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# Taq DNA Polymerases

**Amplibiotherm DNA Polymerase** 

**GOTAQ-Flexi DNA Polymerase Green G2** 

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**GOTAQ-Hot Start Green Master Mix G2** 

Hot Start Proof PCR 2X Master Mix (GC-Rich Buffer)

**PFU DNA Polymerase** 

**GC-Platinum Power TAQ 2X Master Mix** 

#### **DESCRIPTION:**

Amplibiotherm DNA Polymerase is a thermostable 94 kDa DNA Polymerase purified from E.coli PVG-AI recombinant strain expressing *Thermus aquatiqus* polymerase gene. The enzyme catalyzes polymerisation of nucleotides into duplex DNA in the 5'-3' direction in presence of Mg++ ions. The enzyme possesses also a 5'-3' exonuclease activity. Amplification of target DNA fragments <100 b.p. up to 10.000 b.p. can be achieved with this enzyme.

#### **CONCENTRATION:**

5 units/ul

Description	FS-T-002
Amplibiotherm Taq DNA Polymerase	250 U
10X Reaction Buffer	1 vial
25mM MgCl2 separately	1 vial

#### **UNIT DEFINITION:**

**One unit** is defined as the amount of enzyme that incorporates 10 nmoles of dNTPs into acid-insoluble form in 30 minutes at 72°C.

#### STORAGE AND DILUTION BUFFER:

20 mM Tris-HCl , 1 mM DTT; 0.1 mM EDTA; 100 mM NaCl , Stabilizer ; 50% glycerol pH: 7.5(25°C) buffer is optimized to use with 0.2mM for each dNTPs

#### STORAGE TEMPERATURE:

Store Amplibiotherm DNA Polymerase below 0°C, preferably at -20° C, in a constant temperature freezer.

#### EXPIRY DATE:

1 year upon receipt.

#### **10X REACTION BUFFER:**

100mM Tris-HCl, 500mM KCl, pH 9.0 (25°C).

REACTION BUFFERS	
<b>10X Reaction Buffer</b> (contains 15mM MgCl <sub>2</sub> ; included)	Cat. No. <b>FS-B-006</b>
<b>10X Reaction Buffer</b> (without MgCl <sub>2</sub> ; plus 25 mM MgCl <sub>2</sub> separately)	Cat. No. <b>FS-B-007</b>

#### Protocol for routine *Taq* PCR reaction

On ice, prepare each of following master mixes, combine, and place in heated (to 95°C) thermal cycler:

For 50 µl PCR Reaction	Volume	Final Conc.
Amplibiotherm DNA Polymerase (5U/ul)	0.25 ul	1.25 U
10X PCR Buffer	5 ul	1 X
dNTP mix (2.5 mM each)	4 ul	200 uM each
Template	< 500 ng	< 500 ng
Forward Primer	5 ~ 50 pmol	0.1~1 uM
Reverse Primer	5 ~ 50 pmol	0.1~1 uM
Distilled water	up to 50 ul	

Gently mix the reaction and spin down in microcentrifuge.

If the termocycler does not have a heated cover, add one drop of mineral oil to the reaction tube to prevent evaporation.

#### Cycling conditions for a routine PCR reaction:

Step	Temp (°C)	Time	Cycle
Initial Denaturation	95	5 min.	1
Denature	95	10 ~ 30 sec.	
Anneal	50~65	10 ~ 30 sec.	25 ~40
Extend	72	10 ~ 60 sec.	
Final Extension	72	5 min.	1

Cycling conditions may need to be optimized, depending on different primer and template combinations. For example, raise the annealing temperature to prevent non-specific primer binding, increase extension time to generate longer PCR products.

# GOTAQ FLEXI DNA POLYMERASE GREEN G2

#### **Description**

GOTAQ Flexi DNA Polymerase Green G2 is a thermally stable, processive, 5'- 3' DNA polymerase. The 94 kDa protein possesses an inherent 5'-3' nick-translation moiety and lacks a 3'- 5' proofreading function.

GOTAQ Flexi DNA Polymerase Green G2 is provided with 5X Green Buffer, 5X Colorless Buffer and MgCl2 Solution. The 5X Green Buffer contains PCR enhancers and loading dye for direct electrophoresis. And the 5X Colorless Buffer contains PCR enhancers and without loading dye. And the 5X Colorless Buffer is used when direct fluorescence or absorbance readings are required without prior purification of the the Amplified DNA from the PCR. The reaction buffers do not contain MgCl2.

\*Equivalent to GoTaq G2 Flexi DNA Polymerase

Description	FS-T-0531
GOTAQ Flexi DNA Polymerase Green G2	500 U
5X Green Buffer	4 ml
5X Colorless Buffer	4 ml
25mM MgCl2 Solution	4 ml

#### **Storage Buffer**

20mM Tris-HCl, 1mM dithiothreitol, 0.1mM EDTA, 100mM NaCl, Stabilizer, 50% glycerol, pH 8.0 (<u>25°C)</u>.

#### **5X Green Buffer**

Proprietary formulation supplied at pH8.5 containing blue dye and orange dye. The buffer contains Tris-HCI, KCI and PCR enhancers and do not contain Mg.

#### **5X Colorless Buffer**

Proprietary formulation supplied at pH8.5 containing Tris-HCl, KCl and PCR enhancers and do not contain Mg.

#### **Unit Definition**

1 unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid-insoluble material in 30 minutes at  $75^{\circ}$ C.

#### **Storage Conditions**

Store all components at -20°C in a non-frost-free freezer.

#### **Quality Control**

No endonuclease activity, nicking activity, exonuclease activity, or priming activity has been detected.

#### **Recommended Protocol**

On ice, prepare each of following master mixes, combine, and place in heated (to  $95^{\circ}$ C) thermal cycler:

For 50µl PCR Reaction	Volume	Final Conc.
GOTAQ Flexi DNA Polymerase Green	0.25 µl	1.25 U
5X Green Buffer (or Colorless Buffer)	10 µl	1 X
25mM MgCl2 Solution	2~8 µl	1~4 mM
dNTP mix (2.5 mM each)	0.25 µl	0.2 mM each
Template	< 500 ng	< 500 ng
Forward Primer	5 ~ 50pmol	0.1~1 µM
Reverse Primer	5 ~ 50pmol	0.1~1 µM
Distilled water	up to 50 µl	

#### **General Cycling Conditions :**

Step	Temp (°C)	Time	Cycle
Initial Denaturation	95	5 min.	1
Denature	95	10 ~ 30 sec.	
Anneal	50~65	10 ~ 30 sec.	25 ~ 40
Extend	72	10 ~ 60 sec.	
Final Extension	72	5 min.	1

Notice:

Cycling conditions may need to be optimized, depending on different primer and template combinations. For example, raise the annealing temperature to prevent non-specific primer binding, increase extension time to generate longer PCR products.

#### Applications:

- Routine PCR
- Genotyping
- Library construction
- TA Cloning
- Primary Extension
- Colony PCR Multiplex PCR

Hot Start-Taq DNA Polymerase is an antibody mediated hotstart Taq DNA polymerase and quite suitable for high-specific hot-start PCR, real-time PCR and multiplex PCR. The enzyme is a highly processive 5' $\rightarrow$ 3' DNA polymerase that lacks 3' $\rightarrow$ 5' exonuclease activity and lacks a 3' $\rightarrow$ 5' proofreading function.

Hot Start-Taq DNA Polymerase is supplied with a separate vial of "GC Rich" buffer, which minimizes nonspecific amplification products, primer dimers, and background.

GC Rich buffer is a novel additive that enables efficient amplification of "difficult" (e.g., GC rich) templates, is also provided.

#### **Kit Contents**

Contents	FS-T-2131-1	FS-T-2131-5
Hot Start-Taq Polymerase (5U/µl)	100 µl*	500 µl
10X GC Rich Buffer	0,5 mL	3 x 1 mL
ddH2O	1 mL	3 x 1 mL

\*100 µl = 500 untis

#### 10X GC Rich Buffer:

#### **Unit Definition**

1 unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid-insoluble material in 30 minutes at  $75^{\circ}$ C.

Activity detection conditions: 50 mM Tris-Hcl (pH 9.0, 25°C), 50 mM NaCl, 5 mM MgCl2, 0.2 mM each dNTPs (including [3H]-Dttp), 200  $\mu$ g/ml activated calf thymus DNA and 0.1 mg/ml BSA.

#### **Storage Conditions**

Store all components at -20°C in a non-frost-free freezer.

#### **Quality Control**

No endonuclease activity, nicking activity, exonuclease activity, or priming activity has been detected.

#### **Recommended Protocol**

On ice, prepare each of following master mixes, combine, and place in heated (to 95°C) thermal cycler:

For 50µl PCR Reaction	Volume
Hot Start -Taq DNA Polymerase(5U/µl)	1 µl
10X GC Rich Buffer	5 µl
dNTPs (10 mM each)	1 µl
Forward Primer (10 µM)	1 µl
Reverse Primer (10 µM)	1 µl
Template	-
Distilled water	up to 50 µl

#### **General Cycling Conditions :**

Step	Temp (°C)	Time	Cycle
Pre-Denaturation	94	3 min.	1
Denature	94	30 sec.	
Annealing	50~60	30 sec.	30
Extend*	72	30 sec./kb	
Final Extension	72	5 min.	1

\*Subjected to the actual best annealing temperature

#### Notice:

Cycling conditions may need to be optimized, depending on different primer and template combinations. For example, raise the annealing temperature to prevent non-specific primer binding, increase extension time to generate longer PCR products.

# **GOTAQ GREEN 2X MASTER G2**

Cat No.	Size
FS-T-5041	500 reactions 1000 reactions

#### **Description**

GOTAQ Green 2x Master G2 is ready-to-use PCR pre-mixes are the innovation for convenience of your routine PCR. The PCR Green 2X Master is an optimized, ready-to-use PCR mixture of GOTAQ Green 2x Master G2, PCR buffer, MgCl<sub>2</sub> and dNTP's, except DNA template and primers. The mixture is suitable for amplification of most of the DNA templates and highly processive 5' $\rightarrow$ 3' DNA polymerase that lacks 3' $\rightarrow$ 5' exonuclease activity and lacks a 3' $\rightarrow$ 5' proofreading function. PCR reactions contains two dyes (blue and yellow) can be directly loaded onto an agarose gel without the additional need of loading buffer and dyes.

#### Kit Contents

Contents	FS-T-5041
GOTAQ Green 2x Master G2	1 ml/ 100 reactions

#### **Applications**

GOTAQ Green 2x Master G2 is suitable and tested for amplification of genomic targets ranging from 100 bp to 4 kb and of episomal targets (lambda phage; plasmids) up to 10 kb under various reaction conditions.

- High through-put PCR
- Hot-start PCR
- Routine diagnostic PCR requiring high reproducibility
- DNA sequencing template preparation

#### **Storage Conditions**

Store all components at -20°C in a non-frost-free freezer.

#### Note

Do not contaminate the GOTAQ Green 2x Master G2 Master with primers and template DNA used in individual reactions. Thaw and mix all components thoroughly, spin down shortly and chill on ice.

#### **Quality Control**

No endonuclease activity, nicking activity, exonuclease activity, or priming activity has been detected.

#### \* Equivalent to GoTaq G2 Green Master Mix

#### **Recommended Protocol**

This standard protocol applies to a single reaction where only template, primers, and water need to be added to the GOTAQ Green 2x Master G2 For multiple reactions, scale-up volume of reaction components proportionally. All reagents should be thawed on ice, gently mixed and briefly centrifuged before use.

- **1.** Thaw reagents at room temperature. Mix thoroughly and then place on ice immediately after thawing.
- 2. Assemble reaction tubes on ice whenever possible to avoid premature, nonspecific polymerase activity.
- 3. The following table shows recommended component volumes:

#### **Reaction Conditions**

Component	20 µl reaction	Final Conc.
PCR Green 2X Master	10 µl	1X
10µM Forward Primer	0.2 ~ 2.0 µl	0.1~1.0 µM
10µM Reverse Primer	0.2 ~ 2.0 µl	0.1~1.0 µM
Template DNA	1 ~ 5 µl	< 250 ng
Water, RNase-Free	up to 20 µl	

NOTE: In general, use greater than 0.5  $\mu$ M primers for sensitivity and less than 0.5  $\mu$ M for specificity.

NOTE: Recommended amount of template per PCR reaction:

- < 50 ng plasmid or
  - < 500~1000ng genomic DNA or
  - 2µl of a 100µl single plaque eluate or
  - one single bacterial colony
- **4.** Ensure reactions are mixed thoroughly by pipetting or gentle vortexing followed by a brief spin in a microcentrifuge.
- **5.** Optional-Overlay reactions with one-half volume PCR-grade mineral oil when not using heated lid on thermal cycler.
- 6. Transfer tubes on ice into a thermal cycler pre-warmed. The following table shows recommended cycling conditions:

#### **PCR Conditions**

Step	Temp (°C)	Time	Cycle
Initial Denaturation	95	2 min.	1
Denature	95	10~60 sec.	
Anneal	50 ~ 65	10~60 sec.	25~40
Extend	72	60 sec./kb	
Final Extension	72	5 min.	1

<u>NOTE</u>: Cycling conditions may need to be optimized, depending on different primer and template combinations. For example, raise the annealing temperature to prevent non-specific primer binding, increase extension time to generate longer PCR products.

**7.** After cycling, maintain the reactions at 4°C or store at -20°C until ready for analysis.

Cat No.	Size
FS-T-5141	500 reactions
F3-1-3141	1000 reactions

GOTAQ Hot Start Green Master Mix G2 is 2X Ready-to-Use Hot- start PCR pre-mixes are the innovation for convenience of your routine PCR.

The GOTAQ Hot Start Green 2X Master Mix G2 is an optimized, Ready-to-Use PCR mixture of GOTAQ Hot Start Green, PCR buffer, MgCl<sub>2</sub> and dNTP's, except DNA template and primers.

The mixture is suitable for amplification of most of the DNA templates and highly processive 5' $\rightarrow$ 3' DNA polymerase that lacks 3' $\rightarrow$ 5' exonuclease activity and lacks a 3' $\rightarrow$ 5' proofreading function. PCR reactions contains two dyes (blue and yellow) can be directly loaded onto an agarose gel without the additional need of loading buffer and dyes.

Contents	FS-T-5141
GOTAQ HS -PCR Green 2XMaster Mix G2	1 ml/100 reactions

#### Applications

GOTAQ Hot Start Green 2X Master Mix G2 is suitable and tested for amplification of genomic targets ranging from 100bp to 4kb and of episomal targets (lambda phage; plasmids) up to 10 kb under various reaction conditions.

- High through-put PCR
- Hot-start PCR
- Routine diagnostic PCR requiring high reproducibility
- DNA sequencing template preparation

#### **Storage Conditions**

Store all components at -20°C in a non-frost-free freezer.

#### Note

Do not contaminate the GOTAQ Hot Start Green 2X Master Mix G2 with primers and template DNA used in individual reactions. Thaw and mix all components thoroughly, spin down shortly and chill on ice.

#### **Quality Control**

No endonuclease activity, nicking activity, exonuclease activity, or priming activity has been detected.

\*Equivalent to GoTaq G2 Hot Start Polymerase

#### **Recommended Protocol**

This standard protocol applies to a single reaction where only template, primers, and water need to be added to the GOTAQ Hot Start Green 2X Master Mix G2. For multiple reactions, scale-up volume of reaction components proportionally. All reagents should be thawed on ice, gently mixed and briefly centrifuged before use.

- **1.** Thaw reagents at room temperature. Mix thoroughly and then place on ice immediately after thawing.
- 2. Assemble reaction tubes on ice whenever possible to avoid premature, nonspecific polymerase activity.
- 3. The following table shows recommended component volumes:

#### **Reaction Conditions**

Component	20 µl reaction	Final Conc.
GOTAQ HS Green 2XMaster Mix G2	10 µl	1X
10µM Forward Primer	0.2~2.0 µl	0.1~1.0 µM
10µM Reverse Primer	0.2~2.0 µl	0.1~1.0 µM
Template DNA	1 ~ 5 µl	< 250 ng
Water, RNase-Free	up to 20 µl	

**NOTE:** In general, use greater than 0.5  $\mu$ M primers for sensitivity and less than 0.5  $\mu$ M for specificity. **NOTE:** Recommended amount of template per PCR reaction:

- < 50 ng plasmid or
- < 500~1000 ng genomic DNA or
- 2 µl of a 100µl single plaque eluate or
- one single bacterial colony
- **4.** Ensure reactions are mixed thoroughly by pipetting or gentle vortexing followed by a brief spin in a microcentrifuge.
- 5. Optional-Overlay reactions with one-half volume PCR-grade mineral oil when not using heated lid on thermal cycler.
- 6. Transfer tubes on ice into a thermal cycler pre-warmed. The following table shows recommended cycling conditions:

#### PCR Conditions

Step	Temp (°C)	Time	Cycle
Initial Denaturation	95	5 min.	1
Denature	95	10~60 sec.	
Anneal	50~65	10~60 sec.	25~ 40
Extend	72	60 sec./kb	
Final Extension	72	5 min.	1

<u>NOTE</u>: Cycling conditions may need to be optimized, depending on different primer and template combinations. For example, raise the annealing temperature to prevent non-specific primer binding, increase extension time to generate longer PCR products.

 After cycling, maintain the reactions at 4°C or store at -20°C until ready for analysis.

# HOT START PROOF 2X MASTER MIX (GC-Rich Buffer)

#### Long Range PCR, High-Fidelity PCR, Fast PCR

#### Description

**Host Start Proof** DNA polymerase is an ideal enzyme for high fidelity PCR with excellent processivity. It is a novel engineered enzyme with unique structure.

Hot Start Proof 2X Master Mix DNA polymerase contains a recombinant synthesis enhancement domain to increase fidelity and extension speed. The antibody-mediated hot-start feature significantly inhibits non-specific amplifications at room temperature. Hot Start Proof is one of the thermostable DNA polymerases with strong 3'-5'exonuclease activity (**proofreading** activity), which results in its extreme **high fidelity.** The **Hot Start Proof** 2X Master Mix is an ideal product with good amplification efficiency for diversity templates including animals, plants, cDNA, etc.

#### **Kit Contents**

Contents	FS-T-1141-5
Hot Start Proof 2X Master Mix GC Rich Buffer	5 ml

1 ml= 100 Reactions (20 µl volume)

#### Applications:

- Long range PCR
- High-fidelity PCR and primer-extension reactions
- Genotyping
- Library construction
- High GC amplification
- Next-generation DNA sequencing
- TA cloning

#### Storage Buffer:

20 mM Tris-HCl, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA,200µg/mL BSA, 50% Glycerol, 1X Stabilizers, pH 7.4 @ 25°C

- Thermal Inactivation: No
- 5 -3 exonuclease activity: No
- 3'-5' exonuclease activity: Yes
- Product End: Blunt end

#### Standard Protocol :

-It is recommended to prepare all reaction components on ice, and then quickly transfer the reaction system to a thermocycler preheated to 98°C.

-All components should be mixed and collected at the bottom of a tube with a quick spin before use. Add Hot Start Proof DNA polymerase at the end to prevent primer degradation by its strong 3 ´-5 ´ exonuclease activity.

-Note: The Hot Start Proof DNA polymerase requires special reaction conditions different from other polymerase protocols. Please refer to the recommended reaction conditions below for the better amplification yields

#### Note

Do not contaminate the Hot Start Proof 2X Master Mix with primers and template DNA used in individual reactions. Thaw and mix all components thoroughly, spin down shortly and chill on ice.

