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AMPLIBIOTHERM DNA POLYMERASE FS-T-002

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	
10X Reaction Buffer (contains 15mM $MgCl_2$; included)	Cat. No. **FS-B-006**
10X Reaction Buffer (without $MgCl_2$; plus 25 mM $MgCl_2$ separately)	Cat. No. **FS-B-007**

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.	25 ~40
Anneal	50~65	10 ~ 30 sec.	
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 FS-T-0531

Description

GoTaq Flexi DNA Polymerase Green G2 is new generation of Taq polymerase that gives robust amplification and high DNA yield in shorter PCR running time (15-30 s/kb extension). GoTaq Flexi DNA polymerase supports the robust and reliable amplification of a wide range of DNA templates up to 6 kb. GoTaq Flexi is provided with 5× Green Reaction Buffer allowing reactions to be loaded directly into gels without the extra adding of loading dye and with a 10x Colorless Buffer. GoTaq Flexi lacks 3' 5'exonuclease activity. Resulting PCR products have an A-overhang and suitable for cloning.

Description	FS-T-0531
GOTAQ Flexi DNA Polymerase Green G2	500 U
5X Green Reaction Buffer	2 x 2 ml
10 X Colorless Buffer	4X1 ml
50 mM MgCl2 Solution	2X1 ml

Storage Buffer

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

5X Green Reaction Buffer

The 5X Green Reaction Buffer contains 2 dyes (blue & yellow) that separate during electrophoresis to monitor migration progress. The blue dye migrates at the same rate as a 3-5kb DNA fragment in a1% agarose gel,the yellow dye migrates a t a rate faster than primers (<50kb) in a 1% agarose gel.

10X Colorless Buffer

The Colorless Buffer is used when direct fluorescence or absorbance readings are required without prior purification of the amplified DNA from the PCR.
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 74°C.

Storage Conditions

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

Quality Control

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

Genomic DNA contamination:

The product must be free of any detectable DNA contamination as evaluated through PCR. Thus, it is suitable for the amplification of bacterial and fungal DNA based on 16S and 18S rRNA PCR assays.

Nuclease assays

0.2-0.3 µg of pNZY28 plasmid DNA are incubated with 5 U of GoFlexi Taq DNA polymerase, in 1× Reaction Buffer, for 14-16 hours at 37 °C. Following incubation, the DNA is visualised on a Green Gel Safe -stained agarose gel. There must be no visible nicking or cutting of the nucleic acid. Similar tests are performed with buffer and MgCl2 solution.

Functional assay

GoFlexi Taq DNA polymerase is extensively tested for performance in a polymerase chain reaction (PCR) of different-sized DNA fragments (1 and 2.5 kb) from human genomic DNA in the presence of 5× Green Reaction Buffer and MgCl2 solution. The resulting PCR products are visualized as single bands in a Green Gel Safe stained agarose gel

Recommended Protocol

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

PCR using Green reaction buffer

For 50µl PCR Reaction	Volume	Final Conc.
GOTAQ Flexi DNA Polymerase Green	0.25-1 µl	1.25 U
5X Green Reaction Buffer	10 µl	1 X
50 mM MgCl2 Solution	2.5mM	(1.5~4.0) mM
dNTP mix (2.5 mM each)	0.4 µl	(0.25-0.5)
Template DNA **(see below)**	5 pg-0.5ug	0.1-0.5 uM
Forward Primer	0.25 ul	0.1~0.5 µM
Nuclease free water	up to 50 µl	

PCR Reaction using Colorless buffer

For 50µl PCR Reaction	Volume	Final Conc.
GOTAQ Flexi DNA Polymerase G2	0.25-1 µl	1.25 U
10X Colorless Buffer	5 µl	1 X
50 mM MgCl2 Solution	2.5mM	(1.5~4.0) mM
dNTP mix (2.5 mM each)	0.4 µl	(0.25-0.5)
Template DNA **(see below)**	5 pg-0.5ug	0.1-0.5 uM
Forward Primer	0.25 ul	0.1~0.5 µM
Nuclease free water	up to 50 µl	

General Cycling Conditions:

Step	Temp (°C)	Time	Cycle
Initial Denaturation	95	3 min.	1
Denature	94	30 sec.(**)	25 ~ 35
Anneal	50~65(*)	30 sec.	
Extend	72	15 ~ 30 sec./kb**	
Final Extension	72	5 min.	1

* Annealing temperature should be optimized for each primer set based on the primer Tm; typically, it should be Tm- 5 °C. ** For DNA fragments higher than 3 kb to 6 kb in size, it may be beneficial to use 20 sec for denaturation and 30-60 sec/kb for extension

Primer Design

PCR primers generally range in length from 15–30 bases and are designed to flank the region of interest. Primers should contain 40–60% GC, and care should be taken to avoid sequences that might produce internal secondary structure. The 3'-ends of the primers should not be complementary to avoid the production of primer- dimers. Primer-dimers unnecessarily remove primers from the reaction and result in an unwanted polymerase reaction that competes with the desired reaction. Avoid three G or C nucleotides in a row near the 3'-end of the primer, as this may result in non- specific primer annealing. Ideally, both primers should have nearly identical melting temperatures (Tm), allowing their annealing with the denatured template DNA at roughly the same temp.

DNA template

The optimal amount of starting material may vary depending on its quality and complexity. In general, we recommend using 10 ng to 500 ng of genomic DNA templates, although the enzyme is sensitive enough to amplify fragments from as little as 5 pg of human gDNA,for example. Lower amounts of template may be used for amplification of less complex DNA (typically 1-20 ng). When using a cDNA synthesis reaction as template do not exceed 10% of the final PCR reaction volume.

