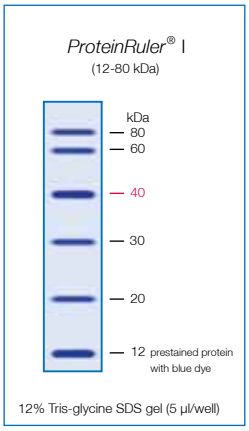
Lane 3: 100 µl Horse Serum with 20 µl Protein G

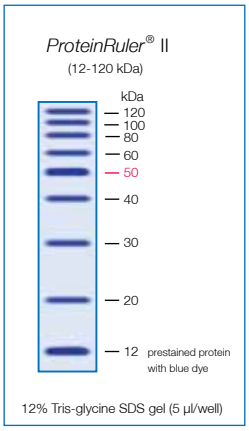
Affinity of Protein A/G for IgG Types

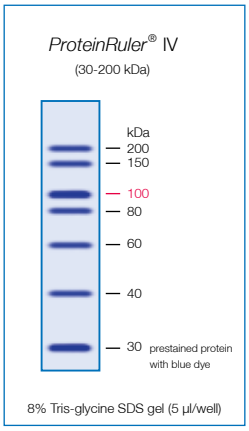
Sources	IgG subtypes	Protein A binding capacity	Protein G binding capacity
human	IgG_1	++++	++++
	IgG_2	++++	++++
	IgG_3	-	++++
	IgG_4	++++	++++
mouse	IgG_1	+	++++
	IgG_{2a}	++++	++++
	IgG_{2b}	+++	+++
	IgG_3	++	+++
rabbit	IgG	++++	+++
goat	IgG	-	++
horse	IgG	++	++++
dog	IgG	++	+
cattle	IgG	++	++++
pig	IgG	+++	+++
monkey	IgG	++++	++++

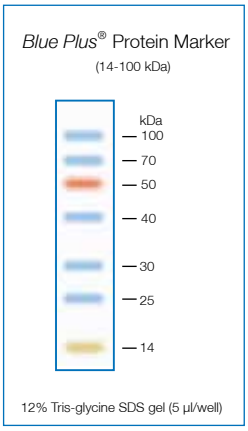
Protein Marker Selection Guide

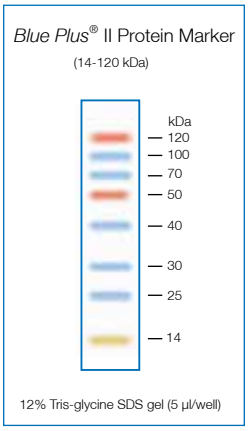
Type	Name	Cat. No.	SDS-PAGE	Western Blot	Monitor migration in SDS-PAGE	MW Range
Unstained Protein Marker	*ProteinRuler*® I	DR101	√	-	√	12-80 kDa
	ProteinRuler® II	DR201	√	-	√	12-120 kDa
	ProteinRuler® IV	DR401	√	-	√	30-200 kDa
Prestained Protein Marker	*Blue Plus*® Protein Marker	DM101	√	√	√	14-100 kDa
	Blue Plus® II Protein Marker	DM111	√	√	√	14-120 kDa
	Blue Plus® III Protein Marker	DM121	√	√	√	14-160 kDa
	Blue Plus® IV Protein Marker	DM131	√	√	√	10-180 kDa
Western Protein Marker	*EasySee*® Western Marker	DM201	√	√	√	25-90 kDa
	EasySee® II Western Marker	DM211	√	√	√	30-150 kDa

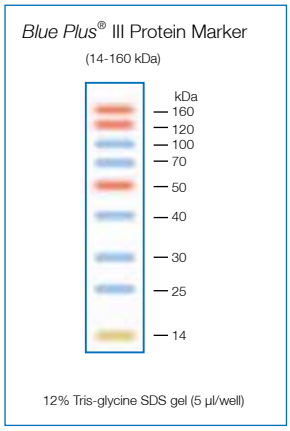


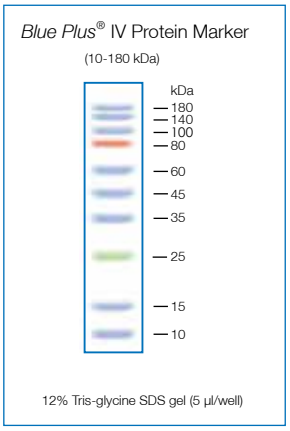


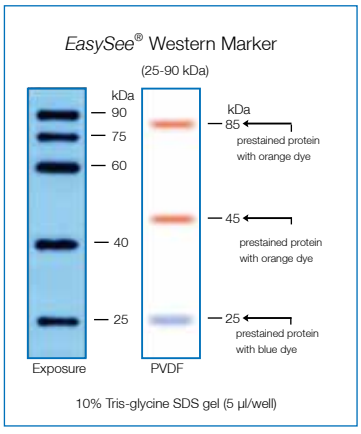


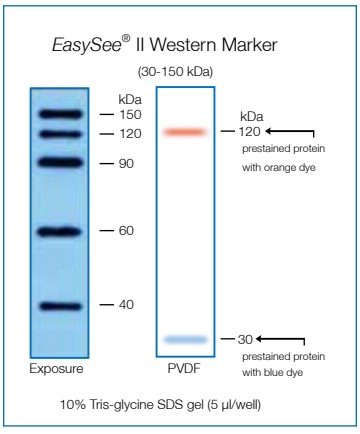












ProteinRuler® I
(12-80 kDa)

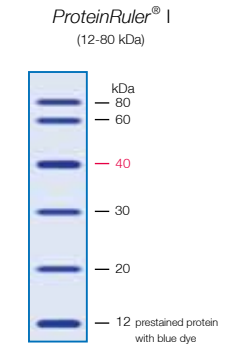
DR101-01	250 µl
DR101-02	500 µl

Concentration
2 µg/5 µl for 12 kDa band; 1 µg/5 µl for 40 kDa band; 0.5 µg/5 µl for each of other bands

Storage
at -20°C for two years

Description
ProteinRuler® I is composed of five unstained recombinant proteins (20 kDa, 30 kDa, 40 kDa, 60 kDa, 80 kDa) and one blue prestained recombinant protein (12 kDa). The prestained band allows monitoring electrophoresis and membrane transfer. The 40 kDa band has doubled intensity to serve as a reference band.

- MW range from 12 to 80 kDa.
- Ready-to-use format, direct load on gels without heating.



12% Tris-glycine SDS gel (5 µl/well)

ProteinRuler® II
(12-120 kDa)

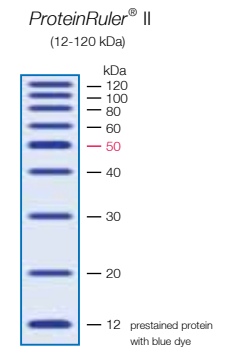
DR201-01	250 µl
DR201-02	500 µl

Concentration
2 µg/5 µl for 12 kDa band; 1 µg/5 µl for 50 kDa band; 0.5 µg/5 µl for each of other bands

Storage
at -20°C for two years

Description
ProteinRuler® II is composed of eight unstained recombinant proteins (20 kDa, 30 kDa, 40 kDa, 50 kDa, 60 Da, 80 kDa, 100 kDa, 120 kDa) and one blue prestained recombinant protein (12 kDa). The prestained band allows monitoring electrophoresis and membrane transfer. The 50 kDa band has doubled intensity to serve as a reference band.

- MW range from 12 to 120 kDa.
- Ready-to-use format, direct load on gels without heating.



12% Tris-glycine SDS gel (5 µl/well)

ProteinRuler® IV

(30-200 kDa)

DR401-01	250 µl
DR401-02	500 µl

Concentration

2 µg/5 µl for 30 kDa band; 1 µg/5 µl for 100 kDa band; 0.5 µg/5 µl for each of other bands

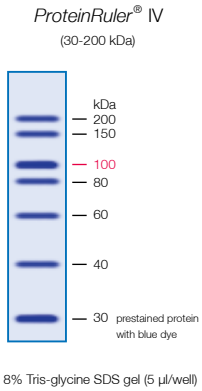
Storage

at -20°C for two years

Description

ProteinRuler® IV is composed of six unstained recombinant proteins (40 kDa, 60 kDa, 80 kDa, 100 kDa, 150 kDa, 200 kDa) and one blue prestained recombinant protein (30 kDa). The prestained band allows monitoring electrophoresis and membrane transfer. The 100 kDa band has doubled intensity to serve as a reference band.

- MW range from 30 to 200 kDa.
- Ready-to-use format, direct load on gels without heating.



8% Tris-glycine SDS gel (5 µl/well)

Blue Plus® Protein Marker

(14-100 kDa)

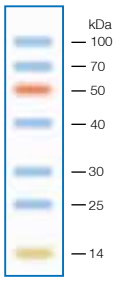
DM101-01	250 µl
DM101-02	500 µl

Concentration
about 2 µg/5 µl for each band
Storage
at -20°C for two years

Description
Blue Plus® Protein Marker is composed of seven prestained proteins ranging from 14 to 100 kDa. The protein of 50 kDa band is covalently coupled to orange dye. The protein of 14 kDa band is covalently coupled to yellow dye. The other five bands are covalently coupled to blue dye. This prestained protein marker is designed for monitoring the electrophoresis and membrane transfer.

- Five blue bands, one orange band and one yellow band.
- MW range from 14 to 100 kDa.
- Ready-to-use format, direct load on gels without heating.

Blue Plus® Protein Marker
(14-100 kDa)



12% Tris-glycine SDS gel (5 µl/well)

Blue Plus® II Protein Marker

(14-120 kDa)

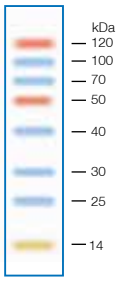
DM111-01	250 µl
DM111-02	500 µl

Concentration
about 2 µg/5 µl for each band
Storage
at -20°C for two years

Description
Blue Plus® II Protein Marker is composed of eight prestained proteins ranging from 14 to 120 kDa. The proteins of 50 kDa and 120 kDa bands are covalently coupled to orange dye. The protein of 14 kDa band is covalently coupled to yellow dye. The other five bands are covalently coupled to blue dye. This prestained protein marker is designed for monitoring the electrophoresis and membrane transfer.

- Five blue bands, two orange bands and one yellow band.
- MW range from 14 to 120 kDa.
- Ready-to-use format, direct load on gels without heating.

Blue Plus® II Protein Marker
(14-120 kDa)



12% Tris-glycine SDS gel (5 µl/well)

Blue Plus® III Protein Marker

(14-160 kDa)

DM121-01	250 μl
DM121-02	500 μl

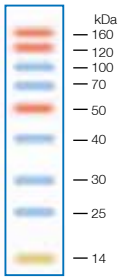
Concentration
about 2 μg/5 μl for each band
Storage
at -20°C for two years

Description

Blue Plus® III Protein Marker is composed of nine prestained proteins ranging from 14 to 160 kDa. The proteins of 50 kDa,120 kDa and 160 kDa bands are covalently coupled to orange dye. The protein of 14 kDa band is covalently coupled to yellow dye. The other five bands are covalently coupled to blue dye. This prestained protein marker is designed for monitoring the electrophoresis and membrane transfer.

- Five blue bands, three orange bands and one yellow band.
- MW range from 14 to 160 kDa.
- Ready-to-use format, direct load on gels without heating.

Blue Plus® III Protein Marker
(14-160 kDa)



12% Tris-glycine SDS gel (5 μl/well)

Blue Plus® IV Protein Marker

(10-180 kDa)

DM131-01	250 μl
DM131-02	500 μl

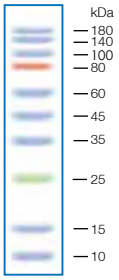
Concentration
about 2 μg/5 μl for each band
Storage
at -20°C for two years

Description

Blue Plus® IV Protein Marker is composed of ten prestained proteins ranging from 10 to 180 kDa. The protein of 80 kDa band is covalently coupled to orange dye. The protein of 25 kDa band is covalently coupled to green dye. The other eight bands are covalently coupled to blue dye. This prestained protein marker is designed for monitoring the electrophoresis and membrane transfer.

- Eight blue bands, one orange band and one green band.
- MW range from 10 to 180 kDa.
- Ready-to-use format, direct load on gels without heating.

Blue Plus® IV Protein Marker
(10-180 kDa)



12% Tris-glycine SDS gel (5 μl/well)

EasySee® Western Marker

(25-90 kDa)

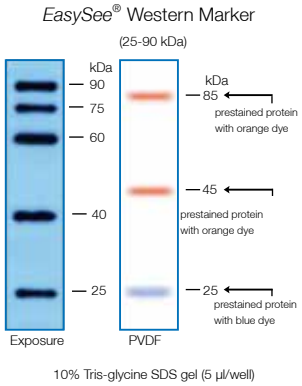
without *EasySee*® Western Blot Kit	DM201-01	250 µl
	DM201-02	500 µl
with *EasySee*® Western Blot Kit	DM201-11	250 µl+100 ml
	DM201-12	500 µl+200 ml

Storage
at -20°C for two years

Description

EasySee® Western Marker is composed of eight proteins ranging from 25 to 90 kDa. The 25 kDa, 45 kDa and 85 kDa bands are prestained allowing easy identification and monitoring electrophoresis and membrane transfer. Other five bands contain several IgG binding sites, allowing marker visualization using the same reagents and protocol for your target proteins. These no-dye-attached proteins provide more accurate molecular weight estimation.

- Three prestained bands for monitoring electrophoresis and membrane transfer.
- No label, no dye attached to other five recombinant protein bands.
- Five recombinant protein bands contain IgG binding sites, which can be developed with the standard Western blot substrates.
- Ready-to-use format, direct load on gels without heating.



10% Tris-glycine SDS gel (5 µl/well)

EasySee® II Western Marker

(30-150 kDa)

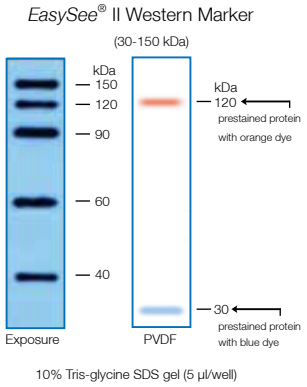
without *EasySee*® Western Blot Kit	DM211-01	250 μl
	DM211-02	500 μl
with *EasySee*® Western Blot Kit	DM211-11	250 μl+100 ml
	DM211-12	500 μl+200 ml

Storage
at -20°C for two years

Description

EasySee® II Western Marker is composed of seven proteins ranging from 30 to 150 kDa. The 30 kDa and 120 kDa bands are prestained allowing easy identification and monitoring electrophoresis and membrane transfer. Other five bands contain several IgG binding sites, allowing marker visualization using the same reagents and protocol for your target proteins. These no-dye-attached proteins provide more accurate molecular weight estimation.