Components	20µL	Total Conc.
Hot Start Proof 2X Master Mix (GC-Rich) Master Mix	10µL	1X
Forward Primer (10 μM)	0.4µL	0.2 µM
Reverse Primer (10 µM )	0.4µL	0.2 µM
DNA Template*	Variable	<300 ng
Nuclease-free Water	to 20µL	N/A

\*Note : The optimal reaction concentration varies with

different DNA templates. Please refer to the basic principles of PCR below

#### **Recommended PCR Program**

Step	Temp.	Time	Cycles
Initial Denaturation	98°C	45s	1
Denaturation	98°C	10 sec.	
Annealing	55~65°C	20-30 sec.	25-35
Extension	72°C	10-30 sec.	
Final Extension	72°C	1-5 min.	1
Hold	4-12°C	∞	1

#### **PCR** Principle

#### 1. Template:

High-quality purified DNA templates are important to high-fidelity PCR reactions. The recommended DNA template amounts with different complexity are listed below (For a 20µL reaction).

#### \*DNA TEMPLATE

DNA	20 µL reaction
Plants, animals and human gDNA	4 ng - 40 ng
E.coli, lambda gDNA	200 pg-75 ng
Plasmid DNA	0,4 pg-4 ng

**Note**: If the DNA template is obtained from a cDNA synthesis reaction, the template volume should be less than 10% of the total reaction volume. If long fragments are amplified, the amount of template input should be increased appropriately

#### 2. Primers :

Oligonucleotide primers are typically 20-40 nucleotides in length with a **GC content of 40-60%**. Primers can be designed and analyzed using software such as Primer 3 The final oncentration of each primer in the PCR reaction system should be in the range of 0.1-1  $\mu$ M.

#### 3. Denaturation:

**98°C pre-denaturation** for 45 seconds can fully denature most DNA templates. In the case of high complexity DNA templates, the pre-denaturation time should be extended up to 3 minutes for fully denaturation.

Generally, the recommended denaturation condition for low-complexity DNA templates is  $98^{\circ}C$ , 5-10 s

#### 4. Annealing:

The annealing temperature of **Hot Start Proof TAQ 2x Master Mix** is usually higher than other PCR polymerases.

Generally, primers longer than 20 nt are annealed at (lower primer Tm+3)°C for 10-30 seconds;

When the primers are shorter than 20 nt, an annealing temperature equivalent to the lower primer Tm.

When using a new primer set for PCR reaction, we recommend a gradient PCR to determine the optimal annealing temperature. In a two-step amplification protocol, the annealing temperature should be set to the extension temperature.

#### 5. Extention:

The recommended extension temperature is 72°C. The extension time depends on the length and complexity of the amplicon. For the low-complexity amplicons (plasmid DNA), the extension conditions is 10 s/kb. For high-complexity amplicons, it is recommended to increase the extension time to 20-30 s/kb. In some cases, the extension time for cDNA templates should be less than 1 min/kb

#### 6. Cycles:

To obtain enough yield of PCR products, 25-35 cycles are recommended.

# **PFU DNA POLYMERASE**

#### **High Fidelity**

#### Description

Pfu DNA polymerase, derived from the hyperthermophilic archae Pyrococcus furiosus, has superior thermostability and proofreading properties compared to other thermostable polymerase. Its molecular weight is 90 kDa. It can amplify DNA target up to 2 kb (simple template). The elongation velocity is 1kb/min(70~75°C). Pfu DNA polymerase possesses 3' to 5' exonuclease proofreading activity that enables the polymerase to correct nucleotide-misincorporation errors. This means that Pfu DNA polymerase-generated PCR fragments will have fewer errors than Taq-generated PCR inserts. Using Pfu DNA polymerase in your PCR reactions results in blunt-ended PCR products, which are ideal for cloning into blunt-ended vectors. Pfu DNA polymerase is superior for techniques that require high-fidelity DNA synthesis.

Description	FS-T-004
Pfu DNA Polymerase (5 U/ul)	1000 U
10X Pfu Buffer (MgCl2)	4x 1,25 ml
6x Loading Buffer	1ml

#### Activities detection conditions:

#### **Unit Definition**

1 unit of the enzyme catalyzes the incorporation of 10 nanomoles of deoxyribonucleotides into a polynucleotide fraction in 30 min at 70°C.

#### Storage Buffer

20mM Tris-HCl (pH 8.0), 100mM KCl, 3mM MgCl2, 1mM DTT, 0.1% NP-40, 0.1% Tween20, 0.2mg/ml BSA, 50% (V/V) glycerol.

#### 10× Pfu Buffer with MgCl<sub>2</sub>:

200mM Tris-HCl (pH 8.8), 100mM KCl, 100mM (NH4)2SO4, 20Mm MgSO4, 1% Triton X-100, 1mg/ml BSA.

#### Concentration:5 u/ul

#### **Quality control**

Free of detectable, non-specific nucleases.

#### **Storage Conditions**

Store all components at -20°C in a non-frost-free freezer.

#### Applications

- High-fidelity PCR and primer-extension reactions
- PCR cloning and blunt-end amplification product generation
- Site-directed mutagenesis
- Blunt-end PCR cloning

#### **Recommended amount of template DNA:**

Human genomic DNA	0.1µg-1µg
Plasmid DNA	0.5ng-5ng
Phage DNA	0.1ng-10ng
E.coli genomic DNA	10ng-100ng

#### **Recommended Protocol**

**1. Add the following** components to a sterile microcentrifuge tube placing on ice:

#### General PCR reaction mixture for 50 ul Reaction :

For 50 ul PCR Reaction	Volume (µl)	Concentration
Pfu DNA Polymerase	0.25-0.5 µl	1.25-2.5U/50 µl
10X Pfu Buffer	5 µl	1x
dNTP mix (10 mM each)	1 ul	0.2 mM each
Template DNA	variable	10 pg -1 ug
Forward Primer (10µM)	variable	04- 1µM
Reverse Primer (10 µM)	variable	04- 1µM
Distilled water	up to 50 ul	

**2. Mix contents of tube.** Cap tubes and centrifuge briefly to collect the contents to the bottom. When using a thermal cycler that does not contain a heated lid, overlay the reaction mixture with  $25 \,\mu$ l mineral oil.

#### **Recommended PCR Cycling Conditions:**

Step	Temp (°C)	Time (min)	Cycle
Initial Denaturation	94	3	1
Denature	94	30 sec.	
Anneal	55-68	30 sec.	30
Extension*	72	1-3 min	
Final Extension	72	10	1

**3.Incubate for an additional 10 min** at 72°C and maintain the reaction at 4°C. The samples can be stored at -20°C until use.

#### 4. Analyze the amplification products by agarose gel

electrophoresis and visualize by nucleic acid dye staining. Use appropriate molecular weight standards.

Notice :

Cycling conditions may need to be optimized, depending on different primer and template combinations. For example, raise the annealing temperature to prevent non-specific primer binding, increase extension time to generate longer PCR products. **Quality Control Assay** 

#### Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to a nicked DNA was observed after incubation of 10U Pfu DNA Polymerase with 1 $\mu$ g pBR322 DNA for 4 hours at 37°C and 70°C.

#### Exodeoxyribonuclease Assay

No detectable degradation of lambda DNA-HindIII fragments was observed after incubation of 10U Pfu DNA Polymerase with 1µg digested DNA for 4 hours at 37°C and 70°C.

#### **Ribonuclease Assay**

0% of the total radioactivity was released into trichloroacetic acidsoluble fraction after incubation of 10U Pfu DNA Polymerase with 1µg E.coli [3H]-RNA (40000cpm/µg) for 4 hours at 37°C and 70°C. **Contamination Assay** 

Pfu DNA Polymerase was passed from quality control assay for contamination of bacterial host DNA using sequence-specific primer set from host bacterial genomic DNA.

#### **Functional assay**

Pfu DNA Polymerase was functionally tested for PCR amplifications using the various size from human genomic DNA

GC-Platinum Power TAQ 2X Master MIX is a ready-to-use and complete system for rapid, consistent, and accurate amplification of long PCR products (>5~20 kb). This kit optimized for PCR amplification of genomic DNA templates up to 10 kb and lambda DNA up to 20 kb. With its enhanced processivity, yield, speed and excellent 3' $\rightarrow$ 5' exonuclease and 3' $\rightarrow$ 5' proofreading activity, this enzyme is able to consistently deliver accurate and reliable amplification of long templates. This product is the ideal choice for long DNA templates unable to be amplified in conventional PCR, and is highly suitable for multiple downstream applications including complex cloning and genotyping experiments. The PCR product amplified with this mixture has one A added at 3'-end, so the product can be directly used for TA cloning.

The kit already contains blue loading dye, therefore the PCR reactions can be directly loaded onto an agarose gel without the additional need of loading buffer and dyes.

#### **Kit Contents**

Contents	FS-T-1642-10	FS-T-1642-25
GC-Platinum Power TAQ 2X Master Mix	10 ml	25 ml

1 ml= 40 Reactions (50 µl volume)

1 ml= 80 Reactions (25 µl volume)

#### Applications

- Long range PCR
- High-fidelity PCR and primer-extension reactions
- Genotyping
- Library construction
- High GC amplification
- Next-generation DNA sequencing
- TA cloning

#### **Storage Conditions**

Store all components at -20°C in a non-frost-free freezer.

#### Note

Do not contaminate the GC-Platinum Power TAQ 2X Master Mix with primers and template DNA used in individual reactions. Thaw and mix all components thoroughly, spin down shortly and chill on ice.

#### **Quality Control**

No endonuclease activity, nicking activity, exonuclease activity, or priming activity has been detected.

#### **Template:**

High-quality purified DNA templates are important to high-fidelity PCR reactions. The recommended DNA template amounts with different complexity are listed Below (For a 50µL reaction)

DNA	INPUT Amount
Plants, animals and human gDNA	10 ng~100 ng
E.coli, lambda gDNA	500 pg-200 ng
Plasmid DNA	1 pg~10 ng

#### Primers :

Oligonucleotide primers are typically 20-40 nucleotides in length with a GC content of 40-60%. Primers can be designed and analyzed using software such as Primer 3. The final concentration of each primer in the PCR reaction system should be in the range of 0.1-1  $\mu$ M.

#### **Recommended Protocol**

This standard protocol applies to a single reaction where only template, primers, and water need to be added to the GC-Platinum Power TAQ 2X Master Mix. For multiple reactions, scale-up volume of reaction components proportionally. All reagents should be thawed on ice, gently mixed and briefly centrifuged before use.

- 1. Thaw reagents at room temperature. Mix thoroughly and then place on ice immediately after thawing.
- 2. Assemble reaction tubes on ice whenever possible to avoid premature, nonspecific polymerase activity.
- 3. The following table shows recommended component volumes:

Component	25 µl volume	50 μl volume	Final Conc.
GC-Platinum Power TAQ2X Master Mix	12,5 µl	25 µl	1X
10µM Forward Primer	0.5 µl	1.0 µl	0.2 µM
10µM Reverse Primer	0.5 µl	1.0 µl	0.2 µM
Template DNA	variable	variable	300 ng
Water, RNase-Free	up to 25 µl	up to 50 µl	NA

#### **Reaction Conditions**

NOTE: In general, use greater than 0.5  $\mu M$  primers for sensitivity and less than 0.5  $\mu M$  for specificity.

- 4. Ensure reactions are mixed thoroughly by pipetting or gentle vortexing followed by a brief spin in a microcentrifuge.
- 5. Optional-Overlay reactions with one-half volume PCR-grade mineral oil when not using heated lid on thermal cycler.
- 6. Transfer tubes on ice into a thermal cycler pre-warmed. The following table shows recommended cycling conditions:

#### **PCR Conditions**

Step	Temp (°C)	Time	Cycles
Initial Denaturation	98	45s	1
Denaturation	98	10 sec.	
Annealing	55~	30 sec.	30
Extension	72	20-30 s/kb	
Final Extension	72	5 min.	1
Hold	4-12°C	∞	1

<u>IMPORTANT</u>: Annealing temperature should be 2-6°C lower than the primer melting temperature. Elongation time should be ~1 min/1 kb.

- <u>NOTE</u>: Cycling conditions may need to be optimized, depending on different primer and template combinations. For example, raise the annealing temperature to prevent non-specific primer binding, increase extension time to generate longer PCR products.
- 7. After cycling, maintain the reactions at 4°C or store at -20°C until ready for analysis.

# **Real Time**

# **qPCR** Master Mix

2X Sybr Green qPCR Master Mix

2X Sybr Green Fast qPCR Master Mix

2X Universal Sybr Green Fast qPCR Mix

2X Universal Power Plus Sybr Green qPCR Master Mix (UDG)

2X EVAGreen qPCR Master Mix

2X TAQMAN Probe Fast qPCR Master Mix

2X TAQMAN Probe qPCR 2X Master Mix (UDG)

SYBR Green qPCR Master Mix is a ready-to-use cocktail containing all components except primers and template. The 2X master mix contains Taq DNA polymerase, dNTPs, MgCl2, SYBR Green I. Rox or No Rox and stabilizers.

In the formulation, for Hot Start, Taq DNA Polymerase is chemically modified and its activity is completely blocked until the first denaturation step in PCR program. This eliminates spurious amplification products resulting from non-specific priming events during reaction setup and initiation, and increases overall reaction efficiency.

For easy and avoiding potential error manipulation, the products are provided in three formats:

CAT.#	Description	SIZE
FS-T-1200-NR	Sybr Green qPCR 2x Master Mix No Rox	1 ml
FS-T-1200-LR	Sybr Green qPCR 2x Master Mix Low Rox	1 ml
FS-T-1200-HR	Sybr Green qPCR 2x Master Mix High Rox	1 ml

1 ml: 100 reactions

#### Following table is helpful for choosing right product formats

No ROX	Bio-Rad CFX96 <sup>™</sup> , CFX384 <sup>™</sup> , iCycler iQ <sup>™</sup> , iQ <sup>™</sup> 5, MyiQ, MiniOpticon, Opticon, Opticon 2, Chromo4; Cepheid SmartCycler®; Eppendorf Mastercycler® EP Real plex, Realplex 2 s; Illumina Eco qPCR; Qiagen/Corbett Rotor-Gene® Q, Rotor-Gene® 3000, Rotor-Gene 6000; Roche Applied Science LightCycler <sup>™</sup> 480; Thermo Scientific Piko Real Cycler
Low ROX	Applied Biosystems 7500, 7500 Fast, ViiA7; Stratagene MX4000, MX3005P, MX3000P
High ROX	Applied Biosystems 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast; StepOne, StepOnePlus.

#### Procedure

3. Set up reaction in qPCR tube as follow:

Composition	20 µl reaction system
SYBR Green qPCR 2x Master Mix	10 µl
Primer 1 (10 µM)	0.4 µl
Primer 2 (10 µM)	0.4 µl
Template DNA/cDNA	Χ μΙ
ddH2O	up to 20 µl

#### Suggestions for better results:

1) Generally 0.2  $\mu$ M primer concentration is suitable, but when results are not satisfied, trying primer concentration between 0.1-1.0  $\mu$ M range

2) qPCR method is very sensitive, accuracy of added temple is essential, recommending using diluted templates to reduce to increase aliquot accuracy, and the result is reproducible.

**3)** If templates are undiluted cDNA from standard reverse transcription reaction, the volume of template is no more than 10% of the reaction volume.

**Storage:** at -20°C avoid light, After thawing cycle the Master Mix should be stored at 4 °C for long time.

The Mix should be kept a -20°C , before using just blend the Master Mix.

#### Running qPCR Reaction as follows:

Stage 1	Pre-denatue	Reps: 1	95°C	5-10 min
Stage 2	Cycling	Reps: 40	95°C 60°C	10 sec 30 sec
Stage 3	Melting curve	Reps: 1	95°C 60°C 95°C	15 sec 60 sec 15 sec

**2.1** Pre-denature condition is suitable for most of reactions, if templates are complicated, extend to 10 min.

- **2.2** for less 300 bp fragment amplification, 30 second extending time is enough, for large than 300 bp fragment amplification, 60second extending time is recommended.
- 2.3 Melting curve collecting program depends on instrument's model, please choose acquiescence for the model.

#### **Optimizing reaction**

Best reaction condition should have following characteristic: single melting curve, amplification efficiency is almost 100%, lower Ct value (high amplification efficiency), if reaction is not as expected under acquiescence condition, reaction condition could be optimized as following ways.

**1.** Primer concentration and reaction: when primer concentration is between  $0.1 \sim 1.0 \mu$ M, higher primer concentration leads non-specific amplification, but amplification efficiency is increased.

**2.** Amplification program and reaction: To increase amplification specificity, increase annealing temperature and extending amplification time.

Two step standard program 95°C/10 sec 60°C/30 sec	Increase annealing Temperature(3°C each time) 95°C/10 sec 63°C/30 sec
Two step standard program95℃/10 sec 60℃/30 sec	Increase extending temperature 95°C/10 sec 60°C/60 sec

**TWO STEP program** 

To increase amplification efficacy, change two step amplification to three step and increase extending time.

#### THREE STEP program

Three Step Program In 95℃/10 sec. 56℃/30 sec 72℃/30 sec
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**Quality Control:** Purity detection: all components are analyzed without exo - endo-nuclease and nucleic acid

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Real-time Quantitative PCR (qPCR) is a powerful technique in detecting initial DNA input in a PCR reaction by fluorescence signal accumulation. The DNA double strand bonded dye, SYBR® Green I is the most commonly used dye in qPCR. 2X SYBR Green Fast qPCR Mix is provided in 3 versions: No Rox, Low ROX, High Rox and they are optimized for Real Time machines with no Rox, High Rox and Low ROX mode. It contains all required components in qPCR except primers and template. It is convenient for experiment and suitable for multiple species. The above features make it as an ideal experiment tool for gene quantitative research.

#### **Compatible Instruments**

Following table is helpful for choosing the right product formats

No Rox	Bio-Rad iCycler serious, Roche Light Cycler
Reference Dye I	serious Qiagen/Corbett serious and others
Low Rox	ABI 7500, ABI ViiATM7, ABI QuantaStudio
	serious, Stratagene serious, Corbett Rotor
	Gene 3000 and others
High Rox	ABI 7000/7300/7700/7900, ABI
Reference Dye	StepOne/StepOnePlus, Eppendorf and
	others

#### **Kit Contents**

Cat.n.	Description	Size
FS-T-50212-5	2X SYBR Green Fast qPCR Mix (No ROX)	5 X 1 mL
FS-T-50213-5	2X SYBR Green Fast qPCR Mix (Low ROX)	5 X 1 mL
FS-T-50214-5	2X SYBR Green Fast qPCR Mix (High ROX)	5 X 1 mL

1 ml =100 reactions

#### **Materials Required**

- 1. EP tubes, PCR tubes and other related materials.
- 2. qPCR specific primers and templates.
- 3. qPCR plates and seal membrane.

#### Usage Notes

- Before using 2X SYBR Green Fast qPCR Mix, please make sure that the mix is thawed completely and then placed it on ice for use.
- Mix other qPCR components with Mix thoroughly and gently by pipetting or vortexing. After usage, place the Mix back to -20°C for long time storage or 4°C for short period storage.
- 3. 2X SYBR Green Fast qPCR Mix (Low ROX) contains Hot Start *Taq* polymerase, all operation should be performed on ice.
- 2X SYBR Green Fast qPCR Mix (Low ROX) contains low ROX dye, suits for qPCR instruments that required Low ROX mode.\* See table below.
- 5. To avoid contamination, pipette tips with filters is suggested.
- 6. To guarantee better qPCR results, DNA template in good quality is suggested.