Applications:

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

HOT - START DNA POLYMERASE (with GC-Buffer) FS-T-2131-1

Description

HOT START DNA polymerase is an ideal enzyme for high fidelity PCR with excellent processivity. It is a novel engineered enzyme with comparable performance to Pyrococcusiosus DNA polymerase. With unique structure HS 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 is one of the thermostable DNA polymerases with strong 3 ' -5 ' exonuclease activity which results in its extreme high fidelity, 10-15 times higher than Taq DNA polymerase and 6 times higher than Pyrococcus furiosus DNA polymerase.
The HS PCR Kit is supplied with a 5X HF Buffer and a 2.5X GC Buffer. The 5X HF Buffer is an optimized buffer for general high fidelity amplifications while the 2.5X GC Buffer is used in the amplifications of problematic or GC-rich templates.

Contents	FS-T-2131-200
Hot Start DNA Polymerase (2000U/mL)	200 RNXS
5X HF PCR Buffer	2x 1 mL
2.5X GC PCR Buffer	4X 1 mL
dNTPs (10 mM each)	200 µL

Thermal Inactivation: No
Product End: Blunt end

Standard Protocol

1. It is recommended to prepare all reaction components on ice, and then quickly transfer the reaction system to a thermocycler preheated to 98℃.It is recommended to prepare all reaction.
2. All components should be mixed and collected at the bottom of a tube with a quick spin before use. Add Hot Start 2X Master Mix with Dye at the end to prevent primer degradation by its strong 3´-5´ exonuclease activity.
Note: The Hot Start DNA polymerase requires special reaction conditions different from other polymerase protocols. Please refer to the recommended reaction conditions below for the better amplification yields.

Recommended Protocol

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

5X HF(High Fidelity) Buffer Reaction System

Component	25 µl reaction	50 µl reaction	Final Conc.
Hot Start DNA Polymerase (2000 U/mL)	0.5 ul	1 ul	2U/50ul
5X High Fidelity PCR Buffer	5 ul	10 ul	1 X
dNTP mix (10 mM each)	0.5 ul	1 ul	0.2mM
DNA Template	variable	variable	<300ng
Forward Primer (10µM)	0.5 ul	1 ul	0.2uM
Reverse Primer(10µM)	0.5 ul	1 ul	0.2uM
Distilled water	to 25 ul	to 50 ul	N/A

2.5 X GC BUFFER Reaction System

Component	25 µl reaction	50 µl reaction	Final Conc.
Hot Start DNA Polymerase (2000 U/mL)	0.5 ul	1 ul	2U/50ul
2,5X GC PCR Buffer	10 ul	20 ul	1x
dNTP mix (10 mM each)	0.5ul	1ul	0.2mM
DNA Template	Variable	variable	< 300 ng
Forward Primer (10µM)	0.5 ul	1 ul	0.2uM
Reverse Primer (10µM)	0.5 ul	1 ul	0.2uM
Distilled water	to 25 ul	to 50ul	N/A

General Cycling Conditions:

Step	Temp (°C)	Time	Cycle
Initial Denaturation	98	45 sec.	1
Denature	98	10 sec.	25 ~35
Anneal	55~65	20 ~ 30 sec.	
Extend	72	10 ~ 30 sec. **s/kb***	
Final Extension	72	5 min.	1
Hold	4-12		1

***Note:** Properly extending the extension time can improve the amplification yield. For complex amplification templates, such as genomic DNA, it is recommended to extend at a speed of 60 s/kb, and more recommended conditions please refer to the basic principles of PCR below**.**

PCR Principles

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 50 µL reaction):

DNA	IMPUT Amount
Plants, animals and human gDNA	**10 ng-300 ng**
E.coli, lambda gDNA	**10 ng-100 ng**
Plasmid DNA	**1 pg-10 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 concentration of each primer in the PCR reaction system should be in the range of 0.1-1 µM.

3. Enhancer

The Enhancer solution is an optional component to increase the amplification efficiency for problematic templates, such as GC-rich sequence or genes with strong secondary structure. Note:Since the enhancer is included in the 2.5X GC Buffer, additional enhancer is not recommended with the use of 2.5X GC Buffer. Excess amount of enhancer may be inhibitory.

4. Buffers The HS PCR Kit contains a 5X HF Buffer and a 2.5X GC Buffer.The 5X HF Buffer is designed for general high fidelity PCR amplification , and the 2.5X GC Buffer is optimized for the amplifications of GC-rich templates.

PFU DNA POLYMERASE FS-T-004

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 $MgCl_2$:

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 -20ºC 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 :

PCR Reaction	Volume (50 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 µl mineral oil.

Recommended PCR Cycling Conditions:

Step	Temp (°C)	Time (min)	Cycle
Initial Denaturation	94	3	1
Denature	94	30 sec.	30
Anneal	55-68	30 sec.	
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 1µg 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 acid-soluble 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

GOTAQ GREEN 2X MASTER G2 FS-T-5041

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, $MgCl_2$ and dNTP's, except DNA template and primers. The mixture is suitable for amplification of most of the DNA templates and highly processive 5'→3' DNA polymerase that lacks 3'→5' exonuclease activity and lacks a 3'→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 µM primers for sensitivity and less than 0.5 µM 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.	25 ~ 40
Anneal	50 ~ 65	10 ~ 60 sec.	
Extend	72	60 sec./kb	
Final Extension	72	5 min.	1

NOTE: 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.

BLUE TAQ DNA 2X MASTER MIX FS-T-80602

Description
BlueTaq DNA 2X Master Mix is an optimized PCR premixed solution containing Taq DNA Polymerase, dNTPs, MgCl2, KCl, Bromophenol blue tracking dye, and other stabilizers. users only need to add template and primer to complete experiment. The amplified products were directly used for agarose gel electrophoresis.
It is ideally suited to routine PCR applications on various templates including pure DNA solutions, bacterial colony/ culture, and cDNA products. It can amplify 5 kb DNA from different sources genomic DNA. It is applicable to PCR reaction, colony PCR identification, rough sample amplification.