- Two prestained bands for monitoring electrophoresis and membrane transfer.
- No label, no dye attached to other five recombinant protein bands.
- Five recombinant protein bands contain IgG binding sites, which can be developed with the standard Western blot substrates.
- Ready-to-use format, direct load on gels without heating.



EasySee® Western Blot Kit

DW101-01	100 ml
DW101-02	200 ml

Storage
at 2-8°C in dark for two years

Description

EasySee® Western Blot Kit is optimized for enhanced chemiluminescence detection of western blots. The kit can be used in detection of horseradish peroxidese (HRP) conjugated secondary antibodies and related antigen.

- High sensitivity.
- Extended exposure time.
- High stability.

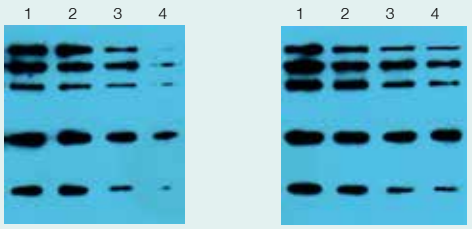
Kit Contents

Component	DW101-01	DW101-02
WB Solution A (Luminol)	50 ml	100 ml
WB Solution B (Oxidant)	50 ml	100 ml
WB Solution C (Light intensifier)	150 µl	300 µl

PROTOCOL

Procedures

1. After electrophoresis, transfer proteins onto PVDF or NC membrane. Probe membrane with primary antibody followed by HRP-conjugated secondary antibody. Wash membrane for three times.
2. Mix equal volume of Solution A with Solution B. Add 0.05%-0.1% (v/v) of Solution C to the mixture (for example, add 1-2 µl of Solution C to 2 ml of Solution A + Solution B).
3. Add the mixed solution to the membrane (0.125 ml of reagent per cm^2 membrane). Incubate at room temperature for 1 minute.
4. Drain off excess solution from membrane. Do not let the membrane dry. Wrap the membrane with plastic wrap.
5. Develop image with x-Ray film.



Company A *Trans*

Lane 1: 5 µl Lane 2: 4 µl Lane 3: 3 µl Lane 4: 2 µl

6×Protein Loading Buffer

DL101-02	5×1 ml

Storage
at -20°C for one year

Description

6×Protein Loading Buffer is especially formulated for protein sample preparation used in SDS-PAGE. Prior to loading, add appropriate volume of 6×Protein Loading Buffer to protein sample to make its working concentration at 1×.

Easy Protein Quantitative Kit (Bradford)

DQ101-01	100 ml

Storage

BSA Standard Solution at -20°C for two years; Coomassie Brilliant Blue Solution at 2-8°C in dark for two years

Description

Easy Protein Quantitative Kit is a ready-to-use modified Bradford Coomassie-binding, colorimetric method for protein quantification. Under acidic condition, Coomassie Brilliant Blue G-250 binds to proteins providing in an immediate shift in absorption maximum from 465 nm to 596 nm and a color change from brown to blue.

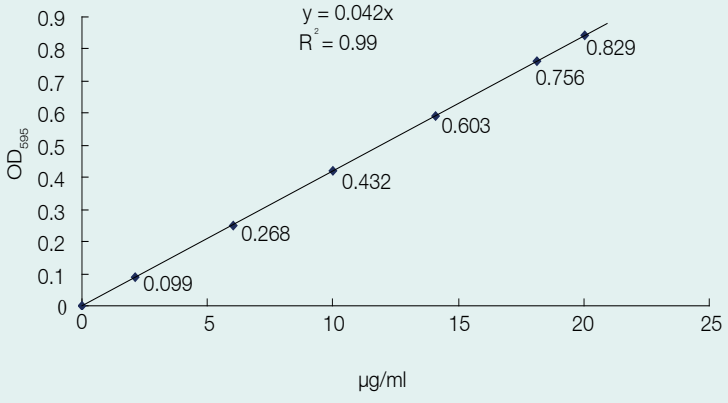
Kit Contents

Component	DQ101-01
Coomassie Brilliant Blue Solution	100 ml
BSA Standard Solution (0.22 mg/ml)	4×1 ml

PROTOCOL

Procedures

1. Prior to use, equilibrate Coomassie Brilliant Blue Solution to room temperature and gently invert to mix well.
2. Transfer 0, 10, 30, 50, 70, 90, 100 µl of BSA Standard Solution (0.22 mg/ml) into seven of 1.5 ml microfuge tubes, and add H_2O to a final volume of 100 µl.
3. Transfer protein sample into a new 1.5 ml microfuge tube, and add H_2O to a final volume of 100 µl.
4. Pipette 1.0 ml Coomassie Brilliant Blue Solution into each tube, mix thoroughly and incubate at room temperature for 5-10 minutes.
5. Measure the absorbance at 595 nm by spectrophotometer and record the value. Use the absorbance of sample without BSA as a blank control.
6. Plot the standard curve and calculate protein concentration in sample. Dilute the sample and re-measure it if the protein concentration falls out of the range of the standard curve.
7. The above procedures can be performed with microtiter-plate with 1/10 of the original volume.



Easy II Protein Quantitative Kit (BCA)

DQ111-01	100 ml

Storage
BSA Standard Solution at -20°C for two years; others at room temperature for one year

Description

The BCA protein assay is one of the most commonly used methods for protein quantification. Under alkaline condition, the reduction of Cu^{2+} to Cu^{+} is realized by peptide bonds in proteins (biuret reaction). The amount of reduced copper is directly proportional to the amount of total proteins.

Kit Contents

Component	DQ111-01
BCA Solution A	100 ml
BCA Solution B	3 ml
BSA Standard Solution (2 mg/ml)	2×1 ml

Interfering Substances

Certain substances are known to interfere with the BCA assay including those substances with reducing potential, chelating agents, and strong acids/bases. The following table shows the highest concentration of these substances in the protein sample buffer without interfering the BCA assay.

Interfering Substances	Tolerant Concentration	Interfering Substances	Tolerant Concentration
Salts/Buffer		Detergents	
HEPES (pH 7.9)	100 mM	NP-40	5%
PIPES (pH 6.8)	100 mM	Triton X-100	5%
Ammonium sulfate	1.5 M	CHAPS, CHAPSO	5%
Sodium chloride	1 M	SDS	5%
Sodium bicarbonate	100 mM	Tween 20	5%
MOPS (pH 7.2)	100 mM	Tween 60	5%
Sodium citrate	200 mM	Tween 80	5%
Tricine (pH 8.0)	25 mM	Mixture/Polar compounds	
Sodium acetate	200 mM	PMSF	1 mM
Guanidine·HCl	4 M	Acetone	10%
Tris	250 mM	Ethanol	10%
Chelating Agents		Glycerol	10%
EDTA	10 mM	Urea	3 M
Reducing Agents		DMSO	10%
DTT	1 mM	Sucrose	40%
2-Mercaptoethanol	0.01%		

PROTOCOL

Procedures

Prepare a serial dilutions of BSA standards

Dilute protein samples

↓

Add into a 96-well-plate or 1.5 ml tubes

↓

Prepare BCA working solution by mixing BCA Solution A & B (50:1 v/v)

↓

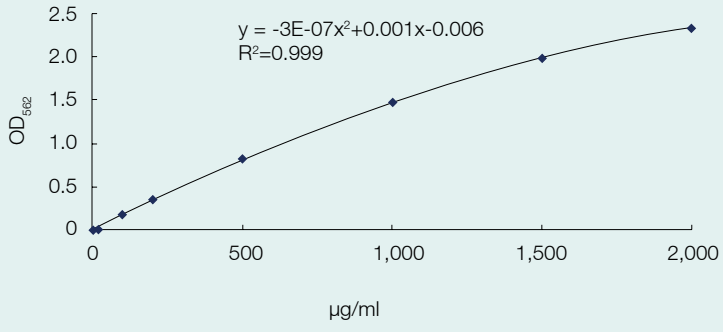
Add Solution A/B mixture into the 96-well plate or the tubes. Mix and incubate at 37°C

↓

Read at 562 nm on a microplate reader or a spectrophotometer

↓

Draw the standard curve and determine the protein concentration



ProteinEle™ Precast Tris-Glycine Gel

DG101-01	8%, 10/Box
DG101-02	10%, 10/Box
DG101-03	12%, 10/Box

Storage
at 2-8°C for one year

Description

ProteinEle™ Precast Tris-Glycine Gel is a polyacrylamide gel used for native and denatured protein electrophoresis. It provides shorter running time and higher transfer efficiency.

Highlights

- High stability: gel shelf life up to one year at 2-8°C.
- High resolution: superior band resolution on a broad range of native and denatured proteins.
- High reproducibility: consistent performance from gel to gel.

Kit Contents

Component	Specification
DG101-01	8%, 1.0 mm, 12 well, 10/box
DG101-02	10%, 1.0 mm, 12 well, 10/box
DG101-03	12%, 1.0 mm, 12 well, 10/box

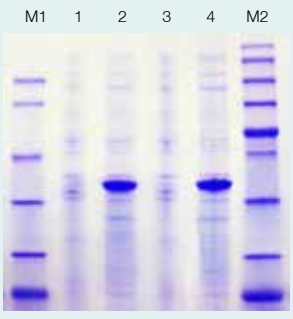
PROTOCOL

Note

If using the mini electrophoresis tank (Bio-Rad) or other similar electrophoresis tank, remove the sealing tape from electrophoresis apparatus, invert it and install again, then place the precast gel in the gel running tank. Otherwise, precast gel cannot be completely sealed with the outer surface of the sealing tape, which may result in electrophoresis buffer leak and therefore affect the result.

Instruction

1. Take out the precast gel, remove the sealing tape near the bottom of the gel cassette. Place the gel in the gel running tank. Fill the gel wells with the electrophoresis buffer to immerse the chamber, gently pull out the comb from the chamber. Load the sample on the gel and run electrophoresis.
2. After electrophoresis is complete. Remove the Gel cassette from the gel running tank. To open the Gel cassette, insert a screwdriver into the gap between the two plastic plates between the gel. Apply pressure to separate them.



M1: *ProteinRuler*® I
Lane 1: Sample 1;
Lane 2: Sample 2;
Lane 3: Sample 3;
Lane 4: Sample 4
M2: *ProteinRuler*® II

12% *ProteinEle*™ Precast Tris-Glycine Gel (5 µl/well)

Chapter 7 Cell Culture and Detection

Cell Cuture

Cell Detection

TransSerum® HQ Fetal Bovine Serum

FS101-02	500 ml

Storage
at -20°C for five years

Description

Fetal Bovine Serum is the most widely used undefined supplement in mammalian cell culture. *TransSerum*® HQ Fetal Bovine Serum is collected from healthy fetal blood, followed by filtration through 0.1 μm filters for three times.

- Low toxicity
- Promoting better cell growth
- Suitable for a broad range of cells

Physical Properties

pH	6.9-7.8
Osmolality	280-340 mOsmol/kg
Total proteins	3.0-3.5 g/dl
Albumin	1.8-2.45 g/dl
Endotoxin	<10 EU/ml
IgG	<100 μg/ml
Hemoglobin	<20 mg/dl
Test of germ, mycete, phage	Negative
Test of mycoplasma	Negative
Test of virus	Negative

Note

This product is not heat inactivated. If needed, incubate thawed FBS at 56°C water bath for 30 minutes to inactivate complement.

Successfully cultured cell types with *TransSerum*® HQ Fetal Bovine Serum

5637	HEK-293T	OV45
7721	HeLa	P815
A2780	Hep G2	PANC-1
A549	HL-60	PC-12
B16-F10	HT-29	PT67
BEL-7402	Huh7	RAW264.7
BHK21	Jurkat	Sf9
BGC-823	K-562	SGC-7901
CEF	L929	SK-OV-3
CEK	LS 174T	Sp2/0
CHO-K1	MCF7	STO
COS-1	MDA-MB-231	SW480
COS-7	MEF	T24
DF-1	MGC803	THP-1
DLD-1	MKN-28	U87
EJ	MKN-45	U937
GLC-82	MRC-5	Vero
HCT 116	NIH3T3	WEHI-3B
HEK-293	NRK	

TransLipid® HL Transfection Reagent

FT111-01	0.75 ml
FT111-02	2×0.75 ml

Storage
at 2-8°C for one year

Description
TransLipid® HL Transfection Reagent is a proprietary formulated cationic lipid that offers superior transfection efficiency and low cytotoxicity across a broad range of mammalian cell lines.

- High efficiency
- Low cytotoxicity
- Transfect DNA, RNA, siRNA
- Adherent or suspension cells
- Can be used in the presence of serum and antibiotics

PROTOCOL

We suggest using DNA(μg)/*TransLipid*® HL (μl) with ratio at 1:2-1:3. To obtain better transfection efficiency, we suggest using high density cell (70%-90% confluency).

Plasmid DNA Transfection
24-well format as an example

Seed cells: plate 0.5-2×10^5 cells in each well so that cells will be 70-90% confluency at the time of transfection

↓

Grow cells for 12-24 hours

↓

Dilute plasmid: dilute 0.8 μg plasmid with 50 μl Opti-MEM medium
Dilute *TransLipid*® HL: dilute 2 μl *TransLipid*® HL with 50 μl Opti-MEM medium

↓

Incubate at room temperature for 5 minutes

↓

Gently mix DNA and *TransLipid*® HL

↓

Incubate at room temperature for 20 minutes

↓

Add the DNA-*TransLipid*® HL complexes to each well containing cells and medium, incubate cells at 37°C in a CO_2 incubator

↓

Change medium 4-6 hours post-transfection (optional). Incubate cells for 18-72 hours

siRNA Transfection

Cells should be 30-50% confluency at the time of transfection. For 24-well plate, use 20 pmol of siRNA and 1 µl of *TransLipid*® HL. The experimental procedure is the same as DNA transfection described above.

Optimization of plasmid DNA and siRNA transfection

In order to achieve optimal combination of high transfection efficiency and low cytotoxicity, the ratio of DNA or siRNA to *TransLipid*® HL as well as the initial cell density for transfection could be optimized. DNA transfection can be optimized within the range of 1:2-1:5, it is recommended to use a range of 10 to 50 pmol of siRNA and 0.5 to 1.5 µl of *TransLipid*® HL.