#### **Before Use**

- 1. Specificity of primers should be checked and a final concentration of  $0.2 \ \mu$ M is suitable for most of primers.
- 2. The length of amplification products is usually range from 70 bp to 200 bp.
- 3. Dilute the template in gradient.
- 4. Add 1 pg-50 ng DNA as PCR templates and a "No Template Control sample" is suggested.
- 5. To ensure the confidence of experiment, at least 2 repeats of each sample is suggested.

#### Procedure

1. Prepare the following reaction systems on ice for a 20 ul

Component	20 ul Reaction
2X SYBR Green Fast qPCR Mix	10 µL
Forward Primer (10 µM)	0,4 µL
Reverse Primer (10 µM)	0,4 µL
gDNA or cDNA (<50 ng)	2 µL
RNase free ddH <sub>2</sub> 0	up to 20 µL

- Dissolve 2X SYBR Green Fast qPCR Mix (No Rox,Low ROX, High ROX) at room temperature then placed it on ice for further usage. Before using, agitate the Mix thoroughly and centrifuge to collect all solution.
- 3. Calculate the amount of mix need, generally a 10% extra amount is suggested.
- 4. Dispense solution in sterile PCR or EP tubes in case of any contamination.
- 5. Add all components listed in above table, agitate the tubes gently to mix thoroughly (avoid bubbles) and centrifuge it.
- 6. Dispense the reaction solution into qPCR plates and seal the plates with optical membrane.
- 7. 2500 rpm centrifuge the qPCR plates to collect all solution.

#### 2. Program qPCR reaction as follows:

•••	r regram dr er redetion de renorie.					
	Stage 1	Denaturation	Reps:1	95°C	3 minutes	
	Stage 2	Cycles	Reps: 40-45	95°C	5 seconds	
				60°C	30-34	
					seconds	
	Stage 3	Melt Curve	Reps: 1	Default		

\*Confirm there is a signal collection step after each extending step. The extending time is varied according to different machines: 30s for StepOne Plus, 31s for 7300 and 34s for 7500

#### Data Analysis :

- Draw a standard curve according to Ct values of endogenous gene. The value of R<sup>2</sup> should be more than 0.98 and the slope of curve should be in the range of -3 to -3.5 which means the PCR amplification efficiency is in the range of 90% to 120%.
- The standard deviation (STD) of Ct values should be less than 0.2 and the variation of Ct value for different experiment should be less than 0.5 (the threshold value of different experiments should be same when comparing Ct values).
- The single melt curve indicate the no non-specific amplification products or primer dimmers, and theTm value in melt curve is usually in the range of 80 to 95°c.

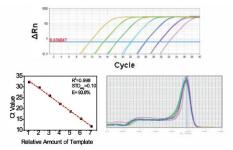


Figure 1. Template: mouse DNA (Mouse GAPDH), 6-log gradient dilution. The target gene GAPDH was detected by Fisher SYBR Green qPCR Fast 2X Master Mix. The experimental results show that the qPCR reagent can be accurately amplified between12-32 Ct, showing good amplification ability.

Real-time Quantitative PCR (qPCR) is a powerful technique in detecting initial DNA input in a PCR reactionby fluorescence signal accumulation. The DNA double strand bonded dye, SYBR<sup>®</sup> Green is the most commonly used dye in qPCR. It contains Hot Start Taq, to avoid unexpected amplification Results. It is an optimized qPCR Mix you need to add primers and template. It isconvenient for experiment and suitable for multiple species. The above features make it as an ideal experiment tool for gene quantitative research.

#### **Kit Contents**

Cat.n.	Description	Size	a
FS-T-50215	2 X Universal SYBR Green Fast qPCR Mix	5 X 1 mL	E

#### **Compatibility:**

2X Universal SYBR Green Fast qPCR Mix contains the novel designed universal reference dye, which can realize higher signal resolution and suits for all qPCR Instruments (including High ROX mode, Low ROX mode and No Rox mode).

#### **Materials Required**

EP tubes, PCR tubes and other related materials. qPCR specific primers and templates. qPCR plates and seal membrane.

#### **Usage Notes**

1.Before using 2X Universal SYBR Green Fast qPCR Mix,please make sure that the mix is thawed completely and then placed it on ice for use.

2.Mix other qPCR components with Mix thoroughly and gently by pipetting or vortexing. After usage, place the Mix back to -20°C for long time storage.

3.2X Universal SYBR Green Fast qPCR Mix (No ROX) contains Hot Start *Taq* polymerase, all operation should be performed on ice.

4. 2X Universal SYBR Green Fast qPCR Mix contains specially reference dye, which suits for all qPCR instruments. No Rox is required.

5.To avoid contamination, pipette tips with filters is suggested. 6.To guarantee better qPCR results, DNA template in good quality is suggested.

#### **Before Use**

a) Specificity of primers should be checked and a final

concentration of 0.2 µM is suitable tor most of primers.

b) The length of amplification products is usually range from 70 bp to 200 bp.

c) Dilute the template in gradient.

d) Add 1 pg-50 ng DNA as PCR templates and a "No Template Control sample" is suggested.

e) To ensure the confidence of experiment, at least 2 repeats of each samples is suggested.

#### Procedure:

Prepare the following reaction systems on ice

Components	20 ul Reaction
2X Universal SYBR Green Fast qPCR Mix	10 μL
Forward Primer (10 μM)	0,4 μL
Reverse Primer (10 μM)	0,4 μL
gDNA or cDNA (<50 ng)	2 μL
ddH20	to 20 μL

1) Dissolve 2X Univeral SYBR Green Fast qPCR Mix (No ROX) at room temperature then placed it on ice for further usage. Before using, agitate the Mix thoroughly and centrifuge to collect all solution.

**2**) Calculate the amount of mix need, generally a 10% extra amount is suggested.

3) Dispense solution in sterile PCR or EP tubes in case of any contamination.

4) Add all components listed in above table, agitate the tubes gently to mix thoroughly (avoid bubbles) and centrifuge it.

Dispense the reaction solution into qPCR plates and seal the

plates with optical membrane.

6)2500 rpm centrifuge the qPCR plates to collect all solution.

#### 2. Program qPCR reaction as follows:

Stage 1	Denaturation	Reps:1	95°C	3 minutes
Stage 2	Cycles	Reps:	95°C	5 seconds
-	-	40-45		
			60°C	30-34
				seconds
Stage 3	Melt Curve	Reps: 1	Default	

\*Confirm there is a signal collection step after each extending step. The extending time is varied according to different machines: 30s for StepOne Plus, 31s for 7300 and 34s for 7500. Data Analysis:

Draw a standard curve according to Ct values of endogenous gene. The value of  $R^2$  should be morethan 0.98 and the slope of curve should be in the range of -3 to -3.5 which means the PCR amplification efficiency is in the range of 90% to 120%. The standard deviation (STD) of Ct values should be less than

0.2 and the variation of Ct value for different experiment should be less than 0.5 (the threshold value of different experiments should be same when comparing Ct values).

The single melt curve indicates the no non-specific amplification products or primer dimmers, and the Tm value in melt curve is usually in the range of 80 to 95°c.

FS-T-50216

Description	FS-T-50216-5	FS-T-50216-25
2X Universal Power Plus SYBR Green qPCR Master Mix with UDG	5 X 1 mL	25 X 1mL

# 1 mL develops: 100 reactions (20µl) **HIGHLIGHTS**:

Specific—minimize primer-dimer and non-specific amplification • Reproducible and sensitive—consistent amplification across a wide dynamic range

- Bright—contains SYBR Green for maximum brightness
- Carry-over contamination control—contains heat-labile UDG
- · Compatible with all quantitative PCR instruments

#### **Product Description**

2X Universal Power Plus SYBR Green qPCR Master Mix contains all the components needed for your real-time PCR reaction, except the template and primers, in a convenient 2X concentration premix designed to be compatible with all types of fluorescence quantitative PCR instruments on the market, including High ROX, Low ROX, and No ROX required instruments.

It utilizes a specially designed reference dye (ROX) for improved sensitivity and resolution. The reagent also incorporates a dUTP/UDG anti-contamination system, which includes UDG to degrade contaminants containing U at room temperature. UDG quickly deactivates when pre-denatured at 95°C without affecting the efficiency and sensitivity of qPCR. Hot start Taq DNA polymerase is used for amplification, which enhances the specificity of the product while ensuring an efficient amplification effect. Overall, this product provides a reliable and versatile solution for SYBR Green-based qPCR experiments.

#### Storage

This product should be stored at -20  $^{\circ}\mathrm{C}$  for long-term storage and should be protected from light.

#### **Materials Required**

- 1. EP tubes, PCR tubes and other related materials.
- 2. qPCR specific primers and templates.
- 3. qPCR plates and seal membrane.

#### Instruments

No additional reference dye is required. Universal Power Plus SYBR Green qPCR Mix with UDG is suited for all currently used qPCR instruments (including high ROX mode, low ROX mode and No ROX mode required machine).

#### qPCR machine Compatibility:

7500 Fast System, 7500 System, QuantStudio<sup>™</sup> 12k Flex, QuantStudio<sup>™</sup> 3, QuantStudio<sup>™</sup> 5, QuantStudio<sup>™</sup> 6 Flex, QuantStudio<sup>™</sup> 7, StepOne<sup>™</sup>, Fast Mode, StepOne<sup>™</sup>, Standard Mode, StepOnePlus<sup>™</sup>, Fast Mode, StepOnePlus<sup>™</sup>, Standard Mode, ViiA<sup>™</sup> 7 System, AB StepOnePlus<sup>™</sup>, Fast Mode, AB StepOne<sup>™</sup>, Standard Mode, AB 7500, Fast Mode, AB 7500, Standard Mode, AB StepOne<sup>™</sup>, Fast Mode, AB StepOnePlus<sup>™</sup>, Standard Mode

#### **Operating instructions**

Preparation before experiment

- 1. It is recommended to choose the amplification product length within the range of 70-200 bp.
- 2. It is recommended to take a reaction volume of 20  $\mu$ L, add 1 pg-50 ng of DNA as a template, and set NTC (no template control).

To ensure the accuracy of the experimental results, it is recommended to weigh each sample and control group three times.

#### **Experimental methods**

Configure qPCR reaction system. It is recommended to prepare a reaction system on ice and quickly transfer the system to a qPCR instrument preheated at 95 °C.

commended Departies 20 vl. sDCD. Departies

Components	Input
2x Universal Power Plus SYBR Green qPCR Mix with UDG*	10 µL
DNA template *	2 µL
Forward primer(10 µM)**	0.4 µL
Reverse primer(10 µM)**	0.4 µL
ddH2O	To 20 μL

\* Note: Using 10 pg-10 ng genomic DNA or 10 pg-100 ng cDNA as the template reference quantity, gradient dilution can be performed on the template to determine the optimal template usage due to the different copy numbers of the target genes contained in the templates of different species. In addition, when using cDNA (RT reaction solution) from the two-step RT qPCR reaction as a template, the addition amount should not exceed 10% of the qPCR reaction system.

\*\*Note: Typically, the final concentration of the primer is 0.2  $\mu$ M,and good results can be obtained , and the final concentration of 0.1-1.0  $\mu$ M can be used as a reference for setting the range. In the case of low amplification efficiency, the concentration of primers can be increased; When non-specific reactions occur, the concentration of primers can be reduced and the reaction system can be optimized.

#### Recommended PCR Program

Steps	Temp	Time	Cycles
UDG Reaction	37°C	2 min	1
Pre Denaturation	95°C	3 min	1
Cycles	95°C	5 sec	40
	60°C	30-34 sec	
Melt Curve	Instrument automatic setting		

\*Confirm there is a signal collection step after each extending step. The extending time is varied according to different machines: 30s for StepOne Plus, 31s for 7300 and 34s for 7500.

# FS-T-1271

#### **Description:**

2X Eva Green qPCR Master Mix is a ready-to-use hot-start mix for qPCR and DNA melt curve analysis of PCR amplicons. It is formulated for qPCR using a fast-cycling protocol, but also can be used for qPCR with regular cycling protocols. Eva Green dye is a unique DNA-binding dye with features ideal for both qPCR and melt curve analysis. Green dye binds to dsDNA via a novel "release-on-demand" mechanism, which permits the use of a relatively high dye concentration in qPCR without PCR inhibition.

2X Eva Green qPCR Master Mix contains our proprietary chemicallymodified Hot-Start Taq DNA Polymerase. Unlike AmpliTaq Gold, which is also a chemically modified Taq but takes 10 minutes or longer to activate, this Taq DNA Polymerase is fully activated in 2 minutes with high activity recovery, making it particularly suitable for fast PCR. HS-Taq is completely inactive at room temperature and largely free of DNA contamination. This makes HS-Taq superior to any antibody-based hotstart Taq, which istypically not completely inactive at room temperature and is prone to DNA contamination due to the nature of antibody production. The Eva Green dye in the Master Mix can act as a DNA pre stain, permitting direct visualization of DNA bands following electrophoresis.

#### **Kit Contents**

Contents	FS-T-1271
2X Eva Green qPCR Master Mix Plus	1 ml*
10x Rox Dye	1 ml

#### \*1 ml = 100 reactions

The product contains two components.

**Component A** contains EVA Green dye, dNTP, PCR buffer (including Tris and MgCl2) and hot-start Taq polymerase.

**Component B** is 10X Rox reference, which may be required on certain ABI instruments (See protocol below).

Spectral Properties :  $\lambda abs/\lambda em = 500/530 \text{ nm}$  (bound DNA)  $\lambda abs = 471 \text{ nm}$  (without DNA)

#### Protocol

- 1. Thaw reagents at room temperature. Mix thoroughly and then place on ice immediately after thawing.
- 2. Assemble reaction tubes on ice whenever possible to avoid premature, non specific polymerase activity.

**Reaction Conditions PCR** 

3. The following table shows recommended component volumes:

Reaction conditions FCR				
Reagents	20ul reaction	Final conc.		
2X Eva Green qPCR Master Mix	10.ul	1X		
ROX Dye (10X)	*(optional)	See note 4		
10 um Forward Primer	X ul each	0.1~0.5 uM		
10 um Reverse Primer	X ul each	0.1~0.5 uM		
Template**	Variable (see note 1 and 2)	NA		
Water RNase Free	Up to 20ul			

# Notes

#### 1. cDNA templates: 2X EVA Green qPCR Master Mix is suitable for mRNA quantitation if a two-step procedure is followed. The first step involves converting the mRNA to cDNA by reverse transcription (components not provided). A portion of the synthesized cDNA can then be quantitated by using 2X EVA Green qPCR Master Mix in the second step. To ensure optimal amplification efficiency, the aliquot of the cDNA sample to be amplified should not exceed 10% of the volume of the PCR reaction. We recommend cDNA synthesis kits For accurate quantitation of transcript levels, a no-RT control is recommended to check for possible genomic DNA contamination.

**2. One-step RT-qPCR** can also be applied for mRNA quantitation. Primer sets must be well characterized to ensure no primer-dimer formation. We recommend that you titrate the amount of reverse transcriptase and the duration of the RT step. If possible, design primers to have Tm at 55 °C, run both RT step and extension step at 55 °C. For accurate quantitation of transcript levels, a no-RT control is recommended to check for possible genomic DNA contamination.

**3. Template concentration**: The optimal amount of template DNA varies by application. Recommended amounts of genomic DNA template per reaction typically range from 50 pg to 50 ng per reaction. Recommended amounts of cDNA typically range from 50 fg to 50 pg, based on the amount of input RNA in the RT reaction.

**4. ROX reference dye**: For certain instruments, ROX is necessary for accurate Ct determination from well to well. Refer to Table 1 (See Page 5-6) for therecommended ROX concentration for your instrument. ROX may add noise to melt curve analysis, which could be mistaken for real peaks. Thus, in case of unexpected peaks, uncheck "ROX" in the "Passive Reference Dye" box in the software so that data is not collected from the ROX fluorescence channel, then re-analyze the data.

#### Following table is helpful for choosing right product formats Using Rox - Dilute 10× ROX 1:10 withdH<sub>2</sub>O to obtain 1× ROX;

add 1 to 2 uL of 1× ROX per 20 uL react.		
No ROX	BioRad: iCycler, MyiQ, MiQ 2, iQ 5, CFX-96,CFX-	
	384, MJ Opticon, Option2, Chromo4, MiniOpticon	
	Qiagen: Roto-Gene Q,Roto-Gene3000,	
	Roto-Gene 6000 Eppendorf: Mastercycler realplex	
	Illumina: Eco RealTime PCR SystemCepheid: SmartCyler	
	Roche: LlghtCycler 480, 96, LightCycler 2.0	
Low ROX	ABI: 7500, 7500 Fast, ViiA 7	
	Stratagene: MX4000P, MX3000P	
	MX3005P, QuantStudio, Illumina Eco, Thmorgan Q6,Q4	
High ROX	Applied Biosystems 5700, 7000, 7300, 7700, 7900, 7900HT,	
-	7900HT Fast; StepOne, StepOnePlus.	

#### **Cycling Protocols**

You may choose one of the following three protocols, depending on the nature of your amplicon and instrument capability.

#### A. Universal cycling protocol

This cycling protocol can be used on nearly all qPCR instruments. The protocol also may be useful for targets that are relatively difficult to amplify underfast cycling conditions.

Cycling Step	Temp.	Holding	N.of
		Time	Cycles
Enzyme Activation	95 ℃	2 min	1
Denaturation Annealing &	95 ℃	15s	45
Extension	<b>60 °</b> C	60s	40

#### B. Two-step fast cycling protocol

This cycling protocol should be applicable to most amplifications where the primer Tm's are designed to be 60°C. Melt curves may be performed by following instructions provided for your instrument.

Denaturation Annealing & S			
g	95 ℃	2 min	1
Extension	95 ℃ 60 ℃	5s (Note 5) 30s	45

#### C . Three-step fast cycling protocol

This cycling protocol can be used if you would like to have the extension step to be performed at a higher temperature than the annealing step. For example, if you have relatively long primers that tend to anneal non-specifically, carrying out the extension step at a higher temperature can reduce nonspecific amplification. Melt curves may be performed by following instructions provided

for your instrument. Cycling Temp. Holding Time N.of Step Cycles Enzyme Activation 95°C 2 min 1 95 ℃ 10 s Denaturation 50~60°C 10s Note 6) 45 Annealing 10s (Note 7) 72 °C Extension

2X TaqMan Fast Probe qPCR Master Mix ready-to-use 2X reagent ideal for most quantitative Real-time PCR applications. The master mix is recommended for use with Labeled Fluorescent Probes, e.g. for 5'-Nuclease Assays or Hybridization probes.

2X TaqMan Fast Probe qPCR Master Mix is an optimized, ready-to-use PCR mixture of Hot-start Taq DNA Polymerase, PCR buffer, Magnesium and dNTPs, except DNA template and primers. The kit includes the components necessary for performing PCR amplification, and have been successfully used to amplify and detect a variety of DNA targets such as genomic DNA, cDNA and plasmid DNA.