Description	FS-T-80602
BLUE TAQ DNA 2X Master Mix	5 ml/ 500 reactions

5´-3´exonuclease activity: Yes
3´-5´exonuclease activity: No
Product End: Single-base 3´ Overhangs

Storage Conditions
Store all components at -20˚C in a non-frost-free freezer.

Recommended protocol:
This standard protocol applies to a single reaction where only template, primers, and water need to be added to the Blue Taq DNA 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:

PCR Reactions

Component	20 µl reaction	Final Conc.
BLUE TAQ DNA 2X Master Mix	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:** The optimal reaction concentration varies with different DNA templates
. Please refer to the basic principles of PCR condition below.

1. Template
Use of high quality, purified DNA templates greatly enhances the success of PCR. Recommended amounts of DNA template for a 50 µL reaction are as follows:

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

2. Primers
Oligonucleotide primers are generally 20–40 nucleotides in length and ideally have a GC content of 40–60%. Computer programs such as Primer3 can be used to design or analyze primers. The final concentration of each primer in a reaction may be 0.05–1 µM, typically 0.1–0.5 µM.

3. Mg2+ and Additives
In the BLUE TAQ DNA 2X Master Mix, the concentration of Mg2+ should be 4 mM, dNTP should be 300 µM. Amplification of some difficult targets, like GC-rich sequences, may be improved with additives, such as DMSO or formamide.

4. Denaturation
An initial denaturation of 3 minutes at 94℃ is sufficient for most amplicons from pure DNA templates. For difficult templates such as GC-rich sequences, a longer denaturation of 5 minutes to fully denature the template. With colony PCR, an initial 5 minutes denaturation at 94℃ is recommended to fully decompose the bacteria. During cycling a 10 seconds denaturation at 98℃ is recommended.

5. Extention
The recommended extension temperature is 65℃. The extension time is related to the length of the amplified fragment. Calculate the extension time at the speed of 1 kb/min. A final extension of 5 minutes at 65℃ is recommended.

6. Cycles
Generally, 25–35 cycles yields sufficient product. Up to 45 cycles may be required to detect low-copy-number targets.

PCR Conditions

Step	Temp (˚C)	Time	Cycle
Initial Denaturation	94	3 min.	1
Denature	98	5~ 10 sec.	30
Anneal	55~ 60	20 ~ 30 sec.	
Extend	65	1 kb/min	
Final Extension	65-68	5 min.	1
Hold	4-12° C	-	1

NOTE:The recommended pre-denaturation time for colony is :2-5 min

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

GC-PLATINUM POWER TAQ 2X MASTER MIX FS-T-1642-5

Description

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'→5' exonuclease and 3′→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-1642-5	FS-T-1642-25
GC-Platinum Power TAQ 2X Master Mix	5 ml	25 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 Conditions

Store all components at -20ºC 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 µM.

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:

Reaction Conditions

Component	20 µl	25 µl	50 µl	Final Conc
GC-Platinum POWER TAQ 2X Master Mix	10 µl	12,5 µl	25 µL	1X
10µM Forward Primer	0,4 µl	0.5 µL	1 µL	0.2 µM
10µM Reverse Primer	0,4 µl	0.5 µl	1 µL	0.2 µM
Template DNA*	Variable	Variable	Variable	>300ng
Water, RNase-Free	up to 20µl	up to 25µl	up to 50 µl	Not available

NOTE: In general, use greater than 0.5 µM primers for sensitivity and less than 0.5 µM 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.	30
Annealing	55 ~	30 sec.	
Extension	72	20-30 s/kb	
Final Extension	72	5 min.	1
Hold	4-12°C	∞	1

IMPORTANT: Annealing temperature should be 2-6ºC lower than the primer melting temperature. Elongation time should be ~1 min/1 kb.

NOTE: 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.

GC-PLATINUM POWER TAQ 2X MASTER MIX (Blue Dye) FS-T-1641-5

Description

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'→5' exonuclease and 3'→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.

This Master Mix 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-1641-5	FS-T-1641-25
GC- Platinum Power TAQ 2X Master Mix (Blue dye)	5 ml	25 ml

1ml = 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

Source: The DNA Polymerase gene was induced and expressed in E.coli and obtained by separation and purification.

Thermal inactivation: No

5'-3' exonuclease activity: No

3-'5' exonuclease activity: Yes

Fast: The amplification speed for simple template is 5-10 s/kb, for complex template is 20-30 s/ kb

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.

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.

Reaction Conditions

Component	20 µl	25 µl	50 µl	Final Conc.
GC-Platinum POWER TAQ 2X Master Mix **(Blue Dye)**	10 µl	12,5 µl	25 µL	1X
10µM Forward Primer	0,4 µl	0.5 µL	1 µL	0.2 µM
10µM Reverse Primer	0,4 µl	0.5 µl	1 µL	0.2 µM
Template DNA*	Variable	Variable	Variable	>300ng
Water, RNase-Free	up to 20µl	up to 25µl	up to 50 µl	Not available

*High-quality purified DNA templates are important to high-fidelity PCR reactions. The recommended DNA template amounts with different complexity are listed below.

Note: The optimal reaction concentration varies with different DNA templates. See table below.

Recommended PCR Program

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

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 50µL reaction).

DNA TEMPLATE

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

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 concentration of each primer in the PCR reaction system should be in the range of 0.1-1 µM.

3. Denaturation:

98°C pre-denaturation for 45 s 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℃, 5-10 s

4. Annealing:

The annealing temperature of GC Platinum Power 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 s;

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 condition 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.