Amount of culture medium, nucleic acid and *TransLipid*® HL in transfection of different cell culture plates

Culture Vessel	Surface Area per Well	Volume of Plating Medium	Dilution Volume	DNA Transfection		siRNA Transfection	
				DNA	*TransLipid*® HL	siRNA	*TransLipid*® HL
96-well	0.3 cm^2	100 µl	2 × 10 µl	0.2 µg	0.4-1 µl	5 pmol	0.25 µl
48-well	1 cm^2	250 µl	2 × 25 µl	0.4 µg	0.8-2 µl	10 pmol	0.5 µl
24-well	2 cm^2	500 µl	2 × 50 µl	0.8 µg	1.6-4 µl	20 pmol	1 µl
12-well	4 cm^2	1 ml	2 × 100 µl	1.6 µg	3.2-8 µl	40 pmol	2 µl
6-well	10 cm^2	2 ml	2 × 250 µl	4 µg	8-20 µl	100 pmol	5 µl
35 mm	10 cm^2	2 ml	2 × 250 µl	4 µg	8-20 µl	100 pmol	5 µl
60 mm	20 cm^2	5 ml	2 × 0.5 ml	8 µg	16-40 µl	200 pmol	10 µl
10 cm	60 cm^2	10 ml	2 × 1.5 ml	24 µg	48-120 µl	600 pmol	30 µl
T 25	25 cm^2	6 ml	2 × 0.625 ml	10 µg	20-50 µl	250 pmol	12.5 µl
T 75	75 cm^2	20 ml	2 × 1.875 ml	30 µg	60-150 µl	800 pmol	40 µl

Successfully transfected cell types with *TransLipid*® HL Transfection Reagent

A549	HCT 116	Huh7	PC3
B16-F10	HeLa	K-562	PANC-1
BEL-7402	Hep G2	MCF7	PT67
BHK21	HEK-293	MDS-L	RAW 264.7
CHO-K1	HEK-293T	MRC-5	STO
COS-1	HMC-1	NIH3T3	Vero
COS-7	HT-29	NRK	

TransIn™ EL Transfection Reagent

FT201-01	0.75 ml
FT201-02	2×0.75 ml

Storage
at 2-8°C for one year

Description
TransIn™ EL Transfection Reagent is a non-liposomal formulation designed to transfect DNA into a wide variety of eukaryotic cell lines with high efficiency and low toxicity. Primary cells and other difficult-to-transfect cells can also be effectively transfected by this reagent.

- Non-liposomal transfection reagent
- High efficiency
- Low cytotoxicity
- Adherent or suspension cells
- Can be used in the presence of serum and antibiotics

PROTOCOL

Plasmid DNA Transfection

24-well format as an example

Seed cells: plate 0.5-2×10^5 cells in each well so that cells will be 70-90% confluency at the time of transfection

↓

Grow cells for 12-24 hours

↓

Dilute plasmid: dilute 0.8 μg of plasmid with 50 μl of Opti-MEM medium

↓

Add 1.6 μl of premixed *TransIn*™ EL Transfection Reagent into the tube of diluted DNA, mix gently

↓

Incubate for 15-20 minutes at room temperature

↓

Add the DNA- *TransIn*™ EL complexes to each well containing cells and medium, incubate cells at 37°C in a CO_2 incubator

↓

Change medium 4-6 hours post-transfection (optional). Incubate cells for 18-72 hours

Optimization of plasmid DNA transfection

In order to achieve optimal combination of high transfection efficiency and low cytotoxicity, the ratio of DNA to *TransIn*™ EL as well as the initial cell density for transfection could be optimized. Ratio of DNA (μg) and *TransIn*™ EL (μl) can be optimized within the range of 1:1-1:3.

Amount of culture medium, nucleic acid and *TransIn*™ EL in transfection of different cell culture plates

Culture Vessel	Surface Area per Well	Volume of Plating Medium	Dilution Volume	DNA	*TransIn*™ EL
96-well	0.3 cm^2	100 μl	10 μl	0.2 μg	0.2-0.6 μl
48-well	1 cm^2	250 μl	25 μl	0.4 μg	0.4-1.2 μl
24-well	2 cm^2	500 μl	50 μl	0.8 μg	0.8-2.4 μl
12-well	4 cm^2	1 ml	100 μl	1.6 μg	1.6-4.8 μl
6-well	10 cm^2	2 ml	200 μl	4 μg	4-12 μl
35 mm	10 cm^2	2 ml	200 μl	4 μg	4-12 μl
60 mm	20 cm^2	5 ml	0.5 ml	8 μg	8-24 μl
10 cm	60 cm^2	10 ml	1 ml	24 μg	24-72 μl
T 25	25 cm^2	6 ml	0.5 ml	10 μg	10-30 μl
T 75	75 cm^2	20 ml	1 ml	30 μg	30-90 μl

Successfully transfected cell types with *TransIn*™ EL Transfection Reagent

A549	HEK-293	MARC-145	
B16-F10	HEK-293T	MCF-7	PT67
BHK-21	HeLa	MEF	SGC-7901
CEF	Hep G2	MIA PaCa-2	SH-SY5Y
CHO	HL-60	NIH/3T3	STO
COS-1	K562	NRK	Vero
HCT-116	L929	P815	

Penicillin-Streptomycin (100×)

FG101-01	100 ml

Storage
-20°C for one year

Description
Penicillin-Streptomycin (100×) contains 10 kU/ml of penicillin and 10 mg/ml of streptomycin. The solution has been filter-sterilized. It can be used for cell culture at a final concentration of 1x.

Note
Aliquots after thawing; avoiding repeated freezing and thawing; store at 2-8°C for two weeks, or at -20°C for one year.

L-Glutamine (100×)

FG201-01	100 ml

Storage
-20°C for one year

Description
L-Glutamine (100×) contains 200 mM of L-Glutamine. The solution has been filter-sterilized. It can be used for cell culture at a final concentration of 1x.

Note
Aliquots after thawing; avoiding repeated freezing and thawing; store at 2-8°C for two weeks, or at -20°C for one year.

Trypsin

FG301-01 (+EDTA)	100 ml
FG301-11 (-EDTA)	100 ml

Storage
-20°C for 18 months

Description
Trypsin contains porcine trypsin (0.25%) , EDTA (+/-), and phenol red. It does not contain calcium and magnesium ion. Trypsin solution has been filter-sterilized and it can be used for cell dissociation.

Note
Aliquots after thawing; avoiding repeated freezing and thawing; store at 2-8°C for two weeks, or at -20°C for one year.

G418

FG401-01	5 ml

Storage
2-8°C for two years

Description
G418 is an aminoglycoside antibiotic, which blocks polypeptide synthesis by interfering with the function of 80S ribosome. Due to its toxicity on prokaryotic and eukaryotic cells (including bacteria, fungi, plants and mammalian cells), it is widely used as a selective reagent for stable cell line construction. The resistance mechanism is based on that resistance gene (Neomycin) specifically express aminoglycoside phosphotransferase, which confers resistance on cells. Thus cells are able to grow in selective culture medium containing G418.

PBS (1×)

FG701-01	500 ml

Storage

at room temperature for two years

Description

PBS (phosphate buffered saline) contains 1.06 mM KH_2PO_4, 155.17 mM NaCl, 2.97 mM Na_2HPO_4 (pH 7.4). PBS has been widely used for a variety of cell culture applications, such as washing, dissociation and dilution. PBS is formulated without calcium, magnesium and phenol red. This product has been filter-sterilized.

TransDetect® Double-Luciferase Reporter Assay Kit

FR201-01	50 rxns
FR201-02	200 rxns

Storage

at -20°C in dark for one year

Description

TransDetect® Double-Luciferase Reporter Assay Kit provides an efficient method to perform dual-reporter assays. In the assay, the activities of firefly (*Photinus pyralis*) and *Renilla* (sea pansy) luciferases are measured sequentially from a single sample. The firefly luciferase reporter is measured firstly by adding Luciferase Reaction Reagent to generate a luminescent signal. After quantifying the firefly luminescence, this reaction is quenched, and the *Renilla* luciferase reaction is initiated by adding Luciferase Reaction Reagent II to the same tube.

Kit Contents

Component	FR201-01	FR201-02
Luciferase Reaction Buffer	5 ml	20 ml
Luciferase Reaction Substrate (Lyophilized)	1 vial	4 vials
Luciferase Reaction Buffer II	5 ml	20 ml
Luciferase Reaction Substrate II (50x)	100 µl	400 µl
Cell Lysis Buffer (5x)	5 ml	20 ml

Procedures

1. Prepare Luciferase Reaction Reagent
   Dissolve the lyophilized Luciferase Reaction Substrate (whole vial) by adding 5 ml of Luciferase Reaction Buffer to the vial. The prepared Luciferase Reaction Reagent can be aliquoted and stored at -20°C for one month or -70°C for one year.
2. Prepare Luciferase Reaction Reagent II
   Mix Luciferase Reaction Substrate II with Luciferase Reaction Buffer II with the ratio of 1:50. The prepared Luciferase Reaction Reagent II can be aliquoted and stored at -70°C for one month. For best results, the Luciferase Reaction Reagent II should be prepared fresh before each use.
3. Prepare 1xCell Lysis Buffer
   Mix 5×Cell Lysis Buffer with ddH_2O with the ratio of 1:4. 1×Cell Lysis Buffer can be stored at 2-8°C for one month.
4. Prepare Cell Lysate
   Wash cells twice with PBS and add appropriate volume of 1×Cell Lysis Buffer. Incubate at room temperature for 10 minutes and then scrape

the cells. Centrifuge at 12,000xg for 10 minutes at 2-8°C. Transfer the supernatant to a new tube.

5. Assay

   Mix 20 µl of lysate with 100 µl of Luciferase Reaction Reagent. Place the tube in luminometer to measure the luminescent signal of firefly luciferase. Then, add 100 µl of Luciferase Reaction Reagent II to the tube, place the tube in luminometer to measure the luminescent signal of *Renilla* luciferase. Record the data.

TransDetect® Cell Counting Kit (CCK)

FC101-01	1 ml
FC101-02	5 ml
FC101-03	10 ml
FC101-04	30 ml

Storage

at 2-8°C in dark for one year or at -20°C in dark for two years

Description

TransDetect® Cell Counting Kit (CCK) is designed for cell proliferation assays as well as cytotoxicity assays by utilizing a water-soluble tetrazolium salt. The salt can be reduced to an orange water-soluble formazan by mitochondrial dehydrogenase in the presence of an electron coupling reagent 1-Methoxy PMS. The amount of the formazan dye generated by dehydrogenases in cells is directly proportional to the number of living cells. Faster cell proliferation, lower cytotoxicity, and more cell number produce deeper color. The depth of the dye is directly proportional to the number of living cells. The toxicity of CCK solution is so low that the same cells can be used for other assays after the CCK assay is completed. Compared with MTT, XTT, MST and WST-1, this method provides higher sensitivity and broader linear range. It is suitable for drug screening, cell proliferation test, cytotoxicity assay and drug sensitivity test.

Kit Content

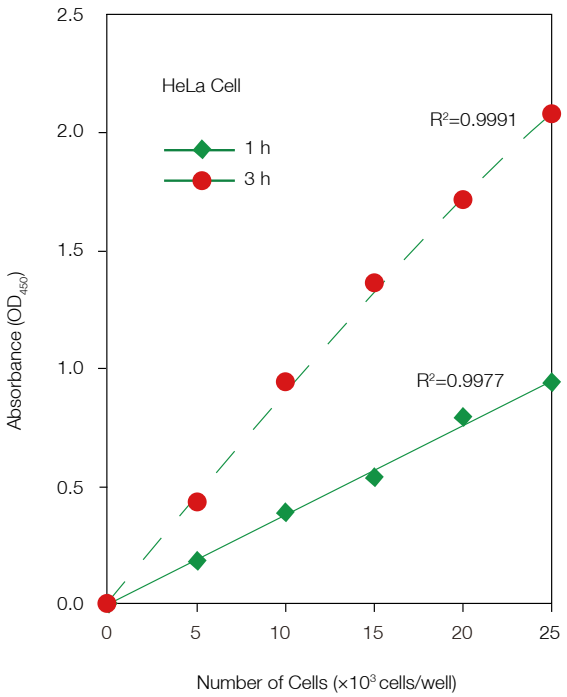
Component	FC101-01	FC101-02	FC101-03	FC101-04
CCK Solution	1 ml	5 ml	10 ml	30 ml

Procedures

1. Inoculate cell suspension in a 96-well plate (100 µl/well). Pre-incubate the plate in a cell incubator at 37°C for 12-24 hours according to experimental need. In general, use 2×10^3 cells per well for cell proliferation assays, use 5×10^3 cells per well for cytotoxicity assays.
2. Add appropriate volume (0-10 µl) of substance to be tested to the plate, incubate for an appropriate length of time in the incubator.
3. Add CCK solution (equal to 1/10 of the media volume) to each well of the 96-well plate (e.g. add 10 µl of CCK solution for 100 µl of the media).
4. Incubate the plate for 1-4 hour in the incubator.
5. Measure the absorbance at 450 nm using a microplate reader.

Notes

1. The presence of phenol red has no effect on the result, but can increase the background absorption. Thus the blank absorbance should be subtracted.
2. The incubation time after adding CCK solution varies by the cell type and density. Perform initial experiments to determine the appropriate incubation time. Generally, lymphocyte has lower sensitivity, which requires longer incubation time or higher cell density.
3. Assays by this kit depend on the catalyzation of dehydrogenase in cells. If the substance to be tested has strong oxidativity or reductivity, fresh media should be changed prior to adding CCK solution.
4. Be careful not to introduce bubbles to the wells since they will interfere with absorbance value.
5. If there is no 450 nm optical filter, the filter with absorbance between 430 nm and 490 nm can be used.
6. For highly turbid cell suspension, 600 nm (or above 600 nm) can be used as a reference to perform dual wavelength measurement.
7. To stop the reaction, add 10 µl of 0.1 M HCl or 1% w/v SDS solution into each well of 96-well plate, and store in dark. The absorbance will not change within 24 hours.
8. The toxicity of CCK solution is so low that the same cells can be used for other assays after the CCK assay is completed.
9. This kit should be stored in dark. For long-term storage, aliquot CCK solution and store at -20°C.



CCK Assay

TransDetect® Annexin V-FITC/PI Cell Apoptosis Detection Kit

FA101-01	25 rxns
FA101-02	50 rxns

Storage

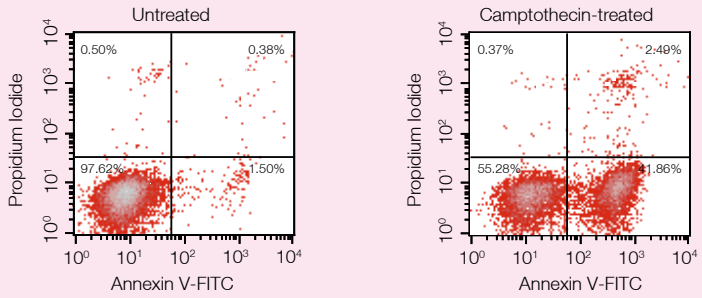
at 2-8°C in dark for one year

Description

The Annexin V-FITC/PI Cell Apoptosis Kit provides a rapid and sensitive method for early apoptosis detection. In normal cells, the membrane phospholipid phosphatidylserine (PS) is located on the cytoplasmic surface of the membrane. In apoptotic cells, PS is translocated from the inner to the outer leaflet of the plasma membrane, thereby exposing PS to the external cellular environment. The FITC-conjugated Annexin V, a Ca^{2+} dependent phospholipid-binding protein, can bind specifically to the exposed PS. Propidium iodide (PI) is a nucleic acid binding dye, which binds tightly to the nucleic acids in the cells and stains the cells with red fluorescence. PI is impermeant to live cells and early apoptotic cells, so the combination of Annexin V-FITC and PI staining allows the differentiation among different stage of apoptotic cells and necrosis cells.