#### Kit Contents

Contents	CAT. N°	Size
2 X TaqMan Fast Probe qPCR Master Mix	FS-T-1072F	100 RX
ROX Dye (1x)		1 vial

\*1 ml = 100 Reactions

#### PCR Machines requiring ROX dye

#### High Rox Dye:

- ABI 7000, 7300, 7700, 7900HT and 7900HT Fast, StepOne, StepOne Plus:
- Amount per 50 ul reaction: 1.0 ul (0.6-1.0 ul)
- Final ROX Concentration: 500nM (300-500nM)

#### Low ROX Dye\*:

ABI 7500, 7500 Fast, Viia 7, QuantStudio; Roche LightCycler; Stratagene Mx3000, Mx3005P and Mx4000 :

- Amount per 50 ul reaction: 0.1 ul (0.06-0.1 ul)
- Final ROX Concentration: 50nM (30-50nM)

#### \*Dilute (1x) Rox : 1:10 with H2O to obtain 0.1X Rox

#### PCR Machines requiring no ROX Dye

BioRad: iCycler, MyiQ, MiQ 2, iQ5, CFX-96, CFX-384, MJ Opticon, Option2, Chromo4, MiniOpticon Qiagen: Roto-Gene Q, Roto-Gene3000, Roto-Gene 6000 Eppendorf: Mastercycle realplex Illumina: Eco RealTime PCR System Cepheid: SmartCyler Roche: LightCycler 480,LightCycler 2.0

Use of the ROX Reference Dye

ROX reference dye is not included in this kit and may be added to compensate for non-PCR related variations in fluorescence. Addition of the reference dye is optional. Optimizing the ROX dye concentration within the qPCR reaction is an important aspect of setup. Too much ROX in the qPCR reaction will reduce background but also makes a low target signal difficult to distinguish from background

#### **Applications**

- Real-time PCR
- Gene expression profiling
- Gene knockdown verification
- Array validation

#### **Storage Conditions**

Upon receipt, store all components at  $-20^{\circ}$ C. Store the Master mix at 4°C after thawing for up to 6 months, depending on the expiration date, without showing any reduction in performance.

#### Note

Do not contaminate the TaqMan Fast Probe qPCR Master Mix mix with primers, probe and template DNA used in individual reactions. Thaw and mix all components thoroughly, spin down shortly and chill on ice.

#### **Recommended Protocol**

Prior to the experiment, it is prudent to carefully optimize experiment conditions and to include controls at every stage. See pre-protocol considerations for details.

This standard protocol applies to a single reaction where only template, primers, probe and water need to be added to the 2X TaqMan Fast Probe qPCR Master Mix. For multiple reactions, scale-up volume of reaction components proportionally. All reagents should be thawed on ice, gently mixed and briefly centrifuged before use.

1- Thaw reagents at room temperature. Mix thoroughly and then place on ice immediately after thawing.

2- Assemble reaction tubes on ice whenever possible to avoid premature, nonspecific polymerase activity.

3- The following table shows recommended component volumes:

#### **Reaction Conditions**

	20ul reaction	Final conc.
2XTaqMan Fast Probe qPCR Master Mix	10.0ul	1X
ROX Dye (1X) *(optional)	0.4ul (0.04ul)	1X (0.1X)
10um Forward Primer	0.2~2.0ul	0.1~1.0uM
10 um Reverse Primer	0.2~2.0ul	0.1~1.0uM
Fluorescence Probe	Variable	≤500ng/reaction
Template**	Variable	NA
Water RNase Free	Up to 20ul	

\*Please note "Use of the ROX Reference Dye"

#### \*\* Recommended amount of template per PCR Reaction:

- < 50 ng plasmid or,
- $< 500 \sim 1,000$ ng genomic DNA or,
- 2ul of a 100ul single plaque eluate or, one single bacterial colony or,
- 100 ng ~ 1 pg of cDNA

<u>NOTE</u>: In general, use greater than 0.5 uM primers for sensitivity and less than 0.5 uM for specificity.

4. Ensure reactions are mixed thoroughly by pipetting or gentle vortexing followed by a brief spin in a microcentrifuge.

5. Transfer tubes on ice into a thermal cycler pre-warmed. The following table shows recommended cycling conditions:

#### **PCR Conditions**

Step	Tem p (°C)	Time	Cycle
Initial Denaturation	95	*20 sec.~5min.	1
Denature	95	1 ~ 10 sec.	35 ~ 40
* Anneal	55~65	20 ~ 50 sec.	00 40

**ATTENTION** :Only \*20 sec ~2min for cDNA, 5 min for genomic DNA

**NOTE:** Cycling conditions may need to be optimized, epending on different primer and template conditions. For example, raise the annealing temperature to prevent non-specific primer binding, increase extension time to generate longer PCR products.

#### **Quality Control Analysis**

Sensitivity and reproducibility in real-time PCR are tested in parallel reactions containing 10-fold dilutions of nucleic acid template

2X TaqMan Probe qPCR Master Mix with UDG is a ready-touse reagent for probe-based qPCR reactions, containing all components except primers, probes and templates. This master mix includes Hot start Taq DNA polymerase modified both chemically and by antibody to inhibit non- specific amplification, which can guarantee high efficiency, high sensitivity and also high reproducibility in qPCR amplification.

At the same time, and has joined the UDG anti- pollution system. The optimization of the Buffer system allows the product to perform multiple fluorescence quantitative experiments, and it is suitable for multiple species and provides a powerful tool for multi-disciplinary experimental need **Kit Contents** 

Contents	CAT. N°	Size
2XTaqMan Probe qPCR Master Mix with UDG	FS-T-50217	500 RX
Rox Dye I (High Rox) 50X		1 Vial
Rox Dye II (low Rox) 50X		1 Vial
1ml = 100 Reactions		

#### **ROX dye - Real Time Machines:**

High Rox Dye: ABI 7000, 7300, 7700, 7900HT and 7900HT Fast, StepOne,stepOne Plus:

Low ROX Dye: ABI 7500, 7500 Fast, Viia 7, QuantStudio; Qiagen: Roto-Gene Q, Roto-Gene3000, Roto-Gene 6000

#### No ROX Dye – Real Time Machines

**BioRad:** iCycler, MyiQ, MiQ 2, iQ5, CFX-96, CFX-384, MJ Opticon, option2, Chromo4, MiniOpticon **Roche**:LightCycler 480, LightCycler 2.0 **Eppendorf**: Mastercycle realplex - **Illumina**: Eco RealTime PCR System Cepheid: SmartCyler

#### **Applications**

• Real-time PCR/Gene expression profiling/Gene knockdown verification/Array validation

Component	50 rxn	250 rxn
2X Taqman Probe qPCR Probe		
Master Mix with UDG*	500 µl	2 x1,25 mL
Rox Dye I (High Rox) conc.50x	22 µL	100 µL
Rox Dye II (Low Rox) conc.50x	22 µL	100 µL

\*Contain hot-start Taq DNA Polymerase,UDG, Mg2+, dNTPs et. al. Note

Do not contaminate the 2X TaqMan Probe qPCR Master Mix with primers, probe and template DNA used in individual reactions. Thaw and mix all components thoroughly, spin down shortly and chill on ice.

#### Additional Material Required but not Supplied

-Optical-grade qPCR tubes, plates, sealing films, and aerosol-resistant pipette tips

-qPCR primers and probes

-DNA or cDNA templates

Storage: Upon receipt, store all components at -20°C.

#### Precautions

1.Fully thaw TaqMan 2X qPCR Probe Master Mix with UDG before use.

2. The TaqMan 2X qPCR Probe Master Mix with UDG contains

glycerin. Mix gently before use without generating air bubbles. Spin briefly to collect all the contents at the bottom.

After use, return it to -20°C immediately.

3.A Hot-start version of Taq polymerase is included in the master mix, allowing reaction. After first thaw, the master mix is stable at 4  $^{\circ}$ C for 1 week

4.Use the ROX reference dye according to the requirement of qPCR instrument to be used.

**5.**If applicable, use aerosol-resistant pipette tips to minimize contamination.

6. High quality DNA templates are recommended for optimal results.

#### Important points before set up:

**1.**A final primer concentration of 0.2  $\mu$ M is recommended for most reactions. However, to optimize individual reaction, a primer titration from 0.1  $\mu$ M to 1.0  $\mu$ M can be performed.

**2.**The length of amplified PCR products should ideally be in the range of 70 - 200 bp.

3.Prepare a serial dilution of the template to access standard curve and test primer efficiency.

**4.**Use 1 pg~50 ng of DNA template in a 20  $\mu$ L reaction. The volume of template should not exceed 10% of the final PCR reaction volume.

5. Always include a no template control (NTC) reaction.

**6**.Triplicates are recommended as technical replicates in realtime PCR reactions.

#### Set up: Prepare the reaction mix.

**1.**Fully thaw the TaqMan Probe 2X qPCR Probe Master Mix with UDG at room temperature, and gently mix well without creating bubbles. Spin down briefly in a microcentrifuge to collect all contents at the bottom.

Reaction Conditions	5
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Reagents	50 µl reaction
2X TaqMan Probe qPCR 2X Master Mix with UDG	10.0µl
(10 μm) Forward Primer	0.4µl
(10 µm) Reverse Primer	0.4µl
Fluorescence Probe(10 µm)	0.4µI
Rox Dye (50X) optional*	0.4µl
DNA Template**	0.4µl(<50ng)
Water RNase Free	Up to 20µl

#### \*Please note "Use of the ROX Dye on Real Time Machines"

2.Calculate the required volume of each components based on the number of reactions to be set up and add extra 10% volume of each component to compensate pipette errors

**3.**Add all the common reaction components (primers and probes) in a master mix and mix thoroughly.

**4.**Dispense appropriate volumes of reaction mix into qPCR plates, and carefully seal it with an optical sealing film.

**5.**Add templates or NTC into wells containing the qPCR reaction mix.

**6**.Centrifuge the qPCR plates (tubes) at 2500 rpm to collect all the contents at the bottom of wells. The samples are ready for thermocycling.

#### **PCR Conditions**

Step	Tem p (°C)	Time	Cycle
UDG	37°	2 min	1
Predenaturation	95°	*20 sec.~5min.	1
Denaturation	95°	5 sec.	
Annealing and * Extention	55°	10 sec.	40

\*To ensure signal acquisition after extension, the extension temperature should be based on the Tm value of the primer probe.Line adjustment. \*\* It is recommended that the shortest pre-denaturation time should not be shorter than 30s, and the longest should not exceed 10 min; the shortest denaturation time during the cyclic reaction is not less than 5s, and the longest is not more than 15s; the cyclic reaction; the shortest extension time in the application is not less than 10s, and the longest can be based on the primer probes and signals used by yourself. The set needs to be adjusted by itself.

# **Reverse Transcription**

# **Enzymes & Kits**

2X One Step Sybr Green RT PCR Mix

ScriptIII One Step RT\_ qPCR Taqman Probe (UDG)

cDNA Synthesis Kit (Plus gDNA Eraser)

miRNA First Strand Synthesis kit

**M-MLV Reverse Transcriptase** 

RNase INHIBITOR (40= U/ul)

**Oligo dT Primer** 

**Random Hexamer Primer 9** 

#### Introduction

This product is a special reagent for Real Time One Step RT-PCR using the probe method. Using this product for Real Time RT-PCR reaction can continuously perform reverse transcription and PCR amplification in the same reaction tube. It is simple to operate and can effectively prevent contamination. Since this reaction system can monitor the amplified products in real time, the detection sensitivity is greatly improved, and the electrophoresis step after PCR reaction is omitted, which is very suitable for the detection of RNA viruses.

This product uses high-efficiency reverse transcriptase and high-specificity hot-start Taq DNA polymerase to perform stable and efficient Real Time One Step RT-PCR reactions. For the fluorescent quantitative PCR instrument that uses ROX as the calibration dye, this product is equipped with a separate ROX dye to correct the fluorescent signal error generated between the wells of the quantitative PCR instrument.

#### **Kit Components**

Components	FS-RT-007-100	FS-RT-007-500
2×One Step RT-PCR Mix	1 ml (100 rxns)	5 x 1 ml (500 rxns)
RT-PCR Enzyme Mix	150 µl	750 µl
50× ROX Dye	100 µl	500ul
RNase-free ddH2O	1 ml	5 x 1 ml
User manual	1 сору	1 сору

#### Reagents and items that users need to prepare

- 1. PCR primers.
- 2. RNA template.
- 3. Suitable for single tube, 8-strip tube, or 96-well PCR tube (plate) for fluorescent quantitative PCR.
- 4. Micropipette and clean tip with filter element.
- 5. Real Time PCR Thermal Cycler.

#### Instructions (recommended reaction system)

1. Prepare PCR reaction solution according to the following components and place on ice.

2. Turn the thawed components upside down and mix them evenly, and add each group to the following table to make a PCR reaction system:

Components	96-W	ells	384-Wells	Concentration
х	50µL reaction system	20µL reaction system	10µL reaction system	X
2×One Step Sybr Green RT PCR Mix	25 µL	10 µL	5 µL	1x
RT-PCR Enzyme Mix	1 µL	0.4 µL	0.2 µL	
PCR Forward Primer (10 µM)	1 µL	0.4 µL	0.2 µL	0.2 µL
PCR Reverse Primer (10 µM)	1 µL	0.4 µL	0.2 µL	0.2 µL
*50 x ROX Dye (optional)	1 µL	0.4 µL	0.2 µL	1x
RNase-free ddH2O	to 50 μL	to 20 μL	to 10 µL	
Template				

Instrument	The amount of ROX required for each 50 $\mu\text{L}$ system reaction	
ABI7300、7900HT、StepOne etc.	5µL	
ABI7500、7500Fast、ViiA7、Stratagene Mx3000™、 Mx3005P™ and Mx4000™ etc.	1µL	
Roche、Bio-Rad,Eppendorf etc.	1	

Script-III One Step RT-qPCR Taqman Probe Kit with UDG a ready-to-use kit allowing reverse transcription and subsequent probe-based gPCR in a single tube. It contains all components for RT-qPCR except primers, probes and RNA templates. The one-step format significantly improves sensitivity and effectively prevent contamination. The heat-liable UDG in this product could degrade U-contained contamination in room temperature, and inactivated in 50°C, which could prevent false positive results without affect the efficiency and sensitivity. The Script Reverse Transcriptase in the kit provides reliable reverse transcription to a wide range of RNA template amount. After reverse transcription, the Hot-start version of Tag polymerase is activated at 95 ° C and the Script Reverse Transcriptase is inactivated simultaneously. In the sequential PCR reaction, the 5'-3' exonuclease activity of Taq polymerase cleaves the hybridized probe, separating the reporter from the quencher and releasing fluorescent signal. The Script-III One Step RTqPCR Probe Kit is an ideal product for high-speed

#### **Kit Contents**

Contents	Cat.#	Size
2xOne Step RT-qPCR Probe Buffer IV*	FS-RT-21402	1.25ml x 2
One Step Probe Enzyme Mix IV**		500µl
50X ROX Dye I (High Rox)***		100µl
50X ROX Dye II (Low Rox)***		100µl
Nuclease Free Water H <sub>2</sub> O		1.25ml x 2

\* Containing dNTP/dUTP Mix, prevent false positive caused by cross contamination with UDG.

\*\* the Taq polymerase is blocked by antibody, containing RNase Inhibitor, Heat-labile UDG

\*\*\* Passive reference dye to normalize the fluorescence signals

#### Applications

- Real-time PCR
- Detection and quantification of DNA and cDNA targets
- Gene expression profiling
- Microbial detection
- Viral load determination
- Array validation
- SNP genotyping

#### **Storage Conditions**

Upon receipt, store all components at -20°C.

#### Use of the ROX Reference Dye:

#### -50x Rox Dye I (High Rox)

Applied Biosystems 7000/7300/7700/7900, Applied Biosystems StepOne<sup>™</sup>/StepOnePlus<sup>™</sup>.

#### -50x ROX Dye II (Low Rox)

Applied Biosystems 7500/ViiA7TM, QuantStudio™, Stratagene Real-time PCR Systems, Rotor-gene™ 3000

#### -NO ROX Dye

Bio-Rad iCyclers/ CFX96/ CFX 384, Roche Light Cyclers®, QIAGEN/Corbett Systems, Eppendor Mastercyclers

#### **Recommended Protocol**

**1**.Fully thaw the 2X One Step RT-qPCR Probe Buffer IV before use. Mix the buffer well and avoid directly sunlight. Determine the total number of reactions required and prepare master mix. Triple replicates for each reaction are recommended.

2. The One Step Probe Enzyme Mix IV contain high concentration of glycerin. Mix gently before use without generating air bubbles. Spin briefly to collect all the contents at the bottom. After use, return it to -20°C immediately.
3. If applicable, use aerosol-resistant pipette tips and microtubes to minimize contamination.

**4.**High quality RNA templates are recommended for optimal results

**5.**Only gene specific primers are recommended. Random primers and Oligo dT primers are NOT recommended in the reverse transcription reaction.

**6.**The optimal length of amplicon is between 70 and 200 bp for general cycling condition.

#### Prepare materials before reaction setup:

Pipette, aerosol-resistant pipette tip, cold blocks and ice. Gene expression primers and probes. RNA templates.

1.5 mL RNase-free EP tubes, Real-time PCR tubes

and plates. 1. Prepare the reaction mix :

Set up the reaction on ice by adding the following components for the number of reactions required. :

#### **Reaction Conditions**

Component	20 µL reaction	25 μL reaction	50 µL reaction
2xOne Step RT-qPCR Probe Buffer IV	10 µl	12,50µl	25µl
One Step Probe Enzyme Mix IV	2 µl	2,5 µl	5µl
10uM Forward Primer*	0.4 µl	0.5-0.6 µl	1µl
10uM Reverse Primer*	0.4 µl	0.5-0.6 µl	1µl
TaqMan Probe (10µM)***	0.4 µl	0.5-0.6 µl	1µl
50X Rox Dye (optional)	0.4 µl	0.5-0.6 µl	1µl
Totatal RNA **	2 µL	2,5 µL	5µl
Water, RNase-Free	Up to 20µl	up to 25 µl	up to 50 µl

\* A final primer concentration of 0.2  $\mu$ M is recommended for most reactions. However, to optimize individual reaction, a primer titration from 0.1  $\mu$ M to 1.0  $\mu$ M can be performed. The length of amplified PCR products should ideally be in the range of 70 - 200bp.

\*\* Use 10 pg~100 ng of RNA template in a 20 µL reaction.

# \*\*\* A Probe concentration of 50-250 nM is recommended. Optimized One Step RT-gPCR Conditions

optimized one step K1-qFCK conditions				
Step	Temp (°C)	Time	Cycle	
UDG Reaction	25°C	5 min.	1	
Reverse Transcription	50°C	5 min.	1	
Polymerase Activation	95°C	3 min.	1	
Denaturation Annealing, and	95° C	5-15 sec.	45	
Extention	60° C	30-34 sec.		

The extension time should be adjusted to the minimum time required for data acquisition according to qPCR instrument guidelines used. (30 s for Applied Biosystems StepOnePlusTM, 31 s for Applied Biosystems 7300, and 34 s for Applied Biosystems 7500)

#### Introduction

The **cDNA Synthesis Kit** is optimized for the synthesis of the First Strand cDNA, from different types of RNA.

The kit contains a mixture with both oligo(dT)18 and pd(N)9 random hexamer primers. It is suitable for the synthesis of cDNA up to 13 kb.

**The gDNA Eraser included in the kit,** can quickly and completely remove genomic DNA. Suitable for reverse transcription of various RNAs such as mRNA, IncRNA and circRNA. The kit can also be used for gene-specific reverse transcription, such as miRNA reverse transcription.