Quality Control

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

Storage Conditions

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

GOTAQ HOT START GREEN 2X MASTER MIX G2 FS-T-5141

Cat No.	Size
FS-T-5141	500 reactions 1000 reactions

Description

GO TAQ 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 GO TAQ Hot Start Green 2X Master Mix G2 is an optimized, Ready-to-Use PCR mixture of GO TAQ Hot Start Green, PCR buffer, $MgCl_2$ and dNTP's , except DNA template and primers. The mixture is suitable for amplification of most of the DNA templates and highly processive 5'→3' DNA polymerase that lacks 3'→5' exonuclease activity and lacks a 3'→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
GO TAQ HS -PCR Green 2XMaster Mix G2	1 ml/100 reactions

Applications

GO TAQ 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.
GO TAQ 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 µM primers for sensitivity and less than 0.5 µM 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.	25 ~ 40
Anneal	50 ~ 65	10 ~ 60 sec.	
Extend	72	60 sec./kb	
Final Extension	72	5 min.	1

NOTE: 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.

HOT START PROOF 2X MASTER MIX FS-T-71702

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-71702-5
Hot Start Proof 2X Master Mix	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

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 2X 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.	25-35
Annealing	55 ~65°C	20-30 sec.	
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 50μL 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 μM.

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℃, 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.

Store at: -20°C – avoid freezing and thawing cycles.

HOT START PROOF 2X MASTER MIX with Red Dye FS-T-82702

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 with Red Dye is an ideal product with good amplification efficiency for diversity templates including animals, plants, cDNA, etc.

Kit Contents

Contents	FS-T-82702-5
Hot Start Proof 2X Master Mix with Red Dye	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

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	25μL	50μL	Total Conc.
Hot Start Proof 2X Master Mix with Red Dye	10μL	12,5 μL	25 μL	1X
Forward Primer (10 μM)	0.4μL	0.5μL	1 μL	0.2 μM
Reverse Primer (10 μM)	0.4μL	0.5μL	μL	0.2 μM
DNA Template*	Variable	Variable	Variable	<300 ng
Nuclease-free Water	to 20μL	To25μl	to50μl	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	3 min	1
Denaturation	98°C	10 sec.	25-35
Annealing	55 ~65°C	20-30 sec.	
Extension	72°C	30-60 sec./kb*	
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 μM.

3. Denaturation:

The DNA templates can fully denaturation when the initial denaturation is set to 3 min.Generally, the recommended denaturation condition for low- complexity DNA templates is 98℃, 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 30 s/kb. For high-complexity amplicons, it is recommended to increase the extension time to 60 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.

Store at: -20°C – avoid freezing and thawing cycles.

HIFI DNA MULTIPLE ASSEMBLY 2X MASTER MIX FS-02012

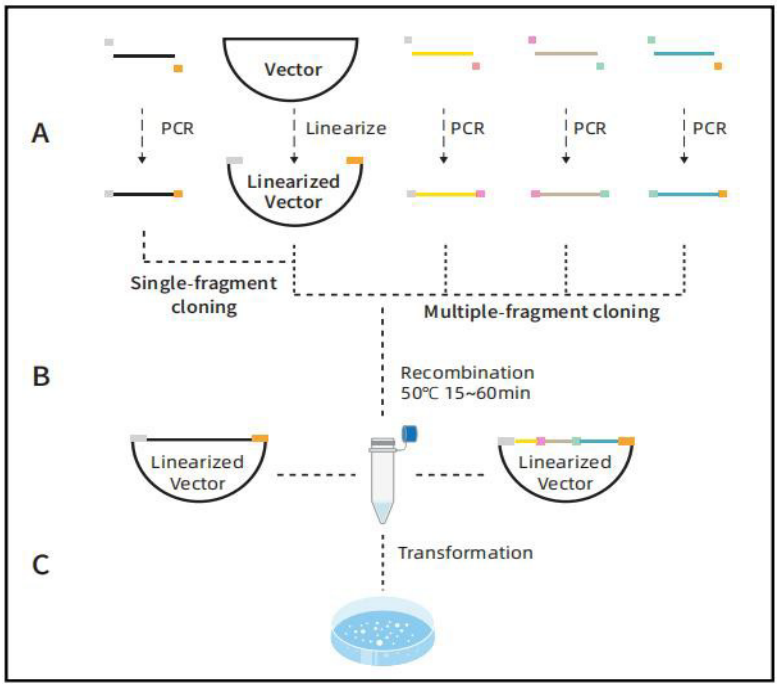
Hi Fidelity DNA Multiple Assembly Master Mix allows for seamless assembly of multiple DNA fragments, regardless of fragment length or end compatibility. It has utility for the synthetic biology community, as well as those interested in one-step cloning of multiple fragments due to its ease of use, flexibility and simple master-mix format.

Hi fi DNA Multiple Assembly 2X Master Mix is a simple, fast, and efficient seamless cloning reagent. It enables targeted cloning of inserts to any site in any vector. Up to 5 inserts can be assembled sequentially at a time, regardless of the digestion site carried by the insert itself. To perform seamless assembly cloning, user needs to linearize the vector, design gene-specific F/R primers with 15-25 homologous bases to vector ends, so that the PCR products 5' and 3 'have the same sequence (15-25 bp) as the two ends of the linearized vector, respectively. Hi fi DNA Multiple Assembly 2X Master Mix works on the inserted fragment(s) and vector DNA, and then they are incubated at 50°C for 15-60 minutes. During incubation, specific enzymatic reactions facilitate a fully assembled DNA construct. ***(Figure 1).***

Hi fi DNA Multiple Assembly 2X Master Mix is the optimization form that significantly improves fragment assembly efficiency and tolerance to impurities. The final product is a fully enclosed, double-stranded DNA that can be directly used for further PCR, RCA, or other molecular biology manipulations ***(e.g., transformation into competent cells).***

Note: Before use, fully thaw the reagents and mix thoroughly. Keep on ice to avoid repeating freeze-thaw cycles.