Kit Contents

Component	FA101-01	FA101-02
Annexin V-FITC	125 µl	250 µl
Propidium Iodide (PI)	125 µl	250 µl
1× Annexin V Binding Buffer	12.5 ml	2×12.5 ml



Apoptosis detection of Camptothecin treated Jurkat cells by Flow Cytometry

TransDetect® Annexin V-EGFP/PI Cell Apoptosis Detection Kit

FA111-01	25 rxns
FA111-02	50 rxns

Storage

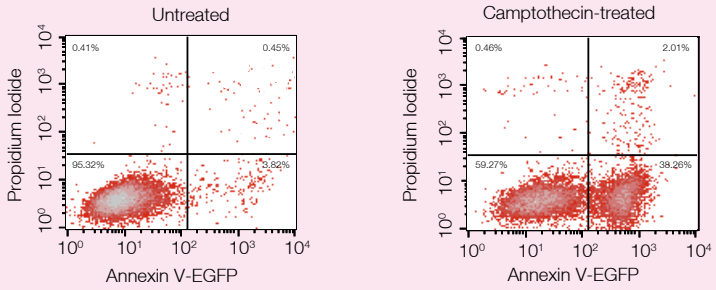
at 2-8°C in dark for one year

Description

The Annexin V-EGFP/PI Cell Apoptosis Kit provides a rapid and sensitive method for early apoptosis detection. In normal cells, the membrane phospholipid phosphatidylserine (PS) is located on the cytoplasmic surface of the membrane. In apoptotic cells, PS is translocated from the inner to the outer leaflet of the plasma membrane, thereby exposing PS to the external cellular environment. The EGFP-conjugated Annexin V, a Ca^{2+} dependent phospholipid-binding protein, can bind specifically to the exposed PS. Propidium iodide (PI) is a nucleic acid binding dye, which binds tightly to the nucleic acids in the cells and stains the cells with red fluorescence. PI is impermeant to live cells and early apoptotic cells, so the combination of Annexin V-EGFP and PI staining allows the differentiation among different stage of apoptotic cells and necrosis cells. Compared with FITC, EGFP is brighter and more photo-stable. Because Annexin V-EGFP is a fusion protein with a 1:1 binding ratio of EGFP to PS, this kit can also be used for quantitative detection.

Kit Contents

Component	FA111-01	FA111-02
Annexin V-EGFP	125 µl	250 µl
Propidium Iodide (PI)	125 µl	250 µl
1× Annexin V Binding Buffer	12.5 ml	2×12.5 ml



Apoptosis detection of Camptothecin treated Jurkat cells by Flow Cytometry

TransDetect® *In Situ* Fluorescein TUNEL Cell Apoptosis Detection Kit

FA201-01	25 rxns
FA201-02	50 rxns

Storage

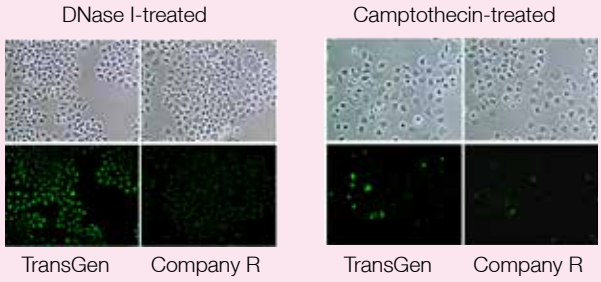
TdT at -20°C for one year, 1×Labeling Solution at -20°C in dark for one year

Description

TransDetect® *In Situ* Fluorescein TUNEL Cell Apoptosis Detection Kit provides a precise, simple and low-toxicity way to detect and quantify apoptotic cell death at single cell level in cells and tissues. TdT-mediated dUTP Nick-End Labeling (TUNEL) reaction preferentially labels DNA strand breaks generated during apoptosis with fluorescein-labeled dUTP. The fluorescein labeled DNA can be detected and quantified by fluorescence microscopy or flow cytometry. This kit can be used to detect apoptosis in paraffin-embedded tissue sections, cryopreserved tissue sections, cells cultured on chamber slides, cell smear and cell suspensions.

Kit Contents

Component	FA201-01	FA201-02
TdT	50 µl	100 µl
1×Labeling Solution	1.25 ml	2×1.25 ml



Apoptosis detection of DNase I or Camptothecin treated HeLa cells by fluorescence microscopy

Chapter 8 Antibodies

Primary Antibodies

Control Antibodies

Secondary Antibodies

Related Products

ProteinFind® Anti-c-Myc Mouse Monoclonal Antibody

HT101-01	50 µl
HT101-02	100 µl

Concentration
1 mg/ml
Storage
at 2-8°C for one month; at -20°C for one year

Description
ProteinFind® Anti-c-Myc Mouse Monoclonal Antibody is a purified monoclonal antibody that detects recombinant proteins containing the c-Myc (EQKLISEEDL) epitope tag.

Suggested Dilution
- Western Blot: 1:500-10,000 dilution.
- ELISA: 1:500-5,000 dilution.
- IF: 1:100-500 dilution.
- IP: 1:100-500 dilution.

M: *EasySee*® Western Marker
Detection of c-Myc fusion protein expression in HEK-293 cell by WB and IF

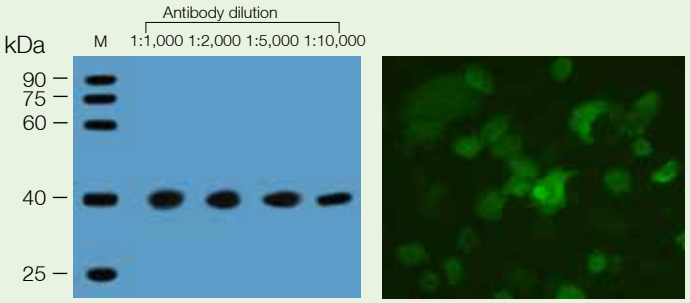
ProteinFind® Anti-DYKDDDDK Tag Mouse Monoclonal Antibody

HT201-01	50 µl
HT201-02	100 µl

Concentration
1 mg/ml
Storage
at 2-8°C for one month; at -20°C for one year

Description
ProteinFind® Anti-DYKDDDDK Tag Mouse Monoclonal Antibody is the same as FLAG antibody from Sigma. It is a purified monoclonal antibody that detects recombinant proteins containing the DYKDDDDK epitope tag.

Suggested Dilution
- Western Blot: 1:500-10,000 dilution.
- ELISA: 1:500-5,000 dilution.
- IF: 1:100-500 dilution.
- IP: 1:100-500 dilution.



M: *EasySee*® Western Marker
Detection of Flag fusion protein expression in HeLa cell by WB and IF

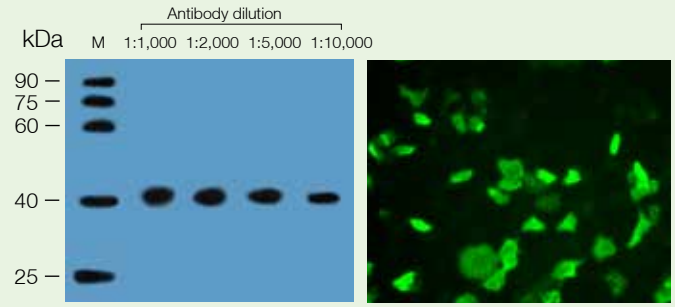
ProteinFind® Anti-HA Mouse Monoclonal Antibody

HT301-01	50 µl
HT301-02	100 µl

Concentration
1 mg/ml
Storage
at 2-8°C for one month; at -20°C for one year

Description
ProteinFind® Anti-HA Mouse Monoclonal Antibody is a purified monoclonal antibody that detects recombinant proteins containing the HA (YPYDVPDYA) epitope tag.

Suggested Dilution
- Western Blot: 1:500-10,000 dilution.
- ELISA: 1:500-5,000 dilution.
- IF: 1:100-500 dilution.
- IP: 1:100-500 dilution.



M: *EasySee*® Western Marker
Detection of HA fusion protein expression in HEK-293 cell by WB and IF

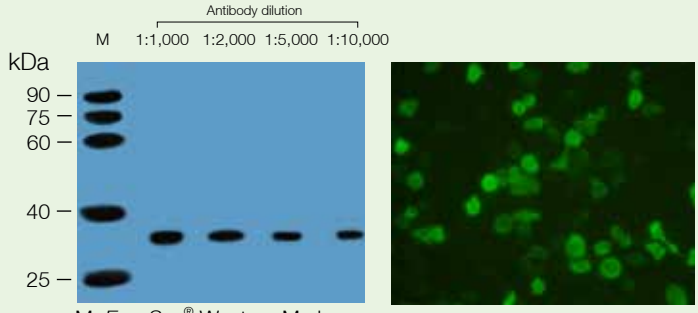
ProteinFind® Anti-V5 Mouse Monoclonal Antibody

HT401-01	50 µl
HT401-02	100 µl

Concentration
1 mg/ml
Storage
at 2-8°C for one month; at -20°C for one year

Description
ProteinFind® Anti-V5 Mouse Monoclonal Antibody is a purified monoclonal antibody that detects recombinant proteins containing the V5 (CGKPIPNPLLGLDST) epitope tag.

Suggested Dilution
- Western Blot: 1:500-10,000 dilution.
- ELISA: 1:500-5,000 dilution.
- IF: 1:100-500 dilution.
- IP: 1:100-500 dilution.



M: *EasySee*® Western Marker
Detection of V5 fusion protein expression in HeLa cell by WB and IF

ProteinFind® Anti-His Mouse Monoclonal Antibody

HT501-01	50 µl
HT501-02	100 µl

Concentration
1 mg/ml
Storage
at 2-8°C for one month; at -20°C for one year

Description
ProteinFind® Anti-His Mouse Monoclonal Antibody is a purified monoclonal antibody that detects recombinant proteins containing the 6xHis (HHHHHH) epitope tag.

Suggested Dilution
- Western Blot: 1:500-10,000 dilution.
- ELISA: 1:500-5,000 dilution.
- IF: 1:100-500 dilution.
- IP: 1:100-500 dilution.

M: *EasySee*® Western Marker
Detection of His fusion protein expression in HeLa cell by WB and IF

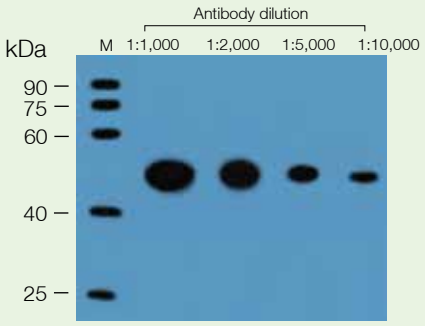
ProteinFind® Anti-GST Mouse Monoclonal Antibody

HT601-01	50 µl
HT601-02	100 µl

Concentration
1 mg/ml
Storage
at 2-8°C for one month; at -20°C for one year

Description
ProteinFind® Anti-GST Mouse Monoclonal Antibody is a purified monoclonal antibody against yeast *Y258* GST recombinant proteins that detects GST fusion proteins.

Suggested Dilution
- Western Blot: 1:500-10,000 dilution.
- ELISA: 1:500-5,000 dilution.
- IP: 1:100-500 dilution.



M: *EasySee*® Western Marker
Detection of GST fusion protein expression by WB

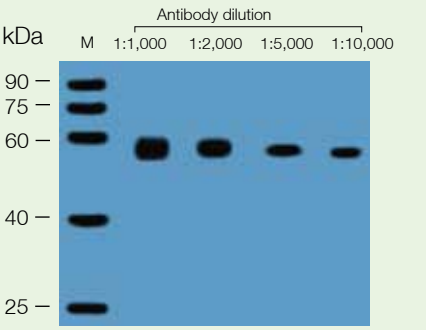
ProteinFind® Anti-MBP Mouse Monoclonal Antibody

HT701-01	50 µl
HT701-02	100 µl

Concentration
1 mg/ml
Storage
at 2-8°C for one month; at -20°C for one year

Description
ProteinFind® Anti-MBP Mouse Monoclonal Antibody is a purified monoclonal antibody that detects MBP fusion proteins.

Suggested Dilution
- Western Blot: 1:500-10,000 dilution.
- ELISA: 1:500-5,000 dilution.
- IP: 1:100-500 dilution.



M: *EasySee*® Western Marker
Detection of MBP fusion protein expression by WB

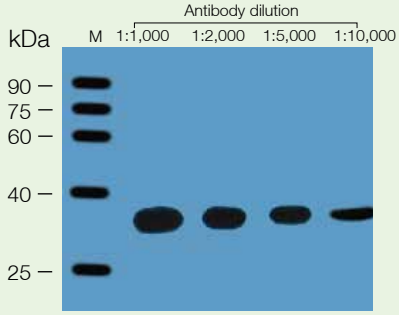
ProteinFind® Anti-GFP Mouse Monoclonal Antibody

HT801-01	50 µl
HT801-02	100 µl

Concentration
1 mg/ml
Storage
at 2-8°C for one month; at -20°C for one year

Description
ProteinFind® Anti-GFP Mouse Monoclonal Antibody is a purified monoclonal antibody that detects GFP fusion proteins.

Suggested Dilution
- Western Blot: 1:500-10,000 dilution.
- ELISA: 1:500-5,000 dilution.
- IP: 1:100-500 dilution.



M: *EasySee*® Western Marker
Detection of GFP fusion protein expression by WB

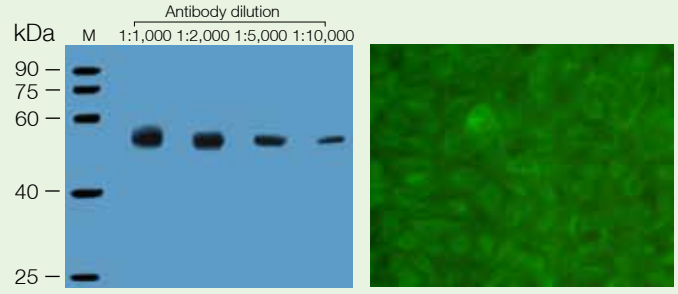
ProteinFind® Anti-β-Tubulin Mouse Monoclonal Antibody

HC101-01	50 µl
HC101-02	100 µl

Concentration
1 mg/ml
Storage
at 2-8°C for one month; at-20°C for one year

Description
Tubulin is an important component of the cytoskeleton. It is widely present in various mammalian cells and mainly consists of α-tubulin and β-tubulin. The expression level of β-tubulin is relatively stable. It is widely used as expression control.
ProteinFind® Anti-β-Tubulin Mouse Monoclonal Antibody is a purified monoclonal antibody that detects β-Tubulin in human, rat, mouse, goat and other species.