#### **Kit Components**

Components	FS-RT-1022 50 RXNS	FS-RT-1023 200 RXNS
Reverse Transcription Primer Mix*	100 µL	400 µL
RNase Inhibitor (40U/uL)	2 x 1,000 units	8 x 1,000 units
gDNA Eraser	50 µL	200 µL
5 X gDNA Eraser Buffer	100 µL	400 µL
Reverse Transcriptase (200 U/ul)	10,000U/ 50ul	40,000U/ 200ul
5 X Reverse Transcriptase Buffer	0.5 mL	1.0 mL
RNase-Free ddH2O	1.5 mL	1.5 mL
dNTPs(10mM each)	50 µL	200 µL

\*(it includes Oligo dT and Random Primer)

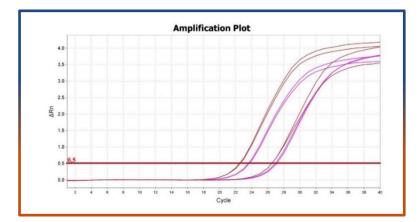
#### **Highlights**

- Full-length first strand cDNA up to 13 kb
- Optimum reaction temperature 42°C
- · Complete kit—all the components for the RT reaction are included

#### **Applications**

- First strand cDNA synthesis for RT-PCR and RT-qPCR
- Construction of full length cDNA libraries
- Primer extension.
- RNA sequencing

CAT.n°	Description	Size
FS-RT-1022	cDNA Synthesis Kit (with gDNA Eraser)	50 RX
FS-RT-1023	cDNA Synthesis Kit (with gDNA Eraser)	200 RX



This test is mainly aimed at the reverse transcription efficiency of the reverse transcription kit.

Fluorescence quantification after reverse transcription of 1ng/10ng total RNA is used to compare the difference of CT. At the same time, it is confirmed that the RNA content of NG level can also be detected after reverse transcription, reflecting the high sensitivity of the kit .The sample is rat muscle tissue

# mi-RNA FIRST STRAND-SYNTHESIS KIT FS-RT-1034

#### Introduction

This kit is suitable for cDNA first strand synthesis using microRNA as template through the tail addition method, where the Poly (A) tail addition reaction and reverse transcription reaction at the 3 'end of miRNA can be efficiently carried out simultaneously.

Script miRNA-A Enzyme Mix contains Poly (A) Polymerase (PAP) and reverse transcriptase. PAP is mainly used to add Poly (A) tails at the 3 'end of RNA molecules, and can also specifically recognize single stranded RNA, effectively avoiding RT reactions of pre-miRNA with double stranded or stem-loop structures. The modified reverse transcriptase lacks of RNase H activity and increases its affinity with RNA, resulting in a significant improvement in the efficiency and sensitivity of miRNA reverse transcription. The obtained cDNA can be directly used for qPCR detection using either SYBR Green dyebase or Taqman probe-base reagent.

#### **Kit Components**

Components	FS-RT-1034
Script miRNA-A Enzyme Mix (20X)	20 µl
miRNA-A Reaction Buffer (2X)	200 µl
Universal RT Primer	60 µl
Universal miRNA-A qPCR Primer R (10 μM)	200 µL
U6 qPCR Primer F (10 μM)	100 µL
Nuclease-free ddH2O	1 mL

\*Universal miRNA-A qPCR Primer R (10 μM) can be used together with designed qPCR forward primers for qPCR detection. \*\*U6 qPCR Primer F, a universal reference forward primer for human, mouse and rat U6, can be used together with Universal miRNA-A qPCR Primer R for qPCR detection.

#### **Product Description**

This kit is suitable for cDNA first strand synthesis using microRNA as template through the tail addition method, where the Poly(A) tail addition reaction and reverse transcription reaction at the 3 'end of miRNA can be efficiently carried out simultaneously. Script miRNA-A Enzyme Mix contains Poly (A) Polymerase (PAP) and reverse transcriptase. PAP is mainly used to add Poly (A) tails at the 3 'end of RNA molecules, and can also specifically recognize single stranded RNA, effectively avoiding RT reactions of pre-miRNA with double stranded or stem-loop structures. The modified reverse transcriptase lacks of RNase H activity and increases its affinity with RNA, resulting in a significant improvement in the efficiency and sensitivity of miRNA reverse transcription. The obtained cDNA can be directly used for qPCR detection using either SYBR Green dye-base or Taqman probe-base reagent.

#### Storage: -20°C

#### **Operation Description**

1. Add the following components to the RNase-free PCR tube on ice, mix well and centrifuge briefly.

Components	20 μL
RNA	10 pg-1 µg Total RNA or 200 ng miRNA
Universal RT Primer	3 µL
Script miRNA-A Reaction Buffer (2X)	10 µL
Script miRNA-A Enzyme Mix (20X)	1 µL
Nuclease-free ddH2O	Up to 20 µL
everse transcription reaction procedure.	

Temp.	Time
37 °C	50 min
85 °C	5 min

The product can be immediately applied to subsequent qPCR detection, or stored at -20°C. It is recommended to store at -80°C for storage longer than six months. Avoid repeated freezing and thawing.

#### Primer design for qPCR detection

Forward primer: It is recommended to design foward primers based on the complete miRNA sequence and replace U with T.

# M-MLV REVERSE TRANSCRIPTASE

**Reverse Transcriptase** is a reverse transcriptase (M-MLV-Reverse Transcriptase) obtained by genetic engineering technology to recombine Moloney murine leukemia virus. It has good heat resistance, can withstand reaction temperatures up to 55 °C, Efficient synthesis of full-length first-strand cDNA up to 13kb, suitable for reverse transcription of complex secondary structure RNA templates, provides broader gene representation and superior qRT-PCR sensitivity.

Cat N°	Size	Storage/Shelf life
FS-RT-1032	10,000 U (50 preps)	-20°C/one year
FS-RT-1033	40,000 U (200 preps)	-20°C/one year

#### **Kit Components**

Component	FS-RT-1032	FS-RT-1033
M-MLV- Reverse Transcriptase	10,000U (50ul)	40,000U (200ul)
5×RT Buffer	0.5 mL	1mL
RNase-Free Water	1.5 mL	1.5 mL

#### Kit application

- 1. First strand cDNA synthesis as a template for RT-PCR and real-time RT-qPCR
- 2. Construction of a full-length cDNA library
- 3. Antisense RNA synthesis

# RNase INHIBITOR (40 U/ul)

#### Introduction

RNase Inhibitor is a recombinant RNase inhibitor expressed in soluble form in Escherichia coli. It has the same application effect as a specific ribonuclease inhibitor present in human placenta. Its essence is a protein with a molecular weight of 51,000 Da, etc. The pH of the electrical point is 4.7.

RNase Inhibitor can specifically bind RNase A, B, and C with a non-covalent bond toform a 1:1 complex to inactivate RNase, and has a broad spectrum of RNase inhibitory activity. RNasin is active in buffers of 0-0.5 M NaCl, pH 5-8, and has the highest activity at pH 7.8. RNasin protects the integrity of mRNA and improves the efficiency of transcription and translation, while avoiding the possible effects of using organic compound inhibitors.

RNase inhibitor is compatible with various reverse transcriptases and DNA Polymerase by RT-PCR and RT-qPCR. Compared with the human RNase inhibitor, the recombinant RNase inhibitor does not contain two cysteines and thus has higher antioxidant activity and is more suitable for experiments sensitive to highDTT (such as qPCR).

Description	FS-RT-1152-1	FS-RT-1152-5
RNase Ihibitor 40U/ul	1,000 units	5,000 units

#### Application

First-strand cDNA synthesis, isolation of polysomes, in vitro translation, in vitrocell-free system transcription, in vitro transcription of SP6 or T7 RNA polymerase.

# FS-RT-1152-1

# Oligo(dT)<sub>18</sub> Primer

Oligo (dT)18 Primer is suitable for use as a primer for first-strand cDNA synthesis with a reverse transcriptase. The primer hybridizes to the poly(A) tail of mRNA

Suitable for cDNA Synthesis kit- First-Strand cDNA Synthesis

• Primer for reverse transcription of polyadenylated RNAs, such as mRNAs

CAT.#	Description	Size
FS-OTD-50	Oligo (dT)18 Primer	50 ul

## RANDOM HEXAMER PRIMER 9

FS-RH-50

High-quality DNA hexamers of randomized sequence

Random Primers are random hexadeoxynucleotides that can be used for first-strand cDNA synthesis and cloning. They are also available as components of the Reverse Transcription System

CAT.#	Description	Size
FS-RH-50	Random Hexamer Primer 9	50 ul

# Nucleic Acid Stains Nucleotides &

# **DNA Ladders**

**Nucleic Acid Stains** 

dNTP SET (High Concentration)

dNTP SET Mix

1 Kb DNA Ladder (RTU)

100 bp DNA Ladder (RTU)

50 bp DNA Ladder (RTU)

# NUCLEIC ACID STAINS

Our Nucleic Acid Stains are ultra sensitive, extremely stable and environmentally safe fluorescent nucleic acid dye designed to replace the highly toxic ethidium bromide (EB) for staining dsDNA, ssDNA or RNA in agarose gels or polyacrylamide gels. Our Nucleic Acid Stains are is far more sensitive than EB without requiring a destaining step.

#### Features:

- Safer than EB: Shown by the Ames test and other tests to be non-mutagenic and noncytotoxic
- Easy disposal: Passed environmental safety tests for direct disposal down the drain or in regular trash
- Ultra-sensitive: Much more sensitive than EtBr
- **Extremely stable**: Available in water, stable at room temperature for long-term storage and microwavable
- Simple to use: Very simple procedures for precast or post-electrophoresis gel staining
- Compatible with a standard UV transilluminator: Replaces EtBr with no optical setting change
- Compatible with downstream applications: Gel purification, restriction digest, sequencing and cloning

Cat.#	Description	Size
FS-02	GREEN GEL SAFE Nucleic Acid Stain conc. 10,000X	1 X 0,5 ML
FS-005	CLEARSIGHT Nucleic Acid Stain conc. 20,000X	1 X 1 ML
FS-GEL01	GREEN GEL PLUS Nucleic Acid Stain High conc. 50,000X	2 X 0,5 ML
FS-33102	SYBR SAFE Nucleic Acid Stain conc. 10,000X	1 X 400 µL
FS-GEL02	EUROSAFE GREEN	1 X 1 ML
FS-31	MIDORI GREEN	1 X 0,5 ML

#### ETHIDIUM BROMIDE DESTROYER

Fisher Molecular Biology Eth Br Destroyer is a specifically designed reagent effectively degrade and destroy the Ethidium Bromide and result in non-fluorescence and non-mutagenic remain. And also it has been demonstrated that its effectiveness of destructing the SYBR dyes. The FMB EtBr Destroyer Sprayer is for the treatment of solid Ethidium Bromide contaminant. The Sprayer can be used for the treatment of solid contaminant waste including electrophoresis gels, glassware, paper towels, gloves, laboratory equipment, bench surface etc

#### Features:

- Effective: EtBr destroyer can destroy EtBr and other SYBR Dyes. This effect can be monitored and confirmed by UV light exposure. Once destroyed, the fluorescence will disappear.
- Safe: The blocking of mutagenic effect of EtBr Destroyer has been demonstrated by Ames Test
- Fast: For general protection of uncontaminated area, spray the EtBr Destroyer on the entire working area, leave for about 5 minutes, then wipe it dry with paper towel.

Cat.#	Description	Size
EDB-30	Ethidium Bromide Destroyer Sprayer (2 X 200 ML) 1 sprayer contains 0.4 ml -1 sprayer can do 600 T.	2 Sprayers/box

FS-013-1

100 mM dNTP Mix is a mixture of 4 deoxynucleotides (dATP, dCTP, dGTP, dTTP) in purified water. Each nucleotide is at a concentration of 100 mM.

100 mM dNTP mix is suitable for use in polymerase chain reaction (PCR), sequencing, fill-in reactions, nick translation, cDNA synthesis, and TdT-tailing reactions, qPCR, RT\_qPCR.

#### Features:

- Chemically synthesized
- pH 7.5
- Free from qPCR, PCR, reverse transcription inhibitors
- Free of DNases and RNases
- Free of human and E. coli DNA

Size	Description	Size
FS-0131-1	dNTP Mix - NUCLEOTIDE SET (High concentration)	4 x 250 µl

#### dNTP Mix – Conc. 10 mM

#### FS-013-2

FS-013-4

dNTP Mix contains premixed aqueous solutions of dATP, dCTP, dGTP and dTTP, each at a final concentration of 10 mM. The nucleotides have greater than 99% purity, are free of nuclease activities, human and E. coli DNA. Mixes offer the possibility to reduce the number of pipetting steps and the risk of reaction set up errors. They are designed for many different molecular biology applications. Standard PCR, High-fidelity PCR, RT-PCR, Real Time PCR (qPCR)

#### Highlights

- Greater than 99% purity confirmed by HPLC
- Free of human and E. coli DNA
- Highly stable

Size	Description	Size
FS-0131-2	dNTP Mix - 10mM	500 µl

#### dNTP Mix – Conc. 20 mM

dNTP Mix contains premixed aqueous solutions of dATP, dCTP, dGTP and dTTP, each at a final concentration of 20 mM.

Applications:PCR, real-time PCR, high fidelity and long PCR, LAMP-PCR, cDNA synthesis, RT-PCR, RDA, MDA, DNA labeling, and DNA sequencing.

Size	Description	Size
FS-0131-4	dNTP Mix- 20mM	500 µl

## 1 kb DNA Ladder 100 bp DNA Ladder

#### **Product Description**

The 1 kb DNA Ladder RTU is suitable for sizing linear doublestranded DNA fragments from 250 bp to 10 kb. The 1 kb and 3 kb bands contain more DNA to provide internal orientation.

The 100 bp DNA Ladder RTU is suitable for sizing linear double-stranded DNA fragments from 100 bp to 1500 bp. The 500 bp and 1,500 bp bands contain more DNA to provide internal orientation.

The ladders are generated from PCR and restriction enzyme digestion of double stranded DNA. The DNA is purified by phenol extraction and diluted in 1X loading buffer. Approximate amounts of DNA per band per 5 uL (100 ng) ladder are listed in Figure 1 for reference, and are not intended for quantification of unknown DNA samples.

The loading buffer provided contains density agents and two blue electrophoresis tracking dyes that run at approximately 1.5 kb and 200 bp in a 1% agarose gel.

#### Protocol

The Ready-to-Use DNA Ladders are supplied in a ready-to-

load format. There is no need to mix with 6X loading buffer prior to loading onto a gel. For agarose gel electrophoresis, load 100-200 ng of DNA ladder (5-10 uL) per 5 mm lane.

#### Storage

Store at 4°C for 6 months or at -20°C for 24 months.

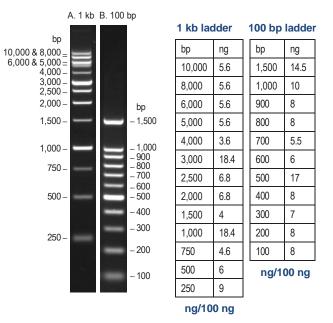


Figure 1. 100 ng of 1 kb DNA Ladder or 100 bp DNA Ladder were run on a 1% agarose/TBE gel containing 1X GelRed Nucleic Acid Gel Stain in 1X TBE at 5 V/ cm for 90 minutes. Gels were imaged using a UVP GelDoc-It imaging system with ethidium bromide filter. Fragment sizes in base pairs (bp) are shown next to each band. Approximate mass per band is shown for 5 uL (100 ng) DNA ladder in the tables at right.

CAT#	Description	Components	Size
FS-MW-600RT	100 bp DNA Ladder <i>RTU</i>	100 bp DNA Ladder in 1 X DNA Loading Buffer	500 ul
FS-MW-500RT	1KB DNA Ladder <i>RTU</i>	1KB DNA Ladder in 1 X DNA Loading Buffer	500 ul

# **READY-TO-USE DNA LADDER**

#### 50 bp DNA Ladder

#### Effective Size Range:

The **50 bp DNA Ladder: 50 to 1200 bp**, 12 fragment, 300 bp and 1200 bp bands have increased intensity.

Recommended Load: from 0.5 ug per lane or 4-6 Ul for Ready-to-Load Ladder.

#### **Concentration:**

500  $\mu$ g/ml in 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA for markers without loading dye; 83 ng/ul for Ready-to-Load Ladders.

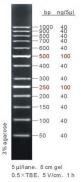
The Ready–to-Load Ladder buffer contains 10 mM Tris HCl (ph 8.0) and 5 mM EDTA,10% glycerol, 0.003% Bromophenol Blue, 0.006% orange G and 0.003% Xylene Cyanol FF

Store : at 4°C for six months or at -20°C for 24 months.

CAT.#	Description	Size
FS-MW-011	50 bp DNA Ladder RTU	500 ul

# 50bp ladder plus

50 bp



# AGAROSE D1-LE MOLECULAR BIOLOGY STANDARD

AS-101

D-1 LE: with Low EEO.

High electrophoresis mobility ideal for DNA and RNA fragments as well as PCR products, for preparation of plasmids, for screening, cloning and blotting techniques.

- Nucleic acid analytical and preparative electrophoresis.
- Blotting

Protein electrophoresis such as radial immunodiffusion.

Size: 500 gr

# AGAROSE MS-8 METAPHOR

An agarose for molecular screening that improves resolution of small DNA fragments and PCR products. Recommended for analytical gels for DNA ≤1,200 bp.

#### Functional Tests:

- DNA resolution: bands appear sharp and finely resolved.
- DNAse/RNAse activity: none detected.
- Gel background: very low after EtBr staining.
- DNA binding: very low

Size: 100 gr

AGAROSE AS-101		
Moisture	4.62%	
Ash	≤ 0.43%	
EEO * (pH8.4)	0.12	
Sulfate	≤ 0,097%	
Clarity 1.5% (NTU)	3.89	
Gel Strength 1% (g/cm2)	≤1.180	
Gel Strength 1.5% (g/cm3)	≤2.920	
Gelling temperature 1.5% (°C)	36.7	
Melting Temperature 1.5% (°C)	88.2	
DNase/RNase activity	None detected	
DNA Resolution 1000 bp	Finely Resolved	
Gel Background	Very low	

## AS-109

GAROSE METAPHOR	1,5%	3%
Moisture	=4.36%	
Ash	=0,26%	
EEO*	=0,11	
Sulfate	$\leq$ 0,075%	
Clarity (NTU)	3,83	
Gel Strength (g/cm2)	1,965	3,810
Gelling Temperature (°C)		33,5
Melting Temperature (°C)		73,3
* EEO (electroendosmosis)		

Ranges of separation:

```
1.8% 400-1200 bp
                   3.0% 150-800 bp
                                      4.5% 15-400 bp
```

AGAROSE PULSED FIELD (Gel Electr

# AGAROSE (PULSED FIELD ELECTROPHORESIS

## AS-108

Agarose Pulsed Field Gel Electrophoresis is a linear polymer with a very high	
molecular weight, giving gel structures unlike those of traditional agaroses.	Γ
This characteristic, added to the very low sulfate content, produces a strong	Γ
intercatenary interaction, yielding a gel with very high gel strength and higher	
exclusion limit.	-
<ul> <li>Pulsed Field Gel Electrophoresis: because of its higher exclusion</li> </ul>	

- limit, larger molecules can be separated
  - Separation Range: from ≥1 Kb up to 40 Kb. Blotting.
- Agarose Beads preparation. Cell and enzyme immobilization

Size: 100 gr

# AGAROSE NUSIEVE 3:1

NuSieve 3:1, a standard gelling/melting temperature agarose, is designed for analytical electrophoresis wherehigh resolving capacity is required. Recommended for DNA analytical gels at 2% concentrations, it can separate 30 -1,500 bp fragments The viscosity is low, so it is easy to make gels at high concentrations which have a very high resolving capacity.

Solutions of 4% or higher are feasible because of this low viscosity.