Figure 1. Hifi DNA Multiple Assembly 2X Master Mix Principle



CAT.#	Description	Size	Storage:
FS-02012-100	Hifi DNA Multiple Assembly 2X Master Mix	100 µL (10 reactions)	-20°C
FS-02012-500		500 µL (50 reactions)	-20°C

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 fast qPCR Mix UDG

2X EVA GREEN qPCR Master Mix

2X TAQMAN Probe Fast qPCR Master Mix

2X TAQMAN Probe qPCR 2X Master Mix (UDG)V5

SYBR GREEN qPCR 2x MASTER Mix FS-T-1200

Description

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™, CFX384™, iCycler iQ™, iQ™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™ 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	**X µl**
ddH2O	**up to 20 µl**

Suggestions for better results:

1) Generally 0.2 µM primer concentration is suitable, but when results are not satisfied, trying primer concentration between 0.1-1.0 µM range
2) qPCR method is very sensitive, accuracy of added temple is essential, recommending using diluted templates to reduce toincrease 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 0.1～1.0 µM, 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 program

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

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

THREE STEP program

Three Step Program 95℃/10 sec. 56℃/30 sec 72℃/30 sec	Increase extending time 95℃/10 sec 56℃/30 sec 72℃/60 sec

Quality Control: Purity detection: all components are analyzed without exo - endo-nuclease and nucleic acid

2X SYBR GREEN FAST qPCR MASTER MIX FS-T-50212

Description

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.

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

Compatible Instruments

Following table is helpful for choosing the right product formats

No Rox Reference Dye I	Bio-Rad iCycler serious, Roche Light Cycler 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 Reference Dye	ABI 7000/7300/7700/7900, ABI StepOne/StepOnePlus, Eppendorf and others

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

1. 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.
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 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.
4. 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 μM 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_20	up to 20 μL

2. 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:

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 :

1. Draw a standard curve according to Ct values of endogenous gene. The value of R^2 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%.
2. 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).
3. 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.

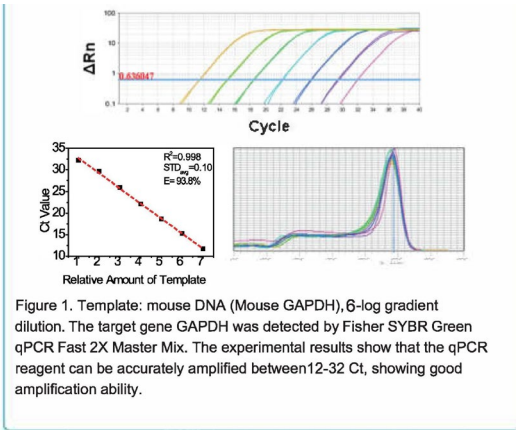


Figure 1. Template: mouse DNA (Mouse GAPDH),6-log gradient dilution. The target gene GAPDH was detected by Fisher SYBR Green qPCR Fast 2X Master Mix. The experimental results show that the qPCR reagent can be accurately amplified between12-32 Ct, showing good amplification ability.

2X UNIVERSAL SYBR GREEN FAST qPCR MASTER MIX FS-T-50215

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® 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
FS-T-50215	2 X Universal SYBR Green Fast qPCR Mix	5 X 1 mL

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.
5) 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: 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^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.

2X UNIVERSAL POWER PLUS SYBR GREEN qPCR MASTER MIX UDG 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℃ 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℃ 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™ 12k Flex, QuantStudio™ 3, QuantStudio™ 5, QuantStudio™ 6 Flex, QuantStudio™ 7, StepOne™, Fast Mode, StepOne™, Standard Mode, StepOnePlus™, Fast Mode, StepOnePlus™, Standard Mode, ViiA™ 7 System, AB StepOnePlus™, Fast Mode, AB StepOne™, Standard Mode, AB 7500, Fast Mode, AB 7500, Standard Mode, AB StepOne™, Fast Mode, AB StepOnePlus™, 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 μL, 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 ℃.

Recommended Reaction 20 μL qPCR Reaction

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 μM,and good results can be obtained , and the final concentration of 0.1-1.0 μM 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℃	2 min	1
Pre Denaturation	95℃	3 min	1
Cycles	95℃	5 sec	40
	60℃	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.

2X EVA GREEN qPCR MASTER MIX 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 chemically-modified 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 hot-start Taq, which is typically 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 : λabs/λem = 500/530 nm (bound DNA) λabs = 471 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.
3. The following table shows recommended component volumes:

Reaction Conditions PCR

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 ℃, run both RT step and extension step at 55 ℃. 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 with dH_2O 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: LIghtCycler 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 Time	N.of Cycles
Enzyme Activation	**95 ℃**	**2 min**	**1**
Denaturation Annealing & Extension	**95 ℃ 60 ℃**	**15s 60s**	**45**

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℃. Melt curves may be performed by following instructions provided for your instrument.

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

2X TAQMAN PROBE FAST qPCR MASTER MIX FS-T-1072F

Description

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°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 ~ 1,000ng genomic DNA or,
- 2ul of a 100ul single plaque eluate or, one single bacterial colony or,
- 100 ng ~ 1 pg of cDNA

NOTE: 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.	

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 qPCR TAQMAN PROBE MASTER MIX (UDG) V5 FS-T-70225

Size: 100 Rxns (25uL/RXN)

Description	FS-T-70225
2X qPCR TaqMan Probe Master Mix UDG V5	100 Rxns 1.25 mL
10X qPCR Enhancer	250 µl

Introduction:

2X qPCR TaqMan Probe Master Mix with UDG V5 is a ready-to-use 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 needs.