Suggested Dilution
- Western Blot: 1:500-10,000 dilution.
- ELISA: 1:500-5,000 dilution.
- IF: 1:100-500 dilution.
- IP: 1:100-500 dilution.



M: *EasySee*® Western Marker
Detection of β-Tubulin protein expression in HEK-293 cell by WB and IF

ProteinFind® Anti-β-Actin Mouse Monoclonal Antibody

HC201-01	50 µl
HC201-02	100 µl

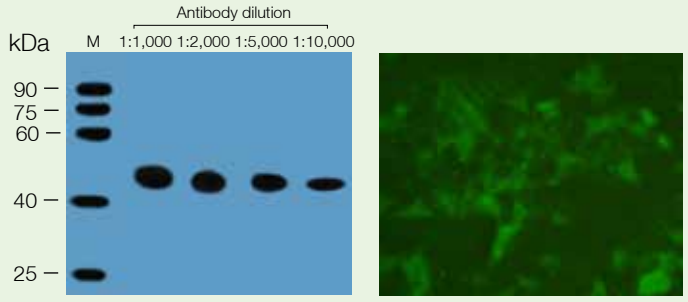
Concentration
1 mg/ml
Storage
at 2-8°C for one month; at -20°C for one year

Description

Actin is an important component of the cytoskeleton. It is widely present in various mammalian cells and mainly consists of the β-Actin. The expression level of β-Actin is relatively stable. It is widely used as expression control. *ProteinFind*® Anti-β-Actin Mouse Monoclonal Antibody is a purified monoclonal antibody that detects β-Actin in human, mouse, rabbit and other species.

Suggested Dilution

- Western Blot: 1:500-10,000 dilution.
- ELISA: 1:500-5,000 dilution.
- IF: 1:100-500 dilution.
- IP: 1:100-500 dilution.



M: *EasySee*® Western Marker
Detection of β-actin protein expression in HEK-293 cell by WB and IF

ProteinFind® Anti-GAPDH Mouse Monoclonal Antibody

HC301-01	50 µl
HC301-02	100 µl

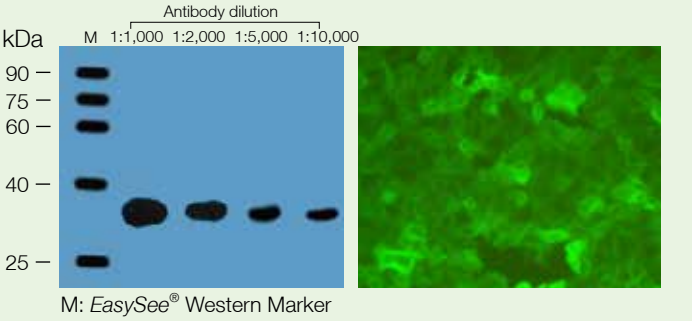
Concentration
1 mg/ml
Storage
at 2-8°C for one month; at -20°C for one year

Description

GAPDH (glyceraldehyde-3-phosphate dehydrogenase) is a key enzyme for the glycolysis process. It is widely present in various cells and has been used as expression control.
ProteinFind® Anti-GAPDH Mouse Monoclonal Antibody is a purified monoclonal antibody that detects GAPDH in human, mouse, rabbit and other species.

Suggested Dilution

- Western Blot: 1:500-10,000 dilution.
- ELISA: 1:500-5,000 dilution.
- IF: 1:100-500 dilution.
- IP: 1:100-500 dilution.



M: *EasySee*® Western Marker
Detection of GAPDH protein expression in HEK-293 cell by WB and IF

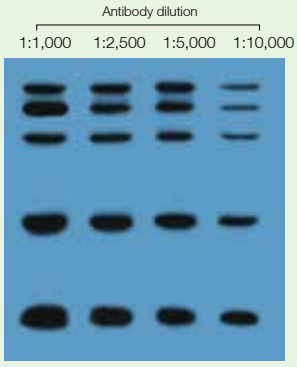
ProteinFind® Goat Anti-Rabbit IgG(H+L), HRP Conjugate

HS101-01 100 µl

Concentration
1 mg/ml
Storage
at 2-8°C for one month; at -20°C for one year

Description
Affinity purified *ProteinFind*® Goat Anti-Rabbit IgG(H+L) Antibody is a horseradish peroxidase (HRP) conjugated secondary antibody for ELISA and Western blot detection.

Suggested Dilution
- Western: 1:1000-10,000 dilution.
- ELISA: 1:1,000-5,000 dilution.



EasySee® Western Marker (5 µl/well)

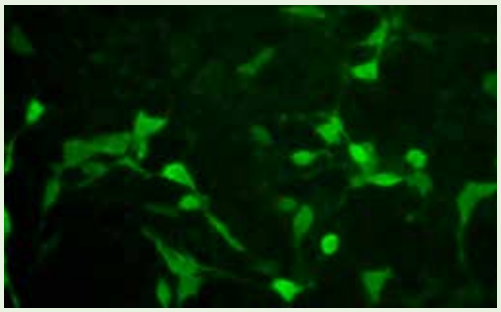
ProteinFind® Goat Anti-Rabbit IgG(H+L), FITC Conjugate

HS111-01 100 µl

Concentration
2 mg/ml
Storage
at 2-8°C in dark for one year

Description
Affinity purified *ProteinFind*® Goat Anti-Rabbit IgG(H+L) Antibody is conjugated with Fluorescein Isothiocyanate (FITC) dye under optimal conditions. FITC dye is a bright, yellow green-fluorescence dye with a maximal absorption wavelength at 490~495 nm and a maximal emission wavelength at 520~530 nm. This product has been optimized for use as a secondary antibody in immunofluorescence and flow cytometry.

Suggested Dilution
- IF: 1:100-500 dilution.
- FCM: 1:100-500 dilution.



Detection of fusion protein expression in HEK-293 cell by IF

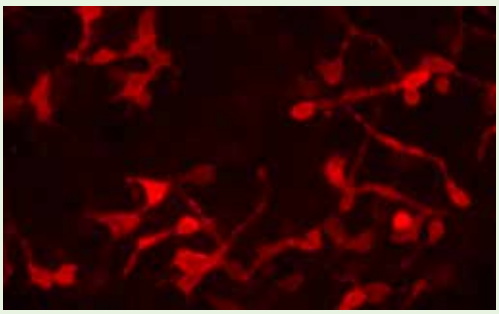
ProteinFind® Goat Anti-Rabbit IgG(H+L), PE Conjugate

HS121-01	100 µl

Concentration
0.4 mg/ml
Storage
at 2-8°C in dark for one year

Description
Affinity purified *ProteinFind*® Goat Anti-Rabbit IgG(H+L) Antibody is conjugated with phycoerythrin (PE) dye under optimal conditions. PE dye is a natural fluorescent dye extracted from red algae with a maximal absorption wavelength at 488 nm and a maximal emission wavelength at 575 nm. This product has been optimized for use as a secondary antibody in immunofluorescence and flow cytometry.

Suggested Dilution
- IF: 1:100-500 dilution.
- FCM: 1:100-500 dilution.



Detection of fusion protein expression in HEK-293 cell by IF

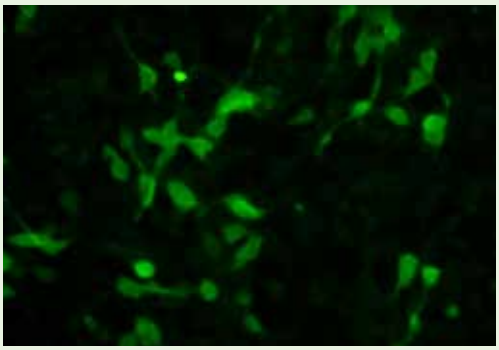
ProteinFind® Goat Anti-Rabbit IgG(H+L), AF488 Conjugate

HS131-01	100 µl

Concentration
1 mg/ml
Storage
at 2-8°C in dark for one year

Description
Affinity purified *ProteinFind*® Goat Anti-Rabbit IgG(H+L) Antibody is conjugated with Alexa Fluor 488 (AF488) dye under optimal conditions. AF488 dye is a bright, green-fluorescence dye with a maximal absorption wavelength at 495 nm and a maximal emission wavelength at 519 nm. This product has been optimized for use as a secondary antibody in immunofluorescence and flow cytometry.

Suggested Dilution
- IF: 1:100-500 dilution.
- FCM: 1:100-500 dilution.



Detection of fusion protein expression in HEK-293 cell by IF

ProteinFind® Goat Anti-Mouse IgG(H+L), HRP Conjugate

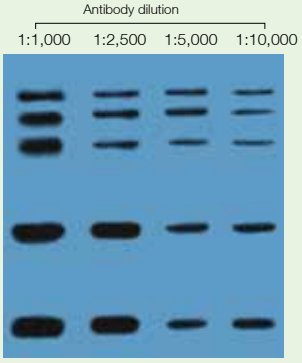
HS201-01	100 µl

Concentration
1 mg/ml

Storage
at 2-8°C for one month; at -20°C for one year

Description
Affinity purified *ProteinFind*® Goat Anti-Mouse IgG(H+L) Antibody is a horseradish peroxidase (HRP) conjugated secondary antibody for ELISA and Western blot detection.

Suggested Dilution
- Western Blot: 1:1,000-10,000 dilution.
- ELISA: 1:1,000-5,000 dilution.



EasySee® Western Marker (5 µl/well)

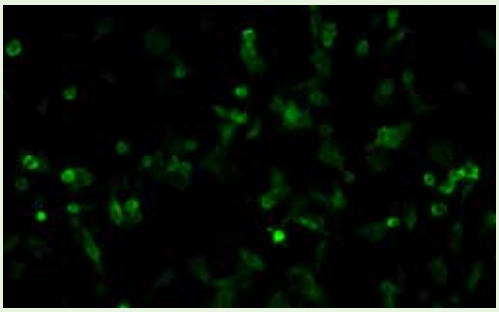
ProteinFind® Goat Anti-Mouse IgG(H+L), FITC Conjugate

HS211-01	100 μl

Concentration
2 mg/ml
Storage
at 2-8°C in dark for one year

Description
Affinity purified *ProteinFind*® Goat Anti-Mouse IgG(H+L) Antibody is conjugated with Fluorescein Isothiocyanate (FITC) dye under optimal conditions. FITC dye is a bright, yellow green-fluorescence dye with a maximal absorption wavelength at 490~495 nm and a maximal emission wavelength at 520~530 nm. This product has been optimized for use as a secondary antibody in immunofluorescence and flow cytometry.

Suggested Dilution
- IF: 1:100-500 dilution.
- FCM: 1:100-500 dilution.



Detection of fusion protein expression in HEK-293 cell by IF

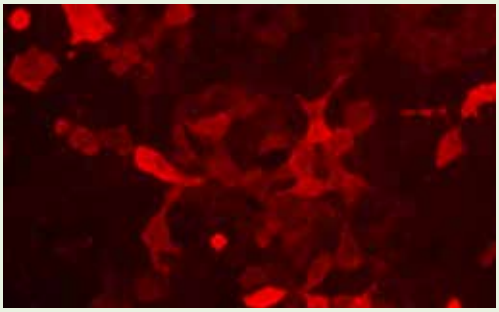
ProteinFind® Goat Anti-Mouse IgG(H+L), PE Conjugate

HS221-01	100 μl

Concentration
0.4 mg/ml
Storage
at 2-8°C in dark for one year

Description
Affinity purified *ProteinFind*® Goat Anti-Mouse IgG(H+L) Antibody is conjugated with phycoerythrin (PE) dye under optimal conditions. PE dye is a natural fluorescent dye extracted from red algae with a maximal absorption wavelength at 488 nm and a maximal emission wavelength at 575 nm. This product has been optimized for use as a secondary antibody in immunofluorescence and flow cytometry.

Suggested Dilution
- IF: 1:100-500 dilution.
- FCM: 1:100-500 dilution.



Detection of fusion protein expression in HEK-293 cell by IF

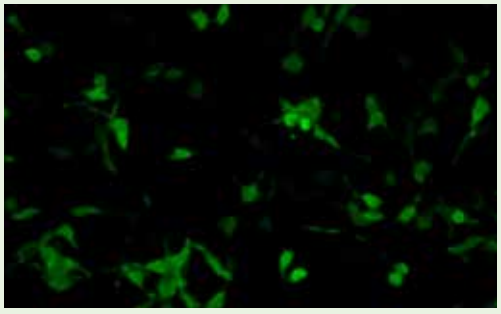
ProteinFind® Goat Anti-Mouse IgG(H+L), AF488 Conjugate

HS231-01	100 µl

Concentration
1 mg/ml
Storage
at 2-8°C in dark for one year

Description
Affinity purified *ProteinFind*® Goat Anti-Mouse IgG(H+L) Antibody is conjugated with Alexa Fluor 488 (AF488) dye under optimal conditions. AF488 dye is a bright, green-fluorescence dye with a maximal absorption wavelength at 495 nm and a maximal emission wavelength at 519 nm. This product has been optimized for use as a secondary antibody in immunofluorescence and flow cytometry.

Suggested Dilution
- IF: 1:100-500 dilution.
- FCM: 1:100-500 dilution.



Detection of fusion protein expression in HEK-293 cell by IF

TMB ELISA Substrate

HE101-01	100 ml

Storage
at 2-8°C in dark for one year

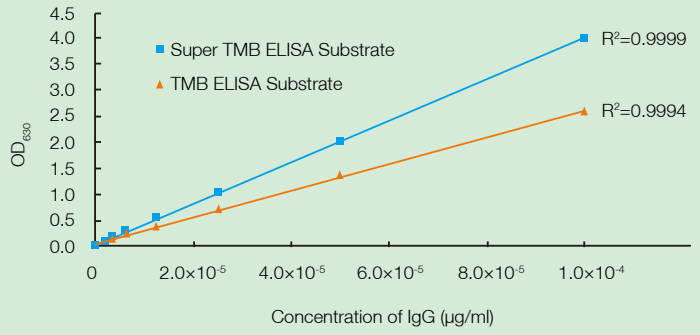
Description
TMB ELISA Substrate is a ready-to-use chromogenic substrate for detection of horseradish peroxidase (HRP) activity. HRP can catalyze 3,3',5,5'-tetramethylbenzidine (TMB) to yield a blue color, the maximal absorbance is at 370 nm or 620-652 nm; however, upon addition of the stop solution, the solution turns to yellow and can be measured at 450 nm.