Because of the high gel strength, gels can also be prepared at lower concentrations, 1.0-1.5%. Gels are strong, flexible and very easy to handle. These features make NuSieve 3:1 gels compatible with blotting of small fragments. Size: 100 gr

# AGAROSE LOW MELTING

The low melting temperature allows for the recovery of undamaged nucleic acids below the denaturation

temperature. The low gelling temperature ensures that the agarose will be in a liquid state at a temperature range where In-Gel manipulations can be performed without prior extraction of the DNA from the gel slice. Applications

LM (Low Melting): with the highest gelling/melting temperatures and gel strength.

- Electrophoresis of DNA fragments ≥ 1000 bp
- Tissue and cell culture.

Viral plaque assays

Size: 25 gr Size 50gr

Moisture	≤ 5,12%	
Ash	$\leq$ 0.22 %	
EEO*	≤ 0.11	
Sulfate	≤0.083%	
Clarity (NTU)	$\leq 4$	
Gel Strength (g/cm2)	≥ 1,910	
Gel Strength 1.5% (g/cm2)	≥ 3,900	
Gelling Temperature (°C)	36,3	
Melting Temperature (°C)	88,5	
DNAse/RNAse activity	None Detected	
DNA resolution	$\geq$ 1 Kb- Up to 40 Kb	
Gel background	Very Low	

# AS-110

AGAROSE NuSieve 3:1		
Moisture	10%	
Ash	0.4%	
EEO*	0.13	
Sulfate	0.15%	
Clarity 4% (NTU)	4	
Gel strength 4% (g/cm2)	1400	
Gelling temperature 4% (°C)	32.5-38	
Melting temperature 4% (°C)	90	
DNase/RNase activity	None detected	
	2%:500 – 1500 bp	
Ranges of separation	4%:150 – 600 bp	

## AS-107

AGAROSE LOW MELTING		
Moisture	≤ 7%	
Ash	≤ 0.4%	
EEO *	≤ 0.12	
Sulfate	≤ 0,10%	
Clarity 1.5% (NTU)	≤ 4	
Gel Strength 1% (g/cm2)	≤ 250	
Gelling temperature 1.5% (°C)	26	
Melting Temperature 1.5% (°C)	≤ 65.5	
DNase/RNase activity	None detected	
Separation Range	≤1 bp	
Inhibitors	none	

#### DITHOTHREITOL (DTT)

FS-0912

Formula : C4H10O2S2 Formula weight : 154.24 CAS #27565-41 -9 Product Specifications: Form : White crystalline powder Assay (S-H): 99.5% Melting point : 40 - 43°C A(280nm,=.1M, 1 cm) :≤0.06 A(260nm,=.1M, 1 cm) :≤0.40 Oxidized DTT : ≤0.2% Storage : -20°C Size: 10 g

#### **EDTA**

FS-03620

## Ethylenediaminetetraacetic Acid

Formula: C10H Assay: ≤ 98.0% Water:<1.0% Heavy Metals (as Pb):<0.001% Sizes: 100g , 500g, 1 Kg

#### GLYCINE

FS-5037G

Assay by titration 99+% anhydrous Purity (by TLC) one spot Water (by Karl Fisher) ≤ 1.0% pH (1.0M)= 6.2± 0.3 A280 < 0.05 (1 .0M in H2O) A260 < 0.05 (1 .0M in H2O) IR: Conforms to known reference Sizes: 1Kg - 5 Kg

## GLYCEROL

**FS-7009** 

FS-3071

**FS-0481** 

Formula: C3H803 MW: 92.09 Purity: 99.5+% Cas# 56-81-5 DNase - Rnase - none detected

Glycerol does not freeze at -20°C Size: 500 MI

#### **HEPES**

Cas No 7365-45-9

(4-(Hydroxyethyl)piperazine-1-ethanesulphonic acid) HEPES may be used as an alternative to PBS. It is the most generally used zwitterionic buffer which improves pH control between pH 6.7 and 8.4 and is obtained when 20-50 mM HEPES is incorporated into culture media. TBS and PBS may be used as washing buffers for alkaline and peroxidase conjugates in Western blotting as well as in various Cell Biology applications. Sizes: 100g - 500g - 1Kg

#### **IPTG**

Isopropyl-b-D (thiogalactopyranoside)

Presented as a white crystalline powder Application: A gratuitous inducer of the E.coli lac + colonies or cells in a colorimetric assay Size: 10gr

#### MOPS

**FS-2071** 

White powder, MW 209.3 Assay (by tritation); 99.5+% Water (by Frank Fisher): ≤ 1.0% Forms a clear, colorless solution in water (10%) ph(1%)=4.0 Size: 1 Kg

## PROTEINASE K (Powder) FS-M-112

Cas No: 39450-01-6 Grade: High purity grade, for Molecular Biology Purity: 99% Specific activity: 35 units/mg of protein DNase - none detected RNase - none detected Endonuclease (nickase) - none detected Store: at -20°C Size: 100 mg

#### SODIUM DODECYL SULFATE FS-0109

#### Ultrapure

Formula: C12H25Na04S CAS # 151-21-3 Moisture : ≤1 % Insolubles : ≤0.003% Chloride (CI) : ≤0.01 Phosphate (PO4) : ≤1 ppm Copper (Cu) : ≤5 ppm Iron (Fe) : ≤1 ppm Lead (Pb) : ≤5 ppm

Formula weight : 288.38 Form : White crystalline flakes A(280nm,3%1cm):≤0.1 A(230nm,3%1cm):≤0.2 Assay : ≥99% (titrimetric) Assay(C1 2) : ≥98% (GC) Storage : Ambient Sizes: 100g , 500g

## SUCROSE

TEMED

Formula: C12H22O11 Formula weight: 342.30 CAS # 57-50-1 Product Specifications: Form: White crystalline powder Identity: IR Purify : ≥99.5% Storage : +20°C Sizes: 1 kg - 5 kg

#### **FS-3009T**

**FS-1503** 

**FS-5393** 

#### N,N,N',N' Tetramethylethyldiamine

Form: clear colorless liquid MW 116.2 Assay (by titration) 97+%

Forms a clear solution in water (20%) pH(0.5%)=10.5±0.5 A400 ≤0.05(20% IN H2O) IR: Conforms to known reference Size : 100 ml

#### TRIS BASE ULTRAPURE

#### equivalent to TRIZMA BASE (Sigma)

M.W. (Tris base /tris HCI) 121.1 / 157.6 Purity: >99.8% Moisture: <1.0% A280(1.0M,water) < 0.05% Insolubles: < 0.005% Arsenic: <0.0005% Copper: <0.0001% Iron: <0.0001%

UREA ULTRAPURE

Formula: NH2-CO-NH2 Formula weight : 60.06 CAS #57-1 3-6 Form:White crystalline powder Identity : By IR Assay : ≥99.5% Melting point : 132 - 135°C Insolubles : Negligible Turbidity : ≤2NTU A(260nm,6M, 1 cm) : ≤0.055 A(280nm,6M, 1 cm) : ≤0.044

Magnesium: < 0.0001% Heavy Metals:<0.0001% DNase, RNase, protease: none detected Storage : Rt Sizes: 1 kg, 5 kg

#### **FS-0114**

Copper (Cu) : ≤0.5 ppm Iron (Fe) : ≤0.5 ppm Lead (Pb) :≤0.5 ppm Chloride (CI) :≤0.0005% Cyanate : None detected Conductivity : ≤1 5 µmho/cm DNase (endo) : None detected Rnase : None detected Protease : Non detected Storage : RT Size: 1 kg/5 kg

# ACRYLAMIDE SOLUTIONS - "READY TO USE"

**Fisher Molecular Biology**'s liquid Acrylamide Solutions are made from highest quality pure material to exact standards. This ensure crystal clear electrophoresis gels which give realiable and reproducible results for separation of DNA and Protein Biomolecules.

CAT. N°	Description	Size	Applications
FS-2600	30% Acrylamide, Acrylamide to Bis-Acrylamide ratio 29:1	500 ml 1,000 ml	Separation of small acrylamide to bis-dsDNA fragments acrylamide (<1 kbps) + proteins
FS-2100	30% Acrylamide, Acrylamide to Bis-Acrylamide ratio 37.5:1	500 ml 1,000 ml	Preparation of protein gels

# **BIOLOGICAL BUFFERS**

Fisher Molecular Biology provides a range of pre-filtered formulated buffer concentrates for a range of Molecular and Cell Biology application. Made from Ultrapure Reagents of molecular Biology Grade. Each lot is tested for DNase , RNase and protease Activity.

CAT. N° Descritipion		Size
FSB-6002-10	TAE Buffer (10X)	1 L
FSB-6000-10	TBE Buffer (10X)	1 L
FSB-7301-10	TBS Buffer (10X)	1 L
FSB-74-10 PBS Buffer (1X)		500 ml
FSB-7415DDulbecco's PBS Buffer (1X)		500ml
FSB-6201TE Buffer (1X)		1 L
FSB-2052-100PBS Buffer Tablets (200 ml/each)		100 tablets

# DNA Extraction & Purification

**GEL Extraction & PCR Clean UP Kit** 

**MicroElute GEL Extraction and PCR Clean UP Kit** 

33

# **GEL EXTRACTION / PCR CLEAN UP KIT**

#### **Description**

The DE-001 Gel Extraction & PCR Clean Up Kit is designed to recover or concentrate DNA Fragment (50bp- 10Kb) from agarose gel, PCR or other enzymatic reaction. The unique dual purpose application and high yield DNA column make this kit exceptional value.

#### **Features**

- With simple steps, quick and easy to use.
- Highly pure DNA (suitable for PCR).
- No phenol/chloroform extraction and ethanol
- precipitation required.

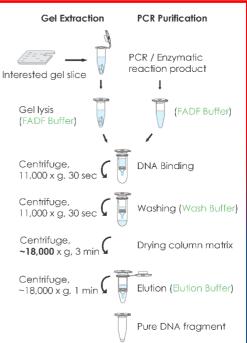
#### **Applications**

- PCR
- Fluorescent or Radioactive Sequencing
- Restriction Digestion
- DNA Labeling
- Ligation and Transformation

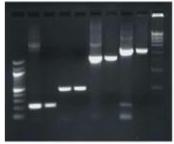
#### **Specification:**

Principle: spin column (silica matrix) DNA Binding capacity of spin column: 20 μg Sample size: up to 300 mg of agarose gel up to 100 μl of reaction solution Recovery: 70% ~ 85% for Gel extraction 90% ~ 95% for PCR clean-up Operation time: 10 ~ 20 min

Elution volume: 40 µl



M1 1 2 3 4 5 6 7 8 M2



The Quality of DNA After Purification DNA fragments before and after extraction with the GEL Extraction and PCR Clean Up Mini Kit Lane 1, 3, 5, 7 before extraction: 200bp, 500bp, 2Kb, 3Kb. Lane 2, 4, 6, 8 after extraction: 200bp, 500bp, 2Kb, 3Kb M1: 1 00bp DNA Ladder M2: 1 Kb DNA Ladder

**Procedure:** The method uses a chaotropic salt, guanidine thiocyanante to dissolve the agarose gel and denature enzymes. The DNA fragment in the chaotropic salt is bond to the glass fiber matrix of the spin column. After washing off the contaminants, the purified DNA fragments are eluted by a low salt elution buffer or water. Salts, enzymes and unincorporated nucleotides can be effectively removed from reaction mixture without phenol extraction and alcohol precipitation.

Cat. N.	Product Name	Size	Kit Components	Store at
DE-001	GEL Extraction & PCR Clean Up Kit	100 preps	Wash Buffer (Conc.)	Store at room temperature
DE-002		300 preps	Elution Buffer FSDF columns 2 ml Collection tubes	for 1 year.

The MicroElute Gel Extraction/PCR Clean UP Kit allows isolation and concentration of DNA fragments, 70bp~4Kb, from agarose gel, PCR reaction or enzymatic reactions. This kit eliminates impurities and salt efficiently from the sample matrix. The purified DNA fragments can be used diectly fro downstream applications and the end elution volume can be as low as 10  $\mu$ l to obtain high concentration of DNA.

#### **Specifications:**

**Principle:** spin column (silica matrix)

DNA Binding capacity of spin column: 5 µg

Sample size:up to 200 mg of agarose gel

up to 100  $\mu$ l of reaction solution **DNA size:** 65 bp ~ 10 kbp

**Recovery:** 70% ~ 85% for Gel extraction 85% ~ 95% for PCR clean-up

Operation time: 10 ~ 20 min

Elution volume: 10 ~12 µl

#### **Applications**

Purified DNA is ready for downstream applicatons such as sequencing, ligation, labeling, amplication and enzymatic digestion.

#### **Procedure**

The DNA fragments in the chaotropic salt, are bonded to the glass-fiber matrix of the spin column. After washing off the contaminants, the purified DNA fragments are eluted by low-salt elution buffer or ddH2O. Salt, enzymes and unincoporated nucleotides can be effectively removed from the reaction mixture without phenol/ chloroform extraction and alcohol precipitation.

#### **Storage Conditions**

Stable for 1 year at room temperature.

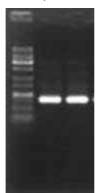
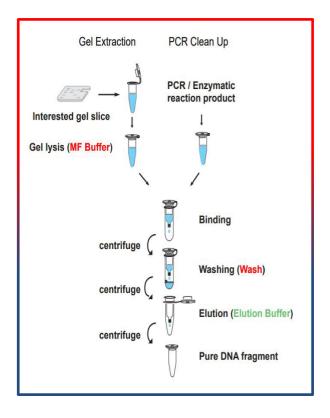


Fig.1: Agarose gel analysis of PCR product before and after purification with MicoElute Gel/PCR Purification.M: 1Kb DNA Ladder. Lane 1: Before purification Lane 2: after purification

#### **Ordering Information**

CAT.N°	Product Name	Size	Kit Components	Storage
DE-020	MicroElute Gel Extraction/ PCR Clean UP Kit	100 preps	GEL Lysis Buffer PCR Binding Buffer Wash Buffer conc. Elution Buffer FAPC-2 Columns 2 ml Collection Tube	Store at RT for 1 year



# Blood & Tissue DNA Extraction &

# Purification

**Blood & Tissue Genomic DNA Extraction Kit** 

**Tissue Genomic DNA Extraction Kit** 

**Tissue Genomic DNA MicroElute Kit** 

Mouse Direct PCR Kit (for genotyping)

Mouse Tails Direct PCR Lysis Buffer (tails, ears, yolk sac, cultured cells)

#### Description

The Blood & Tissue Genomic DNA Extraction Kit is designed for rapid extraction of pure, small-scale genomic & mitochondrial DNA from Whole Blood (not frozen), dried blood spots, buffy coat and several types of tissues (fresh or frozen), fixed tissues (Formalin, Paraffin), Bacteria, Yeast and Amniotic Fluid, sea urchins, marine mollusks and octopus.

#### **Features**

-Rapid isolation cellular DNA from animal tissue (e.g. mouse tails), bacteria, yeast etc.

-Purified DNA is ready for downstream application such as PCR, Southern blotting.

-Centrifugation-based method.

- -Efficiently remove cellular debair and inhibitors
- -No phenol/chloroform extraction and ethanol precipitation.

#### Applications

- PCR
- Southern Blotting
- Forensic Analysis

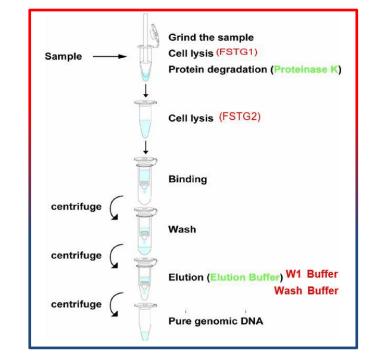
Principle: spin column (silica membrane) Operation time: 30 - 60 min Binding capacity: up to 60 µg/ column Minimum elution volume: 50 µl

#### Sample Sizes:

Up to 200 µl whole blood, serum, plasma, body fluids

- < 25 mg animal tissue
- 1.2 cm mouse tail
- < 10<sup>7</sup> cultured cells

DNA Yield: 4~8 µg/ 200 µl (whole blood) DNA Yield: 15 ~35 µg/ prep (tissues)



Cat.n.	Description	Size	Kit Contents	Storage
DE-047		100 preps	Proteinase K (powder) FSTG1 Buffer FSTG2 Buffer W1 Buffer	Store at room temperature.
DE-049-200	Blood & Tissue DNA Extraction	200 preps	Wash Buffer (concentrated) FSTG Columns 2 mL Collection Tubes 1.5 Elution tubes	Except Proteinase K, store at +4°C.
DE-049-400		400 preps	Micropestles	

#### Description

The Tissue Genomic DNA Extraction Kit is designed for rapid extraction of pure, small-scale genomic & mitochondrial DNA from several types of tissues: fixed tissues (Fresh, Frozen, Formalin, Paraffin) whole Blood, buffy coat, bacteria, yeast, fungi and ,saliva dry blood spots, viral, hair, bone tissue, dental tissue, insects, amniotic fluid, sea urchins, marine mollusks, octopus and insects.

#### Sampling

- Principle: mini spin column (silica matrix)
- Operation time: 30 ~ 60 minutes
- Binding capacity: up to 60 ug DNA/column
- Typical yield: 15 ~35 ug/ prep
- Column applicability: centrifugation and vacuum
- Minimum elution volume: 50 ul
- Sample size: < 25 mg animal tissue
  - 1.2 cm mouse tail
    - < 10<sup>7</sup> cultured cells

Rapid isolation cellular DNA from animal tissue (e.g. mouse tails), bacteria, yeast etc.

Purified DNA is ready for downstream application such as PCR, Southern blotting.

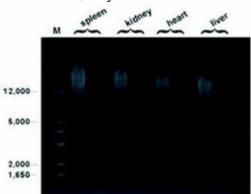
Centrifugation-based method.

Efficiently remove cellular inhibitors

No phenol/chloroform extraction and ethanol precipitation.

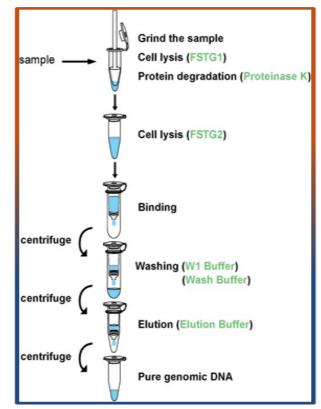
#### Applications PCR Southern Blotting Forensic Analysis

The Quality of DNA after Purification



Genomic DNA Extracted from the indicated mouse tissue by the Tissue Genomic DNA Extraction Kit

For each tissue, the amount of undigested (left) and EcoRI digested (right) are equivalent M1: 1Kb DNA Ladder (100bp-12,000bp)



Cat. N.	Product Name	Size	Store at
DE-012	Tissue genomic DNA Extraction Mini Kit	100 preps	Store at room temperature for 1 year. (Except Proteinase K:store at -20°C)
DE-013		200 preps	

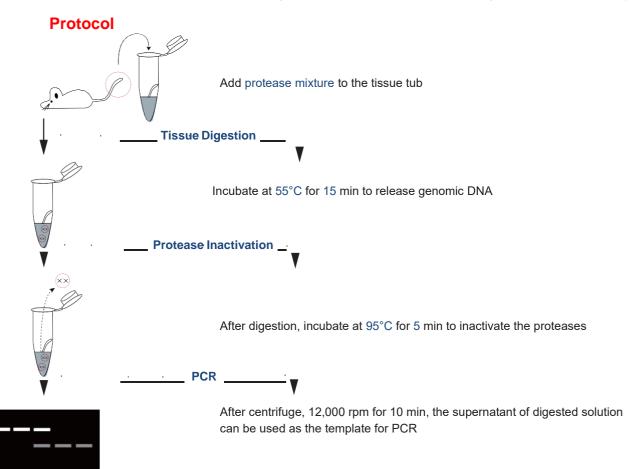
# **MOUSE DIRECT PCR KIT**

#### **Description:**

The Mouse Direct PCR Kit provides a fast preparation and PCR amplification that is specifically designed for mouse genotyping. The Buffer L and Protease Plus rapidly digest mouse **tail**, **ear** and **toe** to release intact genomic DNA that can be used directly as the template for PCR amplification. By using this kit, the digestion process only takes **15 min**. In addition, the 2x PCR Master Mix (which includes an optimized Taq Polymerase) ensures high amplification efficiency of target DNA.