Compatible Instruments

Reference Dye	Instruments
No ROX	Bio-Rad iCyclers/ CFX96/ CFX 384, Roche Light Cyclers® QIAGEN/Corbett Systems Eppendor Mastercyclers®
ROX Reference Dye I	Applied Biosystems 7000/7300/7700/7900,Applied Biosystems StepOne™/StepOnePlus™,
ROX Reference Dye II	Applied Biosystems 7500/ViiA7™, QuantStudio™ Stratagene Real-time PCR Systems, Rotor-gene™ 3000

Additional Material Required but not Supplied

1. Optical-grade qPCR tubes, plates, sealing films, and aerosol-resistant pipette tips
2. qPCR primers and probes
3. DNA or cDNA templates

Precautions

(1) Ensure that the reagent is fully thawed before use. Gently mix before use to avoid creating bubbles, then thoroughly and centrifuge before use. After use, immediately return it to -20°C storage.
(2) This product contains polymerase. During use, keep it on ice. If multiple uses are required within a short period, it can be temporarily stored at 4°C. Repeated freeze-thaw cycles should be avoided as much as possible.
(3) Choose an appropriate reference dye based on the qPCR machine model you are using.
(4) When preparing and dispensing the reaction mixture, use sterile pipette tips, preferably those with filters.
(5) To increase the success rate of the reaction, it is recommended to use high-quality DNA templates. If you need to pre-mix primers and probes for stability testing at different environmental temperatures, the final primer concentration can be adjusted between 0.4-1 µM.

Important points before reaction Step

Ensure the correctness and specificity of primer design. Generally, a final primer concentration of 0.2 µM yields good results. If amplification efficiency is suboptimal, the primer concentration can be adjusted within a range of 0.1-1.0 µM.
(1) It is recommended that the length of the amplification product be in the range of 70-200 bp.
(2) Perform gradient dilution of the template and successively establish a standard curve.
(3) In a 25 µL reaction system, it is recommended to add 1 pg-50 ng of DNA as a template and design a NTC.
(4) To ensure the accuracy of the experimental results, it is recommended to perform each sample and control group in triplicate.
(6) **For viral detection and more complex templates**, you can add 250 µL of 10X qPCR Enhancer to 1.25 mL of 2X qPCR Taqman Probe Master Mix with UDG V5, mix thoroughly by shaking, or calculate and add according to the actual usage amount.

Protocol

1.Prepare the reaction mix.
(1)Fully thaw the TaqMan 2X qPCR Probe Master Mix with UDG V5 at room temperature, and gently mix well without creating bubbles. Spin down briefly in a microcentrifuge to collect all contents at the bottom.

Recommended protocol Component	Volume 20µL	Volume 25µl
2X qPCR TaqMan Probe Mater Mix with UDG V5	10 µL	12.5 µL
Forward Primer (10 µM)	0.4 µL	0.5 µL
Reverse Primer (10 µM)	0.4 µL	0.5 µL
Probe (10 µM)	0.4 µL	0.5 µL
50X ROX Dye (as required by instrument guidelines)	0.4 µL	0.5 µL
DNA Template	4µL<50ng)	5µL<50ng)
Nuclease-free Water	Up to 20uL	Up to 25µL

Reverse Transcription Enzymes & Kits

qRT-PCR Master Mix

cDNA First Strand Synthesis Kit

MMLV R-Transcriptase (RNase H+)

RNase Inhibitor (40 U/µl)

Oligo dT Primer

Random Hexamer Primer 9

mi-RNA cDNA FIRST STRAND-SYNTHESIS KIT

T7 HIGH YIELD RNA SYNTHESIS KIT

SCRIPT-III ONE STEP RT- qPCR TAQMAN PROBE (UDG) V5

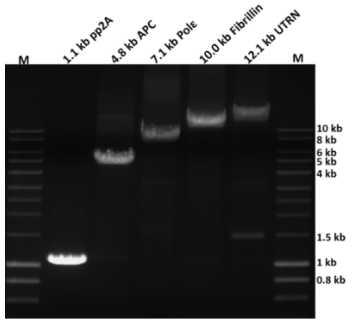
2X One Step Sybr Green RT-PCR Mix

cDNA First Strand Synthesis Kit FS-RT-4020

Introduction

cDNA First Strand Synthesis Kit features two optimized mixes: cDNA ***Enzyme Mix*** and cDNA ***Reaction Mix.*** The enzyme mix combines Reverse Transcriptase and RNase Inhibitor, and the reaction mix contains an optimized buffer. The Reverse Transcriptase is a recombinant M-MuLV reverse transcriptase with reduced RNase H activity and increased thermostability.
It can be used to synthesize first strand cDNA at higher temperatures than the wild type M-MuLV.
The enzyme is active up to 48°C, which provides higher specificity and higher yield of cDNA.
The kit also provides two optimized primers for reverse transcription and nuclease-free water.
An anchored Oligo-dT primer [$d(T)_{23}VN$] forces the primer to anneal to the beginning of the poly A tail.
The optimized Random Primer Mix provides random and consistent priming sites covering the entire RNA templates including both mRNAs and non-polyadenylated RNAs. The first strand cDNA product generated is up to 10 kb.

Kit components	FS-RT-4020 100 RXN	FS-RT-4021 200 RXN
cDNA Enzyme Mix (10X)	200 µL	2X 200 µL
cDNA Reaction Mix (2X)	1 ml	2X 1 ml
Oligo d(T)23VN * (50 µM) **	200 µL	2x200 µL
Random Primer Mix (60 µM)**	200 µL	2x200 µL
dNTPs (10 mM each)	100 µL	2x 100 µL
Nuclease-free H2O	1,25 ml	2x 1,25 ml



Using 1µg of HeLa cell total RNA as template, reverse transcription was performed with cDNA First-Strand Synthesis Kit, and ***1µL of cDNA*** was used as template to obtain clear and specific electrophoresis bands for genes of different length

* V = A, G or C; N = A, G, C or T.
** Contains 1 mM dNTP.