Super TMB ELISA Substrate

HE111-01	100 ml

Storage
at 2-8°C in dark for one year

Description
Super TMB ELISA Substrate is a ready-to-use chromogenic substrate for detection of horseradish peroxidase (HRP) activity. HRP can catalyze 3,3',5,5'-tetramethylbenzidine (TMB) to yield a blue color, the maximal absorbance is at 370 nm or 620-652 nm. Upon addition of the stop solution, the solution turns to yellow and can be measured at 450 nm. This one-component method is 40-50% more sensitive than the traditional TMB ELISA method.



Comparison of sensitivity between Super TMB and TMB ELISA Substrates

Chapter 9 Other Products

T4 DNA Ligase

FL101-01	10,000 units
FL101-02	20,000 units

Concentration
200 units/μl

Contents
- T4 DNA Ligase
- 5×T4 DNA Ligase Buffer

Storage
at -20°C for one year

Description
T4 DNA Ligase catalyzes the formation of a phosphodiester bond between juxtaposed 5'-phosphate and 3'-hydroxyl termini in duplex DNA or RNA with blunt or cohesive end. The enzyme repairs single-strand nicks in duplex DNA, RNA or DNA/RNA hybrids but has no activity on single-strand nucleic acids. T4 DNA Ligase requires ATP as a cofactor.

Source
E.coli strain carrying T4 DNA ligase gene

Unit Definition
One unit is the amount of enzyme required to give 50% ligation of *Hind* III fragments of λDNA (5´ DNA termini concentration of 0.12 μM, 200 μg/ml) in a total reaction volume of 20 μl in 30 minutes at 16°C in 1×T4 DNA Ligase Buffer.

Quality Control
Functional absence of endonucleases and exonucleases activities

Applications
- Cloning blunt end or cohesive end fragments.
- Ligation of synthetic linkers or adaptors.

PROTOCOL

Note
It is recommended to use a molar ratio of insert to vector at 3:1-10:1.

Reaction Components

Component	Volume	Final concentration
Vector	Variable	as required
Insert	Variable	as required
5×T4 DNA Ligase Buffer	2 μl	1×
T4 DNA Ligase	0.5-1 μl	100-200 units
ddH_2O	Variable	-
Total volume	10 μl	-

Reaction Conditions
- Cohesive ends ligation: incubate at 25°C for 10 minutes.
- Blunt ends ligation: incubate at 25°C for 2 hours, or overnight at 16°C.
- Cohesive and blunt ends ligation: incubate at 25°C for 2 hours.

DMT Enzyme

GD111-01	200 units

Concentration
10 units/μl
Storage
at -20°C for two years

Description

DMT cuts the sequence GmATC (G is methylated) but does not cut the sequence GATC (G is not methylated). This enzyme cuts DNA prepared from most commonly used *E.coli* strains (dam^+ strain), but does not cut PCR products.

Source

An *E.coli* strain that carries the cloned DMT enzyme gene from *Diplococcus pneumoniae*.

Unit Definition

One unit is the amount of enzyme required to completely digest 1 μg of pBR322 DNA (prepared from dam^+ strain) in 50 μl of reaction mixture in 1 hour at 37°C.

Quality Control

Functional absence of endonucleases and exonucleases activities

Applications

In vitro site-directed mutagenesis, digestion of methylated DNA.

DNase I (RNase-free)

GD201-01	1,500 units

Concentration
3 units/μl
Contents
- DNase I
- 10×DNase I Reaction Buffer
- 200 mM EDTA

Storage
at -20°C for one year

Description

Deoxyribonuclease I (DNase I) is an endonuclease that degrades double- and single-strand DNA and chromatin. It functions by hydrolyzing phosphodiester linkages, producing mono and oligonucleotides with a 5'-phosphate and a 3'-hydroxyl group. Ribonuclease has been reduced to non-detectable levels. Its activity depends on Mg^{2+} or Mn^{2+} ion. DNase I with Mg^{2+} randomly cuts double strand DNA at any sites, DNase I with Mn^{2+} cuts double strand DNA at the same site to form sticky end with 1-2 nucleotide or form blunt end.

Source

Purified from bovine pancreas.

Unit Definition

One unit is the amount of enzyme required to completely degrade 1 μg pBR322 plasmid DNA in 10 minutes at 37°C.

Applications

- DNase I footprinting
- Nick translation
- Remove DNA from RNA preparation

RNase A

GE101-01 1 ml

Concentration
20 mg/ml

Storage
at -20°C for one year

Description
RNase A is a ribonuclease that cleaves single-strand RNA. It has no DNase activity.

Source
Bovine pancreas

Activity
>60 U/mg

Applications
- Remove RNA from DNA samples
- RNase protection assay

Proteinase K

GE201-01 1 ml

Concentration
20 mg/ml

Storage
at -20°C for one year

Description
Proteinase K is a nonspecific serine protease that will hydrolyze a variety of peptide bands. Proteinase K is active in a broad range of temperature and buffers. It cannot be inactivated by metal ions, chelating agents (e.g., EDTA), or detergents such as SDS.
- Active in a wide range of buffers and pH value.
- Incubation temperature: 55-65°C; optimal temperature: 58°C
- Incubation time: 15 minutes to 48 hours; optimal incubation time: 2 hours

Source
Purified from *Tritirachium* album

Applications
- Preparation of DNA and RNA.
- Inactivation of RNase, DNase and enzymes.
- Isolation of genomic DNA.
- Isolation of RNA.

IPTG

GF101-01 1 ml

Concentration
500 mM

Storage
at -20°C for six months

Description
Isopropylthio-β-galactoside (IPTG) is an effective inducer of β-galactosidase activity. It is commonly used with X-gal to detect *lac* gene activity in cloning based on blue/white selection. It is also used as an inducer of protein expression in *lac* or *tac* promoter-regulated expression vectors.

X-Gal

GF201-01	1 ml

Concentration
20 mg/ml

Storage
at -20°C in dark for six months

Description
X-gal is a substrate of β-galactosidase. It is commonly used with IPTG to detect *lac* gene activity in cloning based on blue/white selection.

Ampicillin

GG101-01	1 ml

Concentration
100 mg/ml

Storage
at -20°C for one year

Description
Extremely pure, molecular biology grade Ampicillin from TransGen can be used as a selective antibiotic for resistant bacteria.

Kanamycin

GG201-01	1 ml

Concentration
50 mg/ml

Storage
at -20°C for one year

Description
Extremely pure, molecular biology grade Kanamycin from TransGen can be used as a selective antibiotic for resistant bacteria.

Chloramphenicol

GG301-01	1 ml

Concentration
34 mg/ml

Storage
at -20°C for one year

Description
Extremely pure, molecular biology grade Chloramphenicol from TransGen can be used as a selective antibiotic for resistant bacteria.

6×DNA Loading Buffer

GH101-01	5×1 ml

Storage
at -20°C for two years

Description
6×DNA Loading Buffer is used as loading buffer in nucleic acid electrophoresis. Prior to loading, add appropriate volume of 6×DNA Loading Buffer to DNA sample to make its working concentration at 1×, and then load the DNA samples on the gel for electrophoresis.

2×RNA Loading Buffer

GH201-01	1 ml

Storage
at 4°C for one month, at -20°C for two years

Description
This product is used as loading buffer in RNA electrophoresis. It is suitable for denatured or native agarose gel and polyacrylamide gel electrophoresis. Prior to loading, add equal volume of the Loading Buffer to RNA sample (or RNA marker), heat at 70°C for 10 minutes, immediately chill on ice, and then load on the gel.

ddH_2O

GI101-01	25 ml

Storage
at room temperature for two years

Description
ddH_2O is purified by reverse osmosis method. It is suitable for most molecular and cell biology applications.

RNase-free Water

GI201-01	25 ml

Storage
at room temperature for two years

Description
RNase-free Water is prepared from deionized water incubated with 0.01% DEPC, then autoclaved to remove residual DEPC. It is suitable for RNA-related molecular biology applications.

T7 High Efficiency Transcription Kit

JT101-01 20 μl×25 rxns

Storage
at -20°C for one year

Description

T7 High Efficiency Transcription Kit is designed for *in vitro* RNA synthesis by T7 RNA Polymerase with supercoiled or linearized DNA templates. Up to 150 μg of RNA can be produced from a 20 μl reaction. Synthesized RNA can be used for *in vitro* translation, RNase protection assays, RNA splicing, and hybridization assays.

Kit Contents

Component	JT101-01
T7 Transcription Enzyme Mix	50 μl
5×T7 Transcription Reaction Buffer	100 μl
10 mM NTP Mix	200 μl
DNase I (1 unit/μl)	25 μl
500 mM EDTA (pH 8.0)	25 μl
RNase-free Water	500 μl
Control Transcription Template (0.5 μg/μl)	10 μl

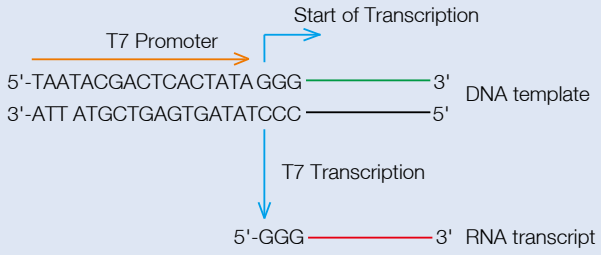
PROTOCOL

Notes

- RNase contamination should be avoided.
- Transcript produced from the control template is 2 kb.

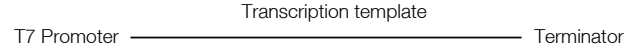
RNA Synthesis

Principle of *In Vitro* Transcription



Template Preparation

- Supercoiled plasmid DNA
  Supercoiled plasmid DNA should contain a T7 promoter and an effective terminator. Termination efficiency varies with terminators. The following sequence is recommended.



T7 Promoter: 5'-TAATACGACTCACTATAGGG#-3' #: G/A
Terminator: 5'-TTCCATCTGTTTTCTTATCTGTTCTTTCATCTGTTCTTTTATCTGTTTGTTT-3'

- Linearized DNA
  Linearized plasmid DNA or PCR product, with T7 promoter and terminal sequences, can be used as template for *in vitro* transcription. We suggest to use 5'-overhang or blunt end restriction enzymes to generate the linearized templates, and avoid to use 3'-overhang restriction enzymes to generate the template. Digested linearized DNA should be purified.

Transcription

- Reaction Components

Component	Volume
Template	1 μg
5×Transcription Reaction Buffer	4 μl
10 mM NTP Mix	8 μl
T7 Transcription Enzyme Mix	2 μl
RNase-free Water	to 20 μl

- Mix thoroughly and incubate at 37°C for 2 hours.
- Add 1 μl of DNase I, incubate at 37°C for 15 minutes. Then add 1 μl of 500 mM EDTA (pH 8.0) to terminate reaction (immediately proceed to the following purification step after termination).

Purification of Synthesized RNA

Please refer to *EasyPure*® RNA Purification Kit.

Quantification and Analysis of synthesized RNA

- RNA concentration can be determined by ultraviolet light spectrophotometer.
- Transcripts of 0.1-1 kb can be run on denatured gel (6% acrylamide, 7 M urea). Use 1×TBE Buffer as the running buffer. (10×TBE Buffer: 0.9 M Tris Base, 0.9 M Boric Acid, 20 mM EDTA.)
- Transcripts of 0.5-5 kb can also be run on 1% formaldehyde denatured gel. Use 1×MOPS Buffer as the running buffer. (10×MOPS Buffer: 0.4 M MOPS (pH 7.0), 0.1 M Sodium Acetate, 10 mM EDTA.)
- For electrophoresis analysis, dilute 0.2-1 μg RNA with RNase-free water to make the total volume to 5 μl, add 5 μl of 2×RNA Loading Buffer and mix thoroughly, incubate at 70°C for 10 minutes and followed by incubation on ice for 2 minutes, then load samples on the gel. After electrophoresis, stain the gel.

shRNA Synthesis Kit

JT111-01	20 μl×25 rxns

Storage
at -20°C for one year

Description

shRNA Synthesis Kit is designed for RNA synthesis by T7 RNA Polymerase with annealed dsDNA as template. Template contains Loop structure, thus synthesized RNA can be formed as shRNA. After RNA synthesis, DNA template can be digested by DNase I, followed by purification. Purified product can be directly used for cell transfection and other shRNA assays. This kit is suitable for production of RNA ≤ 100 nt. Up to 40 μg shRNA can be produced from a 20 μl reaction.

Kit Contents

Component	JT111-01
shRNA Enzyme Mix	50 μl
5×shRNA Synthesis Buffer	100 μl
10 mM NTP Mix	200 μl
Annealing Buffer	450 μl
DNase I (1 unit/μl)	25 μl
500 mM EDTA (pH 8.0)	25 μl
RNase-free Water	500 μl
Universal T7 Promoter Oligonucleotide (20 μM)	30 μl
Control Transcription Template Oligonucleotide (20 μM)	5 μl

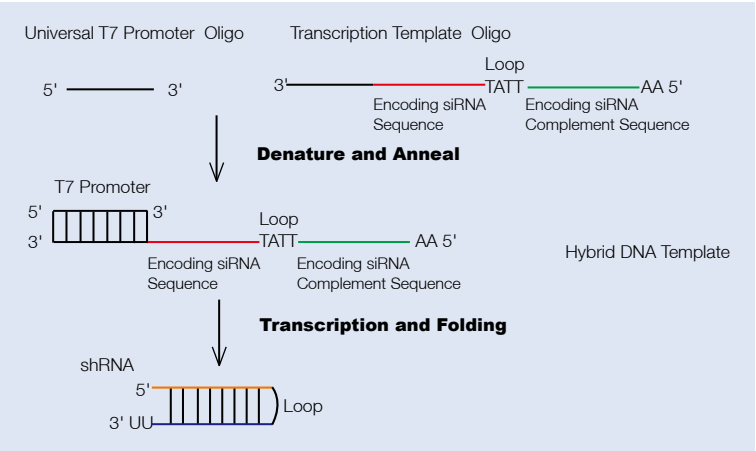
PROTOCOL

Note

RNase contamination should be avoided.

shRNA Synthesis

Principle



Oligonucleotide Template Design

Two Oligos are used for shRNA synthesis: Universal T7 Promoter Oligonucleotide (supplied with the kit) and Transcription Template Oligonucleotide(not supplied with the kit). Transcription Template Oligonucleotide contains sequences complementary to Universal T7 Promoter Oligonucleotide and sequences for shRNA.

Universal T7 Promoter Oligonucleotide (supplied with this kit)

5' -GAGTCCTGCAATTAATACGACTCACTATAG- 3'

Transcription Template Oligonucleotide (provided by users)

3' -CTCAGGACGTTAATTATGCTGAGTGATATCTTCTTCAGCACGACGAAGTATTCTTCGTCGTGCTGAAGAAGAA- 5'

Universal T7 Promoter Complement Sequence | Encoding siRNA Sequence | Loop | Encoding siRNA Complement Sequence

Preparation of Hybrid DNA Template

Dissolve Transcription Template Oligonucleotide with RNase-free Water or TE to a final concentration at 20 μM.