#### Storage

Buffer L should be stored at 4°C. Other reagents should be stored at -20°C. All reagents can be stored for 2 years.



#### Components

Contents	DE-070 (200 rxns)	DE-071 (500 rxns)
Buffer L	20 mL	50 mL
Protease Plus	0.4 mL	1 mL
2 x PCR Master Mix <sup>a</sup>	2 mL	5 mL

a. 2x PCR Master Mix includes more powerful DNA polymerase, dNTPs, Mg<sup>2+</sup>, and DNA Loading Dye.

# MOUSE TAILS DIRECT PCR LYSIS BUFFER

# FLB-1001T

Direct PCR Lysis Buffer was especially developed for the lysis of mouse tail tissue, and other tissues. After a brief heat treatment, the crude lysates are directly used for PCR without time-consuming genomic DNA isolation.

Using Fisher Molecular Biology Mouse Tails-Direct PCR Lysis Buffer, DNA extracts can be easily obtained directly from

- Mouse Tails
- Mouse Ears
- Yolk Sac
- Culture cells



#### No purification of DNA is required

The DNA extracts will be suitable for one-step PCR genotyping and PCR amplifications.

**Fisher Molecular Biology** Direct PCR Lysis Buffer are <u>single-tube systems</u> for rapid, convenient, and reliable preparation of DNA from mouse tails, ears, yolk sacs, and culture cells.

The innovative system developed by **Fisher Molecular Biology** allows the resulting DNA crude extracts to be ready for genomic PCR for genotyping in less time and less hands-on involvement.

Crude extracts of biological samples are not compatible with many molecular biology-grade reactions such as polymerase chain reaction (PCR), in part due to inhibitors contained in crude extracts.

The **Direct PCR Lysis Buffer** not only mediate the *rapid lysis of biological samples* but also contain inhibitors that effectively suppress the inhibitory activities of crude lysates for PCR amplification, while maximally *maintaining the integrity of released genomic DNA*. Our lysis reagents completely eliminate any solution transfer or tube-opening steps, providing you with substantial extra time and less risk.

#### **Brief procedure:**

- 1. Lyse tails in Direct PCR Lysis Buffer
- **2.** Incubate for 45 min at 85°C.
- 3. PCR genotyping with 1 µl lysates.

Detailed protocols: Tails, Ears, Yolk Sac, and Cultured cells.

The Direct PCR Lysis system offers advantages and savings over conventional protocols that include:

- Time: Virtually no hands-on time. Crude tail lysates for PCR.
- · Safety: No organic reagents.
- Environmental: Less waste (organic reagents, tubes, tips, etc...)
- Reliability: Virtually 100% success rate with high yields.

#### **Direct PCR Lysis Reagents**

Cat #	Description
FLB-1001T	Direct Lysis Buffer for mouse tails (100 ml) (500 tails)
FLB-1002E	Direct Lysis Buffer for mouse ears (100 ml) (1000 ears)
FLB-1003Y	Direct Lysis Buffer for Yolk sac (100 ml)
FLB-1004C	Direct Lysis Buffer for Cultured cell (100 ml)

# **Plasmid DNA**

# **Extraction & Purification**

**Plasmid DNA Extraction Mini Prep Kit** 

Plasmid DNA Extraction Midi Prep Kit (Endotoxin Free)

Plasmid DNA Extraction Maxi Filter Kit (Endotoxin Free)

# PLASMID DNA EXTRACTION MINI PREP KIT

The Plasmid DNA Extraction Kit provides a rapid, phenol-free method for the extraction of high-purity plasmid DNA from bacterial cultures such as E. coli , which bacteria is pellet, lysed, and then neutralized. The extracted DNA can be used in a variety of applications such as PCR, cloning, sequencing, in vitro transcription, and labeling. Also, as a column-type tube is utilized in the purification process, extraction is carried out in three simple steps of binding / wa- shing / elution. Once bound, the DNA is washed and then eluted from the column, ready for use.

#### **Features**

- For high yields of plasmid DNA-up to 30µg from 1~5ml overnight cultures.
- Effective purification of DNA fragments ranging from 100bp to <15kb.
- No need for messy resin slurries, extracting with phenol, or concentrating via alcohol precipitation. Superior
- purity-DNA yields quality sequence data using automated or manual methods.
- Optimized buffers are included for maximum DNA purity and yield.
- Versatile protocol-works with all neutral gel buffers and both conventional and low-melting agarose gel.

#### Format

Spin Columns

#### **Specifications**

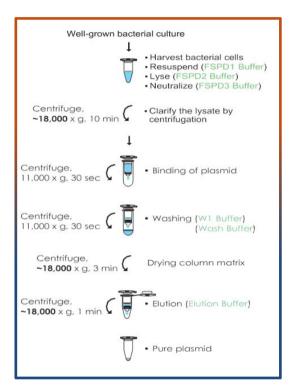
Principle:mini spin column (silica matrix)Sample size:1 ~ 5 mlSize of plasmid or construct:< 15 kb</td>Operation time:25 minutesTypicalYield:20 ~ 30 μg of high copy plasmid 3<br/>~ 10 μg of low copy plasmidBinding capacity:60μg/column

Column applicability: centrifugation and vaccum

#### **Applications**

Fluorescent or radioactive Sequencing

- Ligation
- Restriction enzyme digestion
- Ligation and Transformation
- Library screening



Cat. N.	Product Name	Size	Store at
DE-034	Plasmid DNA Extraction Mini Prep		Store at RT for 1 year
DE-035	Plasmid DNA Extraction Mini Prep		Store FSPD1 Buffer with RNase A included at +4°C. Store the RNAse A vial at -20°C for 1 year.

# PLASMID DNA EXTRACTION MIDI PREP KIT

#### (ENDOTOXIN FREE)

The Plasmid DNA Extraction Midi Kit Endotoxin free, is designed for rapid and efficient extraction of high quality plasmid DNA. With provided filter cartridges the bacteria lysates will be removed without centrifugation. Following a gravity-flow procedure, the plasmid DNA is bound to the resin, and the contaminants can be removed with wash buffer. After using this convenient kit, the purified plasmid DNA is suitable for downstream application such as transfection, in vitro transcription and translation, and all enzymatic modification.

#### **Specifications:**

Technology:<br/>Lysate clarification:Anion-exchange chromatography (gravity-flow column)<br/>centrifugation<br/>up to 60 ml of bacteria for high-copy number plasmid<br/>up to 120 ml of bacteria for low-copy number plasmidPlasmid or constructs range:<br/>Binding Capacity:3kbp ~ 150kbp<br/>650 μg / Midi Column

#### **Important Notes:**

- 1. Store RNase A at -20 °C upon receipit of kit.
- 2. Adding the provided RNase A to PM1 Buffer:
- Add 1 ml of PM1 Buffer to a RNase A tube, vortex the tube to dissolve the RNase A completely.

Transfer the total RNase A mixture back to the PM1 bottle,

- mix well by vortexing and store the PM1 buffer at 4 °C.3. If precipitates have formed in PM2 Buffer, warm the buffer in 37°C waterbath to dissolve preciptates.
- 4. Prechill PM3 Buffer at 4 °C before starting.

#### •Harvest bacterial cells • Resuspend (PM1 Buffer) •Lyse (PM2 Buffer) •Neutralize (PM3 Buffer) •Clarify the lysate by centrifugation Equilibrate PM Midi Column by gravity flow (PEQ Buffer) •Add endotoxin remove Buffer (PTR Buffer), incubate on ice, 30 min l Ţ Binding of plasmid •Column Washing (PW Buffer) Ļ • Plasmid Elution (PEL Buffer) Precipitate DNA by centrifugation Wash plasmid DNA Dissolve plasmid DNA Pure plasmid

Bacterial culture

#### **Additional Requirements:**

- 1. 50 ml tube
- **2.** Refrigerated centrifuge capable of  $\geq$  5,000 x g and the centrifuge tube suitable for the centrifuge rotor
- 3. Isopropanol
- 4. 70% ethanol
- 5. TE buffer or ddH2O

Cat. N°	Product Name	Size	Store at:
DE-051EF	Plasmid Extraction Midi Prep Kit Endotoxin Free	25 preps	At Room Temperature RNase A at -20°C

## PLASMID DNA EXTRACTION MAXI FILTER KIT (ENDOTOXIN FREE)

The Plasmid DNA Extraction Maxi Filter "Endotoxin Free" Kit is designed for rapid and efficient extraction of high quality endotoxin-free purified plasmid DNA with anion-exchange technology. With provided filter cartridges the bacteria lysates will be removed without centrifugation. Following a gravity-flow procedure, the plasmid DNA is bound to the resin, and the contaminants can be remove with wash buffer.PTR Buffer washes away the endotoxins in just one step. This kit is designed for the convenient, easy, and efficient extraction of pure plasmid DNA and makes the endotoxins less than 0.05 EU/µg DNA that is suitable for the transfection of cultured cells.

After using this convenient kit, the purified plasmid DNA is suitable for downstream application such as transfection, in vitro transcription and translation, and all enzymatic modification.

Specifications: bacterial culture

#### Sample Size:

- 120- 240 ml of bacterial for high copy plasmid or low copy plasmid
- Plasmid constructs' range: 3kbp-150 kbp
- Binding Capacity: up to 1.5 mg /Maxi Column

#### **Additional Requirements:**

1.50 ml tubes

2. Refrigerated centrifuge capable of  $\geq$ 5,000 xg and the centrifuge tube suitable for the centrifuge rotor

- 3. Isopropanol
- 3. Isopropanoi
- 4. 70% ethanol
   5. TE buffer or ddH2O

#### **Features**

Time saving: 1,5 hour DNA Yield : up to 1.500ug/column

- High Purity: Equal to that obtained by 2x CsCl gradient centrifugation.
- Safe: Eliminates the use of phenol, chloroform, ethidium bromide, and cesium chloride, minimizing exposure to and disposal of hazardous materials.

#### **Applications**

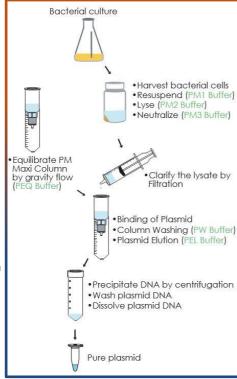
The purified plasmid DNA can be used immediately in downstream application.

- Transfection (non-endotoxin sensitive)
- Microinjection
- · In Vitro transcription
- Restriction Enzyme digestion

#### **Procedure**

In the process, after the modified alkaline lysis, the filter cartridge is used to remove bacteria lysates to obtain cleared sample matrix. Then the plasmid DNA will bind to the onion exchange resin inside the Maxi Column when the sample matrix flowing through. The contaminants can be removed by wash buffer. Finally, the purified plasmid DNA is luted using high-salt buffer and then precipitated with iospropanol for desalting.

Cat. N°	Product Name	Size	Store at:
DE-055EF	Plasmid Extraction Maxi Filter Endotoxin Free Kit	10 preps	At Room Temperature RNase A: at-20°C



# RNA Extraction & Purification

Blood / Tissue Total RNA Extraction Mini Kit

**Tissue Total RNA Extraction Mini Kit** 

**Trizol Ultrapure** 

**RNA-ZOL Direct Clean-up Plus Kit** 

**RNA Stabilization Reagent** 

Viral Nucleic acid (DNA&RNA) Extraction Kit

# BLOOD/TISSUE TOTAL RNA EXTRACTION MINI KIT RE-004 & RE-004B

#### Blood & Tissues Total RNA Extraction Mini Kit is designed for extraction of total RNA from whole blood, animal tissues (fresh and frozen) cultured cells, bacteria and yeast it makes the RNA Clean Up.

This method first lyses cells by using a chaotropic salt, then binds RNA to silica-base membranes washes RNA embranes, with ethanol-contained wash buffer and then elutes purified RNA by RNase-free ddH2O. It takes 30 min for an entire procedure, and the purified RNA is ready for RT-PCR, northern blotting, primer extension and cDNA library construction.

#### Handling time: about 30 minutes

Binding capacity: ≤100 µg RNA/column

Column applicability: centrifugation and vacuum

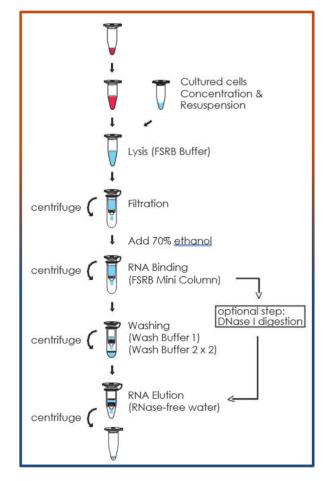
#### Minimum elution volume: 30 µl

#### Sample amount and yield:

- Up to 0.3 ml fresh whole blood
- Up to 30 mg of tissues samples •
- Up to 1 X  $10^6$  animal cultured cells .
- Up to 1 X 10<sup>9</sup> bacterial cells
- Up to 5 X 10<sup>7</sup> yeast .

Sample	Recommended amount of sample used		Yield (µg)
Human whole blood (up to 300 µl )	300 µl		1
Animal calls (up to	NIH/3T3 Hel a		10
Animal cells (up to 5 x 10 <sup>6)</sup>		1 x 10 <sup>6</sup> cells	15
0 X 10	COS-7		30
	LMH		12
	Embryo		25
	Heart		10
Animal Tissue	Brain		10
(Mouse/rat) (up to 30 mg)	Kidney	10 mg	30
(up to 00g)	Liver	To thig	50
	Spleen		35
	Lung		15
	Thymus		45
Bacteria	E. coli		60
	B. subtilis	1 x 10 <sup>9</sup> cells	40
Yeast (up to 5 x 10 <sup>7</sup> )	S.cerevisiae	1 x 10 <sup>7</sup> cells	25

#### **Brief procedure:**



Cat. N°	Product Name	Size	Store at:
RE-004	Blood/Tissues RNA Extraction Mini Kit	100 preps	At Room Temperature
RE-004B	Blood/Tissues RNA Extraction Mini Kit	300 preps	RNase A at -20°C

# **TISSUE & CELLS TOTAL RNA EXTRACTION MINI-KIT**

**RE-006** 

The Tissue & Cells Total RNA Purification Mini Kit is designed for purification of total RNA from: Animal Tissue (fresh, frozen, paraffin) colture Cells, Bacteria, Yeast, Fungi, it makes RNA clean-up, using the chaotropic salt-lysis method without the use of hazardous solvents such as phenol. The Kit can quickly purify total RNA from up to 10mg of tissues within 30 minutes.

The purified RNA is suitable for direct use in RT-PCR,Northern blotting, primer extension and cDNA library construction.

#### Features:

Operation time:  $30 \sim 60$  minutes Binding capacity: up to  $100 \ \mu g$  total RNA/ column Column applicability: centrifugation and vaccum Minimum elution volume:  $40 \ \mu l$ 

#### **Applications**

- · Nothern blotting hybridizations
- Primer extension
- RT-PCR
- RNase protection assays
- Differential display
- · As starting material for purification of mRNA for cDNA synthesis

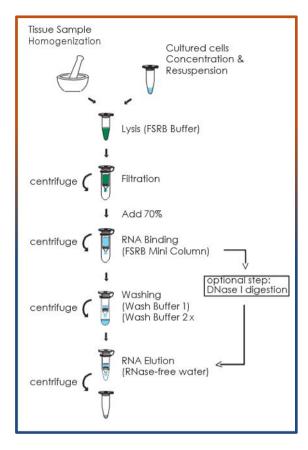
Sample Size: Ani

Animal cells: from 1 x  $10^{6}$  up to 5 x10 cells Animal Tissues: (Mouse/Rat) from 10 mg up to 30 mg Bacteria: 1ml or up to  $1x10^{9}$  cells Yeast: (up to 5 x  $10^{7}$ )

#### **Yield of Purification**

Sample	Recommended amount of sample used		Yield (ug)
Animal Cells (up to 5 x 10 <sup>6</sup> Cells)	NIH/3T3 HeLa COS-7 LMH	1 X 10 <sup>6</sup> Cells	10 15 30 12
Animal Tissue (mouse/rat) (Up to 30 mg)	Embryo Heart Brain Kidney Liver Spleen Lung Thymus	10 mg	25 10 30 50 35 15 45
Bacteria	E.coli B. subtilis	1 x 10 <sup>9</sup> Cells	60 40
Yeast (up to 5 x10 <sup>7</sup> cells)	S. cerevisiae	1 x 10 <sup>7</sup> Cells	25





#### **Procedure**

The method uses detergents and a chaotropic salt to lysis cell and inactivate RNase, then RNA in chaotropic salt is bonded to the glass fiber matrix of column. After washing off the contaminants, the purified RNA is eluted by RNase-free water. The entire procedure can be completed about 30~60 minutes.

#### **Storage Conditions**

Tissue Total RNA Mini Kit can be stored at room temperature (15-25°C). Stable for 1 year at room temperature at 15-25°C.

ĺ	Cat. No.	Product Name	Size	Store at
	RE-006	Tissue & Cells Total RNA Mini Kit	100 preps	At Room Temperature at 15-25°C for 1 year

## TRIZOL ULTRAPURE-RNA / DNA / PROTEIN ISOLATION REAGENT

FS-881

**RE-040** 

TRIzol Reagent is a ready-to-use reagent, designed to isolate high quality total RNA (as well as DNA and proteins) from cell and tissue samples of human, animal, plant, yeast, or bacterial origin, within one hour. TRIzol Reagent is a monophasic solution of phenol, guanidine isothiocyanate, and other proprietary components which facilitate the isolation of a variety of RNA species of large or mall molecular size. TRIzol Reagent maintains the integrity of the RNA due to highly effective inhibition of RNAse activity while disrupting cells and dissolving cell components during sample homogenization. TRIzol Reagent allows for simultaneous processing of a large number of samples, and is an improvement to the single-step RNA isolation method developed by Chomcynski and Sacchi (Chomczynski & Sacchi, 1987).

- · Isolated DNA can be used in PCR, Restriction Enzyme digestion, and Southern Blots.
- · Isolated protein can be used for Western Blots, recovery of some enzymatic activity, and some immunoprecipitation.
- Ready-to-use reagent for the isolation of total RNA or the simultaneous isolation of RNA, DNA and proteins from samples of human, animal, plant, yeast, bacterial and viral origin.
- Single-step method of total RNA isolation
- Performs well with small and large quantities of tissues or cultured cells and allows simultaneous processing of a large number of samples.
  Combines phenol and quanidine thiocyanate in a mono-phase solution to facilitate the immediate and most effective inhibition of RNase
- activity.RNA remains exclusively in the aqueous phase, DNA in the interphase and proteins in the organic phase.