Highlights

- The low activity of RNase H is beneficial to the synthesis of long fragment cDNA, which can synthesize cDNA up to 10 KB.
- The optimum temperature of the enzyme was 42℃ and the enzyme still has high activity at 48℃.
- The specific cDNA yield was higher than that of ordinary M-MLV reverse transcriptase (37℃).
- Suitable for coronavirus detection;
- With a variety of RT primers, both mRNA and ncRNA can be used as reverse transcription templates.

Quality control

The performance of cDNA First Strand Synthesis Kit is tested in an RT reaction using Jurkat total RNA with primer $d(T)_{23}VN$. The length of cDNA achieved is verified by detection of a 9.2 kb amplicon of fibrillin gene.

First Strand cDNA Synthesis Reaction

1. Denaturation of RNA and primer at 65 –70° C for 5 minutes can remove secondary structures that may impede long cDNA synthesis. However, this step can be omitted in many cases (unpublished results).

2. We recommend incubation at 42 ° C for one hour for maximum yield and length. However, many targets can be detected after a much shorter incubation time. For example, 10 minutes incubation can be used for up to 5 kb cDNA synthesis.

M-MLV REVERSE TRANSCRIPTASE FS-RT-1032

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.

Kit Components

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

Applications

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) FS-RT-1152-1

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 weightof 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 theefficiency of transcription and translation, while avoiding the possible effects of using organic compound inhibitors.

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

Applications

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

Oligo(dT)$_{18}$ Primer FS-ODT-50

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 First-Strand cDNA Synthesis Kit, Primer for reverse transcription of polyadenylated RNAs, such as mRNAs

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

Random Hexamer Primer 9 FS-RH-50

High-quality DNA hexamers of randomized sequenceRandom 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

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

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

Introduction

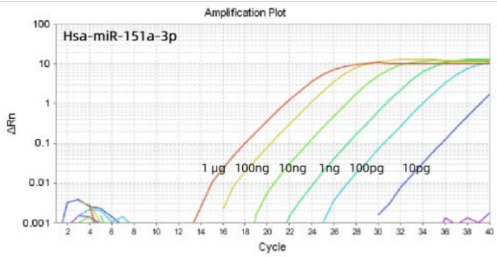
This kit is suitable for cDNA first strand synthesis using microRNA as template through the tail addition method, where the Poly (A) tail addition reaction and reverse transcription reaction at the 3 'end of miRNA can be efficiently carried out simultaneously.

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.

Kit Components

Components	FS-RT-1034
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 (10µM) 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.**

HIGHLIGHTS

- . High specificity: The kit only performs Poly (A) tail addition reaction and reverse transcription reaction on single-stranded miRNAs, avoiding interference from pre-miRNAs with secondary structure;
- Convenient and fast: Poly (A) tail addition reaction and reverse transcription reaction can be completed in one preparation.
- High sensitivity: Total RNA as low as 10 pg can be detected

Ordering Information

Cat.#	Description	Size
FS-RT-1034	miRNA First Strand cDNA Synthesis Kit	20 Reactions (20 µl)

T7 HIGH YIELD RNA SYNTHESIS KIT FS-RT-30220

T7 High Yield RNA Synthesis Kit is a flexible kit for in vitro transcription of RNA using T7 RNA polymerase. Enables the substitution of NTPs for labeling and incorporation of modified bases.

The kit is suitable for synthesis of high yield RNA transcripts and for incorporation of modified nucleotides to obtain biotin labeled, dye labeled or capped RNA. The kit is also capable of synthesizing high specific activity radiolabeled RNA probes.

RNA synthesized from the kit is suitable for many applications including RNA structure and function studies, ribozyme biochemistry, probes for RNase protection assays and hybridization based blots, anti-sense RNA and RNAi experiments, microarray analysis, microinjection, and in vitro translation and RNA vaccines.

The T7 Enzyme Mix in the kit contains RNase inhibitors and inorganic pyrophosphatase, enabling a yield of at least **150 µg of RNA per reaction** from 1 µg of DNA template.

Kit Components (50 reactions)	Size
T7 RNA Enzyme Mix	100 µL
T7 Transcription Buffer (10x)	100 µL
ATP (100 mM)	100 µL
UTP (100 mM)	100 µL
GTP (100 mM)	100 µL
CTP (100 mM)	100 µL
Control Template (0.5 µg/µL)	10 µL
DNase I, RNase-free	100 µL

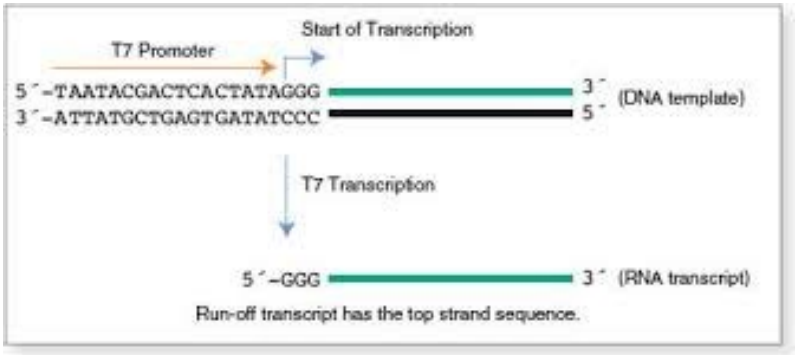
The kit contains sufficient reagents for 50 Reactions of 20 µl each

Note:

1.Prevent RNase contamination: When using this kit, wear a lab coat, disposable latex gloves, disposable masks, and use RNase-free consumables.

2.Template selection: It is recommended to purify the template before in vitro transcription to prevent contamination from RNase, protein, RNA, and salts.