- Reaction Components

Component	Volume
Universal T7 Promoter Oligonucleotide (20 μM)	1 μl
Transcription Template Oligonucleotide (20 μM)	1 μl
Annealing Buffer	18 μl
Total Volume	20 μl

Incubate at 95°C for 5 minutes, then slowly cool down to room temperature. (the prepared Hybrid DNA Template can be stored at 4°C for one week)

shRNA Synthesis

- Reaction Components

Component	Volume
Hybrid DNA Template	2 μl
5×shRNA Synthesis Buffer	4 μl
10 mM NTP mix	8 μl
shRNA Enzyme Mix	2 μl
RNase-free Water	to 20 μl

- Mix thoroughly and incubate at 37°C for 2 hours for shRNA synthesis.
- After shRNA synthesis reaction, add 1 μl of DNase I to the reaction mixture and incubate at 37°C for 15 minutes to digest the templates. Stop the reaction by adding 1 μl of 500 mM EDTA (pH8.0).

Purification of shRNA

Please refer to *EasyPure*® RNA Purification Kit.

Quantification and Analysis of shRNA

- shRNA concentration can be determined by ultraviolet light spectrophotometer.
- shRNA can be analyzed with denatured gel (12% acrylamide, 7 M urea.)

Services

TransGen provides the following services with fast turnaround time and very competitive price. Please contact Customer Service Department for further information.

- PCR and qPCR
- RT-PCR and qRT-PCR
- Cloning
- Vector construction
- Plasmid DNA Mini, Midi, and Maxi Prep
- Genomic DNA Mini, Midi, and Maxi Prep
- Total RNA or/and mRNA Isolation
- Mutagenesis
- Protein expression and purification
- Establishment of stable cell line
- Lentivirus package
- Cell transfection
- Luciferase assay
- Other molecular and cell biology related services

TransGen will keep all information confidential. All data and materials are the property of the purchasers. TransGen will not use, disclose, or publish the materials without the written consent of the purchasers.

PCR, RT-PCR, qPCR and qRT-PCR

Products Name	Catalog Number	Quantity	Page
TransFast® *Taq* DNA Polymerase	AP101-01	500 units	6
	AP101-02	6×500 units	
TransFast® *Taq* DNA Polymerase (with 2.5 mM dNTPs)	AP101-11	500 units	
	AP101-12	6×500 units	
EasyTaq® DNA Polymerase	AP111-01	500 units	7
	AP111-02	6×500 units	
	AP111-03	4×2,500 units	
	AP111-04	10×5,000 units	
EasyTaq® DNA Polymerase (with 2.5 mM dNTPs)	AP111-11	500 units	
	AP111-12	6×500 units	
	AP111-13	4×2,500 units	
EasyTaq® DNA Polymerase for PAGE	AP112-01	2,500 units	9
	AP112-02	4×2,500 units	
EasyTaq® DNA Polymerase for PAGE (with 2.5 mM dNTPs)	AP112-11	2,500 units	
	AP112-12	4×2,500 units	
TransTaq®-T DNA Polymerase	AP122-01	250 units	10
	AP122-02	500 units	
	AP122-03	6×500 units	
TransTaq®-T DNA Polymerase (with 2.5 mM dNTPs)	AP122-11	250 units	
	AP122-12	500 units	
	AP122-13	6×500 units	
TransTaq® DNA Polymerase High Fidelity (HiFi)	AP131-01	250 units	11
	AP131-02	500 units	
	AP131-03	6×500 units	
TransTaq® DNA Polymerase High Fidelity (HiFi) (with 2.5 mM dNTPs)	AP131-11	250 units	
	AP131-12	500 units	
	AP131-13	6×500 units	
TransStart® *Taq* DNA Polymerase	AP141-01	250 units	14
	AP141-02	500 units	
	AP141-03	6×500 units	
TransStart® *Taq* DNA Polymerase (with 2.5 mM dNTPs)	AP141-11	250 units	
	AP141-12	500 units	
	AP141-13	6×500 units	
TransStart® *TopTaq* DNA Polymerase	AP151-01	250 units	16
	AP151-02	500 units	
	AP151-03	6×500 units	
TransStart® *TopTaq* DNA Polymerase (with 2.5 mM dNTPs)	AP151-11	250 units	
	AP151-12	500 units	
	AP151-13	6×500 units	
EasyPfu DNA Polymerase	AP211-01	250 units	18
	AP211-02	500 units	
	AP211-03	6×500 units	

Products Name	Catalog Number	Quantity	Page
EasyPfu DNA Polymerase (with 2.5 mM dNTPs)	AP211-11	250 units	18
	AP211-12	500 units	
	AP211-13	6×500 units	
TransStart® FastPfu DNA Polymerase	AP221-01	250 units	19
	AP221-02	500 units	
	AP221-03	6×500 units	
TransStart® FastPfu DNA Polymerase (with 2.5 mM dNTPs)	AP221-11	250 units	
	AP221-12	500 units	
	AP221-13	6×500 units	
TransStart® FastPfu Fly DNA Polymerase	AP231-01	250 units	21
	AP231-02	500 units	
	AP231-03	6×500 units	
TransStart® FastPfu Fly DNA Polymerase (with 2.5 mM dNTPs)	AP231-11	250 units	
	AP231-12	500 units	
	AP231-13	6×500 units	
TransStart® KD Plus DNA Polymerase	AP301-01	100 units	23
	AP301-02	200 units	
	AP301-03	6×200 units	
TransStart® KD Plus DNA Polymerase (with 2.5 mM dNTPs)	AP301-11	100 units	
	AP301-12	200 units	
	AP301-13	6×200 units	
GC Enhancer	AG101-01	200 μl	25
PCR Stimulant	AG111-01	200 μl	
2×*EasyTaq®* PCR SuperMix (-dye)	AS111-01	1 ml	27
	AS111-02	5×1 ml	
	AS111-03	15×1 ml	
2×*EasyTaq®* PCR SuperMix (+dye)	AS111-11	1 ml	
	AS111-12	5×1 ml	
	AS111-13	15×1 ml	
	AS111-14	6×80 ml	
2×*EasyTaq®* PCR SuperMix for PAGE (+dye)	AS112-11	1 ml	28
	AS112-12	5×1 ml	
	AS112-13	15×1 ml	
2×*TransTaq®*-T PCR SuperMix (-dye)	AS122-01	1 ml	29
	AS122-02	5×1 ml	
2×*TransTaq®*-T PCR SuperMix (+dye)	AS122-11	1 ml	
	AS122-12	5×1 ml	
2×*TransTaq®* High Fidelity (HiFi) PCR SuperMix I (-dye)	AS131-01	1 ml	30
	AS131-02	5×1 ml	
2×*TransTaq®* High Fidelity (HiFi) PCR SuperMix II (-dye)	AS131-21	1 ml	
	AS131-22	5×1 ml	
2×*EasyPfu* PCR SuperMix (-dye)	AS211-01	1 ml	32
	AS211-02	5×1 ml	

Products Name	Catalog Number	Quantity	Page
2×*TransStart*® *FastPfu* PCR SuperMix (-dye)	AS221-01	1 ml	33
	AS221-02	5×1 ml	
TransDirect® Animal Tissue PCR Kit	AD201-01	100 rxns (20 μl per reaction)	34
	AD201-02	500 rxns (20 μl per reaction)	
TransDirect® Plant Tissue PCR Kit	AD301-01	100 rxns (20 μl per reaction)	36
	AD301-02	500 rxns (20 μl per reaction)	
TransDirect® Blood PCR Kit	AD401-01	100 rxns (20 μl per reaction)	37
	AD401-02	500 rxns (20 μl per reaction)	
EasyScript® Reverse Transcriptase	AE101-02	10,000 units	41
	AE101-03	5×10,000 units	
TransScript® Reverse Transcriptase	AT101-02	10,000 units	43
	AT101-03	5×10,000 units	
TransScript® II Reverse Transcriptase	AH101-02	10,000 units	44
EasyScript® First-Strand cDNA Synthesis SuperMix	AE301-02	50 rxns (20 μl per reaction)	45
	AE301-03	100 rxns (20 μl per reaction)	
EasyScript® One-Step gDNA Removal and cDNA Synthesis SuperMix	AE311-02	50 rxns (20 μl per reaction)	47
	AE311-03	100 rxns (20 μl per reaction)	
TransScript® First-Strand cDNA Synthesis SuperMix	AT301-02	50 rxns (20 μl per reaction)	48
	AT301-03	100 rxns (20 μl per reaction)	
TransScript® One-Step gDNA Removal and cDNA Synthesis SuperMix	AT311-02	50 rxns (20 μl per reaction)	49
	AT311-03	100 rxns (20 μl per reaction)	
TransScript® Fly First-Strand cDNA Synthesis SuperMix	AF301-02	50 rxns (20 μl per reaction)	50
	AF301-03	100 rxns (20 μl per reaction)	
TransScript®-Uni One-Step gDNA Removal and cDNA Synthesis SuperMix	AU311-02	50 rxns (20 μl per reaction)	51
	AU311-03	100 rxns (20 μl per reaction)	
TransScript®-Uni Cell to cDNA Synthesis SuperMix for qPCR	AC301-01	25 rxns	53
TransScript® miRNA First-Strand cDNA Synthesis SuperMix	AT351-01	20 rxns (20 μl per reaction)	55
TransScript® II First-Strand cDNA Synthesis SuperMix	AH301-02	50 rxns (20 μl per reaction)	57
	AH301-03	100 rxns (20 μl per reaction)	
TransScript® II One-Step gDNA Removal and cDNA Synthesis SuperMix	AH311-02	50 rxns (20 μl per reaction)	58
	AH311-03	100 rxns (20 μl per reaction)	
TransScript® All-in-One First-Strand cDNA Synthesis SuperMix for PCR	AT321-01	50 rxns (20 μl per reaction)	59
TransScript® All-in-One First-Strand cDNA Synthesis SuperMix for qPCR (One-Step gDNA Removal)	AT341-01	50 rxns (20 μl per reaction)	60
	AT341-02	100 rxns (20 μl per reaction)	
TransScript® II All-in-One First-Strand cDNA Synthesis SuperMix for PCR	AH321-01	50 rxns (20 μl per reaction)	62
TransScript® II All-in-One First-Strand cDNA Synthesis SuperMix for qPCR (One-Step gDNA Removal)	AH341-01	50 rxns (20 μl per reaction)	63
TransScript® Two-Step RT-PCR SuperMix	AT401-01	50 rxns (20 μl per RT reaction) 80 rxns (50 μl per PCR)	64
TransScript® II Two-Step RT-PCR SuperMix	AH401-01	50 rxns (20 μl per RT reaction) 80 rxns (50 μl per PCR)	65
EasyScript® One-Step RT-PCR SuperMix (+dye)	AE411-02	200 rxns (20 μl per reaction)	66
TransScript® One-Step RT-PCR SuperMix (+dye)	AT411-02	200 rxns (20 μl per reaction)	67
TransScript® II One-Step RT-PCR SuperMix (+dye)	AH411-02	200 rxns (20 μl per reaction)	68
Ribonuclease Inhibitor	AI101-01	2,000 units	70
	AI101-02	5×2,000 units	

Products Name	Catalog Number	Quantity	Page
TransStart® Green qPCR SuperMix	AQ101-01	1 ml	71
	AQ101-02	5×1 ml	
	AQ101-03	15×1 ml	
TransStart® Green qPCR SuperMix UDG	AQ111-01	1 ml	73
	AQ111-02	5×1 ml	
	AQ111-03	15×1 ml	
TransStart® Top Green qPCR SuperMix	AQ131-01	1 ml	74
	AQ131-02	5×1 ml	
	AQ131-03	15×1 ml	
	AQ131-04	25×1 ml	
TransStart® Tip Green qPCR SuperMix	AQ141-01	1 ml	75
	AQ141-02	5×1 ml	
	AQ141-03	15×1 ml	
	AQ141-04	25×1 ml	
TransScript® Green Two-Step qRT-PCR SuperMix	AQ201-01	50 rxns (20 μl per RT reaction) 300 rxns (20 μl per qPCR)	76
TransScript® Green miRNA Two-Step qRT-PCR SuperMix	AQ202-01	20 rxns (20 μl per RT reaction) 500 rxns (20 μl per qPCR)	78
TransScript® II Green Two-Step qRT-PCR SuperMix	AQ301-01	50 rxns (20 μl per RT reaction) 300 rxns (20 μl per qPCR)	79
TransScript® Green One-Step qRT-PCR SuperMix	AQ211-01	100 rxns (20 μl per reaction)	80
	AQ211-02	400 rxns (20 μl per reaction)	
TransScript® II Green One-Step qRT-PCR SuperMix	AQ311-01	100 rxns (20 μl per reaction)	82
	AQ311-02	400 rxns (20 μl per reaction)	
TransStart® Probe qPCR SuperMix	AQ401-01	1 ml	84
	AQ401-02	5×1 ml	
	AQ401-03	15×1 ml	
TransScript® Probe One-Step qRT-PCR SuperMix	AQ221-01	100 rxns (20 μl per reaction)	85
	AQ221-02	400 rxns (20 μl per reaction)	
TransScript® II Probe One-Step qRT-PCR SuperMix	AQ321-01	100 rxns (20 μl per reaction)	87
	AQ321-02	400 rxns (20 μl per reaction)	
High Pure dNTPs (2.5 mM)	AD101-01	1 ml	89
	AD101-02	5×1 ml	
High Pure dNTPs (10 mM)	AD101-11	1 ml	
	AD101-12	5×1 ml	

DNA Molecular Weight Standards

Products Name	Catalog Number	Quantity	Page
*Trans*2K® DNA Marker	BM101-01	500 μl	92
	BM101-02	5×500 μl	
*Trans*2K® Plus DNA Marker	BM111-01	500 μl	
	BM111-02	5×500 μl	
*Trans*2K® Plus II DNA Marker	BM121-01	500 μl	
	BM121-02	5×500 μl	

Products Name	Catalog Number	Quantity	Page
*Trans*5K DNA Marker	BM141-01	500 μl	93
	BM141-02	5×500 μl	
*Trans*15K DNA Marker	BM161-01	500 μl	
	BM161-02	5×500 μl	
1Kb DNA Ladder	BM201-01	500 μl	
	BM201-02	5×500 μl	
1Kb Plus DNA Ladder	BM211-01	500 μl	94
	BM211-02	5×500 μl	
100bp DNA Ladder	BM301-01	500 μl	
	BM301-02	5×500 μl	
100bp Plus DNA Ladder	BM311-01	500 μl	
	BM311-02	5×500 μl	
100bp Plus II DNA Ladder	BM321-01	500 μl	95
	BM321-02	5×500 μl	
GelStain	GS101-01	500 μl	
	GS101-02	1 ml	
Agarose	GS201-01	100 g	