Cat. N.	Product Name	Size
FC 004	TRIZOL DNA/RNA	100 ml
FS-881	Protein Isolation Reagent	200 ml



# **RNA-ZOL DIRECT CLEAN-UP PLUS KIT**

The RNA-Zol Direct Clean Up Plus kit provides a streamlined method for the purification of up to 100 µg (per column) of highquality RNA directly from samples in TRIzol®, TRI Reagent® or similar

Total RNA including small RNAs (17-200 nt) is isolated from a variety of sample sources (cells, tissues, serum, plasma, blood, etc.). Simply add ethanol to a TRI Reagent® sample, bind directly to the Column, wash, and elute RNA. No phase separation, precipitation, or post-purification steps are necessary. RNA is high-quality and ready for Next-Gen Sequencing, RT-qPCR, transcription profiling, hybridization, etc.

#### Features

RNA clean up plus can be operated directly after the chloroform extraction without isopropanol

- precipitation.
- Sample Size: Up to 100 µl of RNA sample or enzymatic reaction mixture.
- High purity: OD260/280: 1.9~21.
- Binding Capacity: Up to 100 ug
- · Handling Time: Within 10 minutes
- Expected Recovery: 85~95%
- Format: Spin Column

#### Applications

- Real-Time PCR
- Northern blotting hybridization
- Primer extension
- Differential display
- RNase protection assays
- As starting material for the purification of mRNA for cDNA synthesis

#### Storage Conditions

Stable for 1 year at room temperature

Cat. N.	Product Name	Size	Store at
RE-040	RNA-Zol Direct Clean-Up Plus	50 preps	Store at 15°C for 1 years
RE-041	Kit (Tri-Zol included)	200 preps	
RE-042	RNA-Zol Direct Clean-Up Plus	50 preps	
RE-043	Kit (Tri-Zol not included)	200 preps	



RNA/ater Stabilization Reagent immediately stabilizes RNA in tissues, cell cultures and blood samples to preserve the gene expression profile.

RNA Later makes it possible for researchers to postpone RNA isolation for days, weeks, or even months after tissue collection without sacrificing RNA integrity.

The reagent preserves RNA for up to 1 day at 37°C, 7 days at 18-25°C, or 4 weeks at 2-8°C, allowing processing, transportation, storage, and shipping of samples without liquid nitrogen or dry ice.

Alternatively, the samples can also be placed at -20°C or -80°C for archival storage.

#### Advantages:

In addition for RNA stabilization, RNA Solution can be easily integrated into a modified single-step RNA isolation method. This modified single-step method isolates undegraded RNA from tissues or cells in hours and can be used to process a large number of samples.

#### **Protocol for Tissues**

1. (Solution up to 100 mg tissue add 1 ml RNA Later) Store the tube at -20°C until use.

2. When processing thaw and homogenize tissues in RNA I ater

3. Transfer 0.8 ml of the homogenate mix into a 2 ml tube and add 0.8 ml of the acid-phenol, pH 5.2, and 320 ul of chloroform.

4.Vortex the mixture vigorously by mixing 4 times, 30 sec for each.

5.Centrifuge at 12,000 rpm for 2 min

6. Transfer the upper aqueous phase (containing RNA) to a fresh 2 ml tube, taking care not to disturb the interface (containing DNA/protein).

7. Precipitate the RNA by adding an equal volume (0.8 ml) of isopropanol and 80 ul of 3 M NaAc at -20°C for 30 min. 8. Centrifuge at 12,000 rpm for 15 min and discard the

supernatant.

9. Wash the RNA pellet by using 200 ul of 70% ethanol and gentle inverting the tube for several times.

10.After a brief spin and careful removing the supernatant, let the RNA pellet to air dry for about 5-10 min.

11.Dissolve the RNA pellet in 20 ul DEPC-treated TE.

12. Store the samples at -20°C and used for cDNA synthesis.

#### Protocol for Culture Cells

1. Transfer 107 cells (isolated from cell colture) into 1 ml of **RNA Later Solution** 

Store the tube at -20°C until use.

2. When processing thaw and homogenize tissues in RNA I ater

3. Transfer 0.8 ml of the homogenate mix into a 2 ml tube and add 0.8 ml of the acid-phenol, pH 5.2, and 320 ul of chloroform.

4. Vortex the mixture vigorously by mixing 4 times, 30 sec for each.

5. Centrifuge at 12,000 rpm for 2 min.

6. Transfer the upper aqueous phase (containing RNA) to a fresh 2 ml tube, taking care not to disturb the interface (containing DNA/protein).

7. Precipitate the RNA by adding an equal volume (0.8 ml) of isopropanol and 80 ul of 3 M NaAc at -20°C for 30 min 8.Centrifuge at 12,000 rpm for 15 min and discard the supernatant.

9. Wash the RNA pellet by using 200 ul of 70% ethanol and gentle inverting the tube for several times.

10. After a brief spin and careful removing the supernatant, let the RNA pellet to air dry for about 5-10 min.

11. Dissolve the RNA pellet in 20 ul DEPC-treated TE.

12. Store the samples at -20°C and used for cDNA synthesis. Protocol for Whole Blood

1. Collect fresh human blood in an anticoagulant-treat collection tube.

2. Transfer up to 300 µl fresh blood to a 1.5ml microcentrifuge tube (not provided). If the sample is more than 300 µl (up to

1ml), add the sample to a sterile 15 ml centrifuge tube. 3. Add 3 x the sample volume of RBC Lysis Buffer and mix by inversion. Do not vortex.

4. Incubate at room temperature for 10 minutes. Centrifuge at 3,000 x g for 5 minutes and completely remove the supernatant. 5. Resuspend the pellet with 100 µl of RBC Lysis Buffer.

6. Store 100µl of RBC Lysis Buffer with 1 ml of RNA Stabilization Solution at -20°C until RNA isolation.

7. When processing, thaw and homogenize tissue in RNA Stabilization Solution.

8. Transfer 0.8 ml of the homogenate mix into a 2 ml tube and add 0.8 ml of the acid-phenol, pH 5.2, and 320 ul of chloroform. 9. Vortex the mixture vigorously by mixing 4 times, 30 sec for each.

10. Centrifuge at 12,000 rpm for 2 min.

11. Transfer the upper aqueous phase (containing RNA) to a fresh 2 ml tube, taking care not to disturb the interface (containing DNA/protein).

12. Precipitate the RNA by adding an equal volume (0.8 ml) of isopropanol and 80 ul of 3 M NaAc at -20°C for 30 min 13. Centrifuge at 12,000 rpm for 15 min and discard the supernatant.

14. Wash the RNA pellet by using 200 ul of 70% ethanol and gentle inverting the tube for several times.

15. After a brief spin and careful removing the supernatant, let the RNA pellet to air dry for about 5-10 min.

16. Dissolve the RNA pellet in 20 ul DEPC-treated TE.

17. Store the samples at -20°C and used for cDNA synthesis.

Cat. No.	Product Name	Samples	Size	Store at
FS-883	RNA Later Stabilization Reagent	Tissues Cell Cultures	100 ml	Store at +4°C

# VIRAL NUCLEIC ACID (DNA & RNA) EXTRACTION KIT I

**DR-001** 

Viral Nucleic Acid Extraction Mini Kit I is designed for extraction of Viral DNA or RNA from cell free fluides such as serum, plasma, body fluid and cell cultured supernatant and from transport medium of swabs (covid samples). This method first lyses virus by using a chaotropic salt, then binds nucleic acid to silica-based membranes. After washing with ethanol-contained wash buffer, contaminants and enzyme inhibitors will be removed completely. It takes only 20 min for an entire procedure, the purified nucleic acid is ready for RT-PCR and PCR . gel, up to 200 mg. This kit contains carrier RNA for very low viral load samples.

#### Features:

- Principle: spin column (silica membrane)
- Safe Use: Eliminates the use of phenol, chloroform, ethidium bromide, and cesium chloride, minimizing exposure to, and disposal of hazardous materials.
- High Purity: Complete removal of contaminants and inhibitors for reliable downstream applications

**Sample:** 140 µl cell-free fluid such of plasma, serum, body fluids , cell cultured supernatant and from transport medium of swabs (covid samples.)

Length of recovery nucleic acid: > 200 bp

Recovery rate: 80-90%

Binding capacity: 30 ug

Elution Volume: 40-50 µl

**Operation time:** 20 minutes

Binding capacity: 60 ug RNA/column

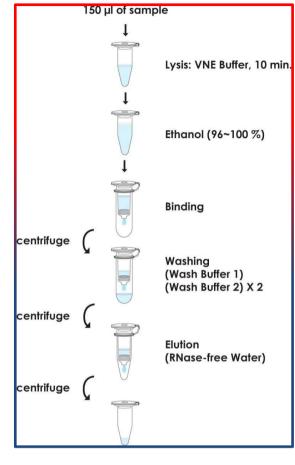
#### **Applications:**

- Real-time PCR
- PCR
- RT-PCR
- Real-time RT-PCR

#### **Quality Control:**

The quality of our Viral RNA/ Viral Nucleic Acid Mini Kit is tested on a lot-to-lot basis according to ISO quality management system.

Cat. N.	Product Name	Size	Store at
DR-002	Viral Nucleic Acid (DNA/RNA) Extraction Kit I	100 preps	Store at room temperature for 1 year



# Enviromental DNA/RNA Extraction

# Kits

**Plant Genomic DNA Extraction Mini Kit** 

Soil DNA Isolation Mini Kit

**Stool DNA Isolation Mini Kit** 

Fungi/Yeast Genomic DNA Extraction Mini Kit

**Plant Total RNA Purification Mini Kit** 

# PLANT GENOMIC DNA EXTRACTION MINI KIT

Plant Genomic DNA Mini Kit provides a fast and simple method to isolate total DNA (genomic DNA, mitochondrial chloroplast and viral DNA) from plant tissue and cells. Plant tissues are ground in liquid nitrogen and lysed by buffer containing detergent. The tissue debris in lysate could be removed by provided filter column. In the presence of a chaotropic salt, the genomic DNA in the lysate binds to the glass fiber matrix in the spin column. After washing off the contaminants, the purified DNA is eluted by low salt elution buffer or waters.

#### **Features**

• High Purity: DNA is immediately suitable for a variety of applications, including amplification, digestion, PCR etc.

• **High Speed:** Using a column type extraction system to allow a more rapid, more convenient methods compared to the conventional mmethods. Rapid speed for the isolation of genomic DNA from various plants, within 40 minutes.

• **Safe:** The kits use a spin column tube and removes proteins, nucleases in cells, it is notnecessary to treat the sample with harmful organic solvents such as phenol and chloroform.

#### **Applications**

- Real-time PCR
- PCR
- RFLP
- Amplification
- Southern blotting

#### **Time Required**

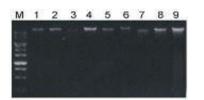
About 30-60 minutes depending upon the sample types.

#### Sample Size

Mini: up to 100mg fresh sample or 20mg dry sample.

**Storage Conditions:** Plant Genomic DNA Extraction Kit can be stored at room temperature (15-25  $^{\circ}$ C). Stable for 1 year at room temperature at 15-25 $^{\circ}$ C.

#### The Quality of DNA After Purification



7: Populus tremula (Aspen)

- 8: Flammulina velutipes
- 9: Oxalis comiculats (Fourleaf clover)

	DNA yield (ug)		
Sample	Mini	Maxi	
	100 mg young leaf	1 g young leaf	
Arabidopsis	3~5	30~50	
Rice	10~15	100~150	
Tomato	10~15	100~150	
Tobacco	20~25	200~250	
Chinese Yam	30~60	300~500	
Maize	15~20	150~200	
Sweet Potato	20~30	200~300	
Orchis	5~10	50~100	
Campor Tree	15~20	150~200	
Spinach	5~10	50~100	
Bamboo	10~15	100~150	

**DNA Yield** 

Cat. N.	Product Name	Size	Store at
DE-021		50 preps	Store at RT for 1 years Store RNAse A at -20°C
DE-022	Plant Genomic DNA Extraction Mini Kit	100 preps	

## SOIL DNA ISOLATION MINI KIT

Our Soil DNA Isolation Mini Kit is suitable to isolate DNA from different environmental samples improving DNA recovery in terms of DNA yield.

The technology operates through our high-quality beads- beating disruption method and is perfect for use with different *soil samples of up to 0.5 g.* The silica membrane technology, and spin column along with beads- beating method guarantee the high-quality purification and isolation of DNA that can be used for PCR, genotyping, arrays, etc. The inhibitors of downstream PCR or enzymatic reactions will be removed with the sequent buffers in this kit. Phenol/chloroform is not required in the whole procedure; all operation can be finished within 60 minutes. The purified DNA is ready-to-use for downstream applications.

#### **Specifications:**

Principle: Spin Column (silica membrane)

#### Sample: 0,25 -0,5 g

**Operation time:** < 60 min

Elution volume: 50~200 µl

#### **Important Notes:**

1. Buffers provided in this system contain irritants. Wear gloves, safety glasses

and lab coat when handling these buffers.

2. Check FSDE1 Buffer before use, Warm FSDE1 Buffer at 60°C for 10 minutes if

any precipitate formd.

3. Add indicated volume of ethanol (96~100%) to Wash Buffer before use.

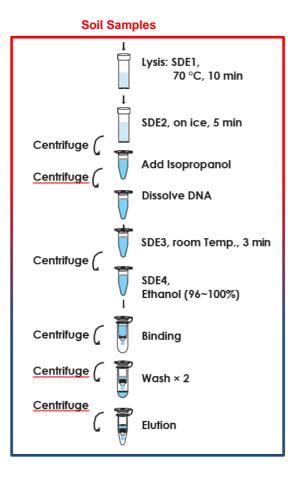
4. Prepare a heating block or a water bath to 70°C. If DNA is isolated from

gram positive bacteria, prepare a heating block or a water bath to  $95^\circ\mathrm{C}$ 

for another incubation.

5. All centrifuge steps are done at full speed (~18,000 xg) in a microcentrifuge.

6. Preheat Elution Buffer or ddH2 O to 60°C for elution step



Cat. N°	Product Name	Size	
DE-025	Soil DNA Isolation kit	50 Reactions	
DE-026		100 Reactions	

## STOOL DNA ISOLATION MINI KIT

Stool DNA Isolation Mini Kit is designed for the isolation of high-quality total DNA from 50~200 mg of fresh or frozen stool samples. The inhibitors, such as polysaccharides and humic acid, will be removed with the sequent buffers in this kit. High quality DNA for sensitive downstream applications including PCR, qPCR, Sequencing and microarray

#### **Specifications:**

Principle: Spin Column (silica membrane)

Sample: 50~200 mg

**Operation time:** < 60 min

Elution volume: 50~200 µl

#### **Important Notes**

 Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
 Check FSDE1 Buffer before use, warm FSDE1 Buffer at

60°C for 10 mins if any precipitate formed. 4. Add indicated volume of ethanol (96~100%) to Wash

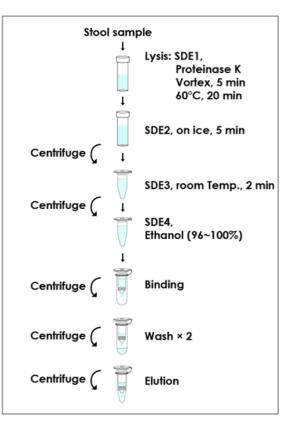
Buffer before use.

5. Prepare a heating block or a water bath to  $60^{\circ}$ C. If DNA is isolated from gram positive bacteria, prepare a heating block or a water bath to  $95^{\circ}$ C

for another incubation.

6. All centrifuge steps are done at full speed (~18,000 x g) in a microcentrifuge.

7. Preheat Elution Buffer or ddH2 O to 60°C for elution step.

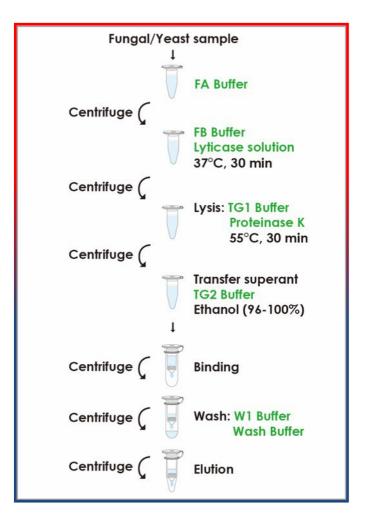


Cat. N°	Product Name	Size
DE-023	Stool DNA Isolation kit	50 Reactions
DE-024		100 Reactions

#### Description

The FavorPrepTM Fungi/Yeast Genomic DNA Extraction Mini Kit is designed for the purification of DNA from fungus and yeast cells. The enzyme teatment (lyticase & proteinase K) and **bead- beating homogenization** are applied to lyse samples efficiently and improving DNA yield. This kit provides the most complete and effective method to extract application-ready pure genomic DNA from fungi and yeast samples.

**Technology:** mini spin column (silica matrix) **Sample size:**1~ 5 x10<sup>6</sup> cell culture fungal/yeast cells Operation time:~ 60 minutes Binding capacity:60 μg/ column Column applicability: centrifugation and vaccum



Cat. No.	Product Name	Size	Store at:
DE-046	Fungi Yeast genomic DNA Extraction Kit	50 preps.	At Room Temperature for 1 year Lyticase : At -20°C.

# PLANT TOTAL RNA PURIFICATION MINI KIT

**RE-007** 

#### Description

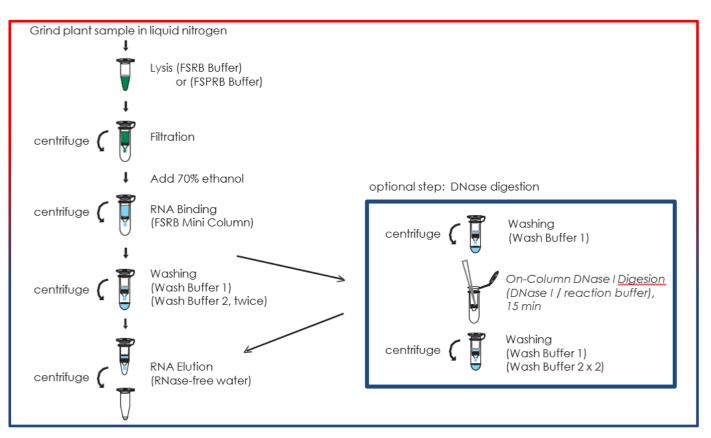
The Plant Total RNA Purification Mini Kit is designed for purification of total RNA from plant tissues and cells using the modified salt precipitation procedure and RNase inhibitors without the use of hazardous solvents such as phenol. Plant RNA is quickly and efficiently isolated and is immediately available for downstream applications, including RT-PCR, Northern blotting, primer extension and cDNA library construction. For RNA Plant Total RNA extraction from woody plant we reccomend RE-015 (50 preps) and RE-016 (100 preps).

#### **Specification:**

Principle: spin column (silica membrane) Sample: up to 100 mg plant tissues or 1x10<sup>7</sup> plant cells Operation time: 30- 60 min Binding capacity: up to 100 ug Total RNA/column Expected Yield: 5-30 ug of Total RNA from 100 mg of young leave Column Applicability: Centrifugation and Vacuum Minimum Volume: 30 ul

#### Applications

- Nothern blotting hybridizations
- Primer extension
- RT-PCR
- RNase protection assays
- Differential display
- · As starting material for purification of mRNA for cDNA synthesis



Cat. No.	Product Name	Size	Store at
RE-007 RE-008	Plant Total RNA Purification Mini Kit	50 preps 100 preps	Store at room temperature at 4-8°C for 1 year.