3.Capped or modified RNA synthesis: If capped RNA is needed, prepare Cap Analog separately. If synthesizing labeled RNA (e.g., biotin, digoxigenin, FITC) or RNA with special modifications, the corresponding modified NTP needs to be prepared additionally.



1. Figure 1 illustrates the minimal T7 promoter sequence and the start of transcription as well as a run-off transcript after T7 transcription

SCRIPT-III ONE STEP RT- qPCR TAQMAN PROBE (UDG) FS-RT-21402

Description

Script-III One Step RT-qPCR Taqman Probe Kit with UDG a ready-to-use kit allowing reverse transcription and subsequent probe-based qPCR 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 Taq 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 RT-qPCR 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_2O		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™/StepOnePlus™.

-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 µM is recommended for most reactions. However, to optimize individual reaction, a primer titration from 0.1 µM to 1.0 µM 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-qPCR 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 Extention	95° C	5-15 sec.	45
	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)

2X ONE STEP SYBR GREEN RT-PCR Mix FS-RT-007

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 copy	1 copy

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 tothe following table to make a PCR reaction system:

Components	96-Wells		384-Wells	Concentration
X	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 µL system reaction
ABI7300、7900HT、StepOne etc.	5µL
ABI7500、7500Fast、ViiA7、Stratagene Mx3000™、Mx3005P™ and Mx4000™ etc.	1µL
Roche、Bio-Rad，Eppendorf etc.	/

NUCLEIC ACID STAINS, Nucleotides & DNA Ladders

Green Gel Safe Nucleic Acid Stain

Clearsight Nucleic Acid Stain

Green Gel Plus Nucleic Acid Stain

Sybr Safe Acid Stain

Eurosafe Green

Midori Green

Ethidium Bromide Destroyer

dNTP SET (High Concentration)

dNTP SET MIX 10

dNTP SET MIX 20

1 Kb DNA Ladder (RTU)

100bp 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

dNTP Mix - NUCLEOTIDE SET (High concentration) 100 mM 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

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 FS-013-4

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

READY-TO-USE DNA LADDERS 1 kb - 100 bp

1 kb DNA Ladder
100 bp DNA Ladder

Product Description
The 1 kb DNA Ladder RTU is suitable for sizing linear double-stranded 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.

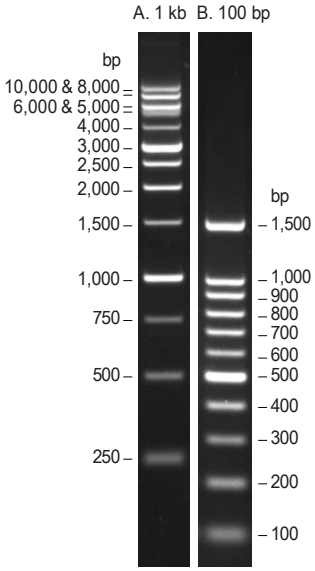
Protocol

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.



1 kb ladder

bp	ng
10,000	5.6
8,000	5.6
6,000	5.6
5,000	5.6
4,000	3.6
3,000	18.4
2,500	6.8
2,000	6.8
1,500	4
1,000	18.4
750	4.6
500	6
250	9

ng/100 ng

100 bp ladder

bp	ng
1,500	14.5
1,000	10
900	8
800	8
700	5.5
600	6
500	17
400	8
300	7
200	8
100	8

ng/100 ng

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 ***RTU***	100 bp DNA Ladder in 1 X DNA Loading Buffer	500 ul
FS-MW-500RT	1KB DNA Ladder ***RTU***	1KB DNA Ladder in 1 X DNA Loading Buffer	500 ul

READY-TO-USE DNA LADDER 50 bp

50 bp DNA Ladder

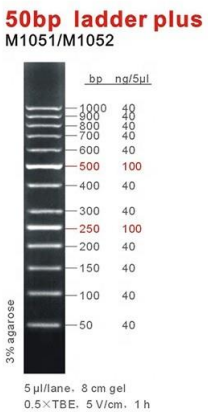
Effective Size Range:

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 µg/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

Molecular Biology Reagents & Buffers

Agarose D1-LE Standard

Agarose MS-8 Metaphor

Agarose (PFGE)

Agarose NuSieve 3:1

Agarose Low Melting

Dithiothreitol

EDTA

GLYCINE

GLYCEROL

HEPES

IPTG

MOPS

PROTEINASE K

Sodium Dodecyl Sulfate

SUCROSE

TEMED

TRIS Base Ultrapure

UREA Ultrapure

Acrylamide Solutions “Ready To Use”

30% Acry-BisAcrylamide ratio 29:1

30% Acry-BisAcrylamide ratio 37.5:1

Biological Buffers

TAE, TBE, TBS, PBS,Dulbecco’s PBS,

TE,PBS Tablets

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 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

AGAROSE MS-8 METAPHOR AS-109

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 METAPHOR	1,5%	3%
Moisture	=4.36%	
Ash	=0,26%	
EEO*	=0,11	
Sulfate	≤ 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 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. This characteristic, added to the very low sulfate content, produces a strong intercatenary interaction, yielding a gel with very high gel strength and higher exclusion limit.

- Pulsed Field Gel Electrophoresis: because of its higher exclusion 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 PULSED FIELD (Gel Electrophoresis)	
Moisture	≤ 5,12%
Ash	≤ 0.22 %
EEO*	≤ 0.11
Sulfate	≤0.083%
Clarity (NTU)	≤ 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	≥ 1 Kb- Up to 40 Kb
Gel background	Very Low

AGAROSE NUSIEVE 3:1 AS-110

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 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
Ranges of separation	2%:500 – 1500 bp 4%:150 – 600 bp

AGAROSE LOW MELTING AS-107

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

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

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 Ml

HEPES FS-3071

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 FS-0481
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 FS-5393

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

TEMED FS-3009T
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 FS-1503
equivalent to TRIZMA BASE (Sigma)