Cloning and Mutagenesis System

Products Name	Catalog Number	Quantity	Page
pEASY®-T1 Cloning Kit	CT101-01	20 rxns	99
	CT101-02	60 rxns	
pEASY®-Blunt Cloning Kit	CB101-01	20 rxns	102
	CB101-02	60 rxns	
pEASY®-T1 Simple Cloning Kit	CT111-01	20 rxns	103
	CT111-02	60 rxns	
pEASY®-Blunt Simple Cloning Kit	CB111-01	20 rxns	104
	CB111-02	60 rxns	
pEASY®-T3 Cloning Kit	CT301-01	20 rxns	105
	CT301-02	60 rxns	
pEASY®-Blunt3 Cloning Kit	CB301-01	20 rxns	106
	CB301-02	60 rxns	
pEASY®-T5 Zero Cloning Kit	CT501-01	20 rxns	107
	CT501-02	60 rxns	
pEASY®-Blunt Zero Cloning Kit	CB501-01	20 rxns	108
	CB501-02	60 rxns	
pEASY®-Uni Seamless Cloning and Assembly Kit	CU101-01	10 rxns	109
*Trans*10 Chemically Competent Cell	CD101-01	10×100 μl	112
	CD101-02	20×100 μl	
*Trans*5α Chemically Competent Cell	CD201-01	10×100 μl	
	CD201-02	20×100 μl	
*Trans*109 Chemically Competent Cell	CD301-02	10×100 μl	113
	CD301-03	20×100 μl	
*Trans*110 Chemically Competent Cell	CD311-02	10×100 μl	

Products Name	Catalog Number	Quantity	Page
*Trans*1-Blue Chemically Competent Cell	CD401-02	10×100 µl	113
	CD401-03	20×100 µl	
*Trans*2-Blue Chemically Competent Cell	CD411-02	10×100 µl	114
	CD411-03	20×100 µl	
*Trans*1-T1 Phage Resistant Chemically Competent Cell	CD501-01	5×100 µl	
	CD501-02	10×100 µl	
	CD501-03	20×100 µl	
DMT Chemically Competent Cell	CD511-01	10×50 µl	
	CD511-02	20×50 µl	
*Trans*Stbl3 Chemically Competent Cell	CD521-01	10×100 µl	115
*Trans*DB3.1 Chemically Competent Cell	CD531-01	10×100 µl	
Fast Mutagenesis System	FM111-01	10 rxns	116
	FM111-02	20 rxns	
Fast MultiSite Mutagenesis System	FM201-01	10 rxns	117

Nucleic Acid Purification

Products Name	Catalog Number	Quantity	Page
BloodZol	EE131-01	for 50 ml blood	121
	EE131-02	for 200 ml blood	
PlantZol	EE141-01	100 ml	122
EasyPure® Genomic DNA Kit (with RNase A)	EE101-01	50 rxns	123
	EE101-02	200 rxns	
EasyPure® Genomic DNA Kit	EE101-11	50 rxns	
	EE101-12	200 rxns	
EasyPure® Plant Genomic DNA Kit (with RNase A)	EE111-01	50 rxns	125
	EE111-02	200 rxns	
EasyPure® Plant Genomic DNA Kit	EE111-11	50 rxns	
	EE111-12	200 rxns	
EasyPure® Blood Genomic DNA Kit (with RNase A)	EE121-01	50 rxns	126
	EE121-02	200 rxns	
EasyPure® Blood Genomic DNA Kit	EE121-11	50 rxns	
	EE121-12	200 rxns	
EasyPure® Marine Animal Genomic DNA Kit (with RNase A)	EE151-01	50 rxns	127
EasyPure® Marine Animal Genomic DNA Kit	EE151-11	50 rxns	
EasyPure® Bacteria Genomic DNA Kit (with RNase A)	EE161-01	50 rxns	128
EasyPure® Bacteria Genomic DNA Kit	EE161-11	50 rxns	
EasyPure® Food and Fodder Security Genomic DNA Kit	EE171-01	50 rxns	129
EasyPure® Micro Genomic DNA Kit	EE181-01	50 rxns	131
EasyPure® Plasmid MiniPrep Kit	EM101-01	50 rxns	132
	EM101-02	200 rxns	
EasyPure® HiPure Plasmid MiniPrep Kit	EM111-01	50 rxns	133
EasyPure® HiPure Plasmid MaxiPrep Kit	EM121-01	10 rxns	134
ArtMedia® Plasmid Culture	EM201-01	95 ml+5 ml	135
EasyPure® PCR Purification Kit	EP101-01	50 rxns	136
	EP101-02	200 rxns	

Products Name	Catalog Number	Quantity	Page
EasyPure® Quick Gel Extraction Kit	EG101-01	50 rxns	137
	EG101-02	200 rxns	
TransZol	ET101-01	100 ml	138
TransZol Up	ET111-01	100 ml	139
TransZol Plant	ET121-01	100 ml	140
EasyPure® RNA Kit	ER101-01	50 rxns	141
EasyPure® Viral DNA/RNA Kit	ER201-01	50 rxns	142
EasyPure® Plant RNA Kit	ER301-01	50 rxns	143
EasyPure® Blood RNA Kit	ER401-01	50 rxns	144
TransZol Up Plus RNA Kit	ER501-01	100 rxns	145
EasyPure® miRNA Kit	ER601-01	50 rxns	146
EasyPure® RNA Purification Kit	ER701-01	25 rxns	147
RNAhold®	EH101-01	100 ml	148

Gene Expression

Products Name	Catalog Number	Quantity	Page
pEASY®-Blunt E1 Expression Kit	CE111-01	10 rxns	151
pEASY®-Blunt E2 Expression Kit	CE211-01	10 rxns	154
ArtMedia® Protein Expression	CP101-01	95 ml+5 ml	155
BL21(DE3) Chemically Competent Cell	CD601-02	10×100 μl	156
	CD601-03	20×100 μl	
BL21(DE3)pLysS Chemically Competent Cell	CD701-02	10×100 μl	
	CD701-03	20×100 μl	
*Trans*etta(DE3) Chemically Competent Cell	CD801-02	10×100 μl	157
	CD801-03	20×100 μl	
*Trans*B(DE3) Chemically Competent Cell	CD811-02	10×100 μl	
BL21 Chemically Competent Cell	CD901-02	10×100 μl	
	CD901-03	20×100 μl	
pEASY®-Blunt M2 Expression Kit	CM211-01	10 rxns	158
pEASY®-Blunt M3 Expression Kit	CM311-01	10 rxns	161

Protein Extraction, Purification and Detection

Products Name	Catalog Number	Quantity	Page
ProteinExt™ Mammalian Total Protein Extraction Kit	DE101-01	100 ml	164
ProteinExt™ Mammalian Nuclear and Cytoplasmic Protein Extraction Kit	DE201-01	50 rxns	165
ProteinExt™ Mammalian Membrane Protein Extraction Kit	DE301-01	50 rxns	166
ProteinExt™ Mammalian Mitochondria Isolation Kit for Cultured Cells	DE401-01	50 rxns	167
ProteinExt™ Mammalian Mitochondria Isolation Kit for Tissue	DE501-01	50 rxns	168
ProteinIso® Ni-NTA Resin	DP101-01	5 ml	169
	DP101-02	25 ml	
ProteinIso® Ni-IDA Resin	DP111-01	5 ml	171
	DP111-02	25 ml	

Products Name	Catalog Number	Quantity	Page
ProteinIso® GST Resin	DP201-01	10 ml	173
ProteinIso® Protein A Resin	DP301-01	5 ml	175
ProteinIso® Protein G Resin	DP401-01	5 ml	177
ProteinRuler® I (12-80 kDa)	DR101-01	250 μl	180
	DR101-02	500 μl	
ProteinRuler® II (12-120 kDa)	DR201-01	250 μl	
	DR201-02	500 μl	
ProteinRuler® IV (30-200 kDa)	DR401-01	250 μl	181
	DR401-02	500 μl	
Blue Plus® Protein Marker (14-100 kDa)	DM101-01	250 μl	182
	DM101-02	500 μl	
Blue Plus® II Protein Marker (14-120 kDa)	DM111-01	250 μl	
	DM111-02	500 μl	
Blue Plus® III Protein Marker (14-160 kDa)	DM121-01	250 μl	183
	DM121-02	500 μl	
Blue Plus® IV Protein Marker (10-180 kDa)	DM131-01	250 μl	
	DM131-02	500 μl	
EasySee® Western Marker (25-90 kDa)	DM201-01	250 μl	184
	DM201-02	500 μl	
EasySee® Western Marker (with *EasySee*® Western Blot Kit)	DM201-11	250 μl+100 ml	
	DM201-12	500 μl+200 ml	
EasySee® II Western Marker (30-150 kDa)	DM211-01	250 μl	185
	DM211-02	500 μl	
EasySee® II Western Marker (with *EasySee*® Western Blot Kit)	DM211-11	250 μl+100 ml	
	DM211-12	500 μl+200 ml	
EasySee® Western Blot Kit	DW101-01	100 ml	186
	DW101-02	200 ml	
6× Protein Loading Buffer	DL101-02	5×1 ml	
Easy Protein Quantitative Kit (Bradford)	DQ101-01	100 ml	187
Easy II Protein Quantitative Kit (BCA)	DQ111-01	100 ml	188
ProteinEle™ Precast Tris-Glycine Gel	DG101-01	8%, 10/Box	190
	DG101-02	10%,10/Box	
	DG101-03	12%,10/Box	

Cell Culture and Detection

Products Name	Catalog Number	Quantity	Page
TransSerum® HQ Fetal Bovine Serum	FS101-02	500 ml	192
TransLipid® HL Transfection Reagent	FT111-01	0.75 ml	193
	FT111-02	2×0.75 ml	
TransIn™ EL Transfection Reagent	FT201-01	0.75 ml	195
	FT201-02	2×0.75 ml	
Penicillin-Streptomycin (100×)	FG101-01	100 ml	197
L-Glutamine (100×)	FG201-01	100 ml	
Trypsin (+EDTA)	FG301-01	100 ml	
Trypsin (-EDTA)	FG301-11	100 ml	
G418	FG401-01	5 ml	

Products Name	Catalog Number	Quantity	Page
PBS (1×)	FG701-01	500 ml	198
TransDetect® Double-Luciferase Reporter Assay Kit	FR201-01	50 rxns	
	FR201-02	200 rxns	
TransDetect® Cell Counting Kit (CCK)	FC101-01	1 ml	199
	FC101-02	5 ml	
	FC101-03	10 ml	
	FC101-04	30 ml	
TransDetect® Annexin V-FITC/PI Cell Apoptosis Detection Kit	FA101-01	25 rxns	201
	FA101-02	50 rxns	
TransDetect® Annexin V-EGFP/PI Cell Apoptosis Detection Kit	FA111-01	25 rxns	202
	FA111-02	50 rxns	
TransDetect® *In Situ* Fluorescein TUNEL Cell Apoptosis Detection Kit	FA201-01	25 rxns	203
	FA201-02	50 rxns	

Antibodies

Products Name	Catalog Number	Quantity	Page
ProteinFind® Anti-c-Myc Mouse Monoclonal Antibody	HT101-01	50 μl	205
	HT101-02	100 μl	
ProteinFind® Anti-DYKDDDDK Tag Mouse Monoclonal Antibody	HT201-01	50 μl	
	HT201-02	100 μl	
ProteinFind® Anti-HA Mouse Monoclonal Antibody	HT301-01	50 μl	206
	HT301-02	100 μl	
ProteinFind® Anti-V5 Mouse Monoclonal Antibody	HT401-01	50 μl	207
	HT401-02	100 μl	
ProteinFind® Anti-His Mouse Monoclonal Antibody	HT501-01	50 μl	
	HT501-02	100 μl	
ProteinFind® Anti-GST Mouse Monoclonal Antibody	HT601-01	50 μl	208
	HT601-02	100 μl	
ProteinFind® Anti-MBP Mouse Monoclonal Antibody	HT701-01	50 μl	
	HT701-02	100 μl	
ProteinFind® Anti-GFP Mouse Monoclonal Antibody	HT801-01	50 μl	209
	HT801-02	100 μl	
ProteinFind® Anti-β-Tubulin Mouse Monoclonal Antibody	HC101-01	50 μl	
	HC101-02	100 μl	
ProteinFind® Anti-β-Actin Mouse Monoclonal Antibody	HC201-01	50 μl	210
	HC201-02	100 μl	
ProteinFind® Anti-GAPDH Mouse Monoclonal Antibody	HC301-01	50 μl	
	HC301-02	100 μl	
ProteinFind® Goat Anti-Rabbit IgG(H+L), HRP Conjugate	HS101-01	100 μl	211
ProteinFind® Goat Anti-Rabbit IgG(H+L), FITC Conjugate	HS111-01	100 μl	
ProteinFind® Goat Anti-Rabbit IgG(H+L), PE Conjugate	HS121-01	100 μl	212
ProteinFind® Goat Anti-Rabbit IgG(H+L), AF488 Conjugate	HS131-01	100 μl	
ProteinFind® Goat Anti-Mouse IgG(H+L), HRP Conjugate	HS201-01	100 μl	213
ProteinFind® Goat Anti-Mouse IgG(H+L), FITC Conjugate	HS211-01	100 μl	214
ProteinFind® Goat Anti-Mouse IgG(H+L), PE Conjugate	HS221-01	100 μl	
ProteinFind® Goat Anti-Mouse IgG(H+L), AF488 Conjugate	HS231-01	100 μl	215
TMB ELISA Substrate	HE101-01	100 ml	216
Super TMB ELISA Substrate	HE111-01	100 ml	

Other Products

Products Name	Catalog Number	Quantity	Page
T4 DNA Ligase	FL101-01	10,000 units	218
	FL101-02	20,000 units	
DMT Enzyme	GD111-01	200 units	219
DNase I (RNase-free)	GD201-01	1,500 units	
RNase A	GE101-01	1 ml	220
Proteinase K	GE201-01	1 ml	
IPTG	GF101-01	1 ml	
X-gal	GF201-01	1 ml	221
Ampicillin	GG101-01	1 ml	
Kanamycin	GG201-01	1 ml	
Chloramphenicol	GG301-01	1 ml	
6×DNA Loading Buffer	GH101-01	5×1 ml	222
2×RNA Loading Buffer	GH201-01	1 ml	
ddH_2O	GI101-01	25 ml	
RNase-free Water	GI201-01	25 ml	
T7 High Efficiency Transcription Kit	JT101-01	20 μl×25 rxns	223
shRNA Synthesis Kit	JT111-01	20 μl×25 rxns	225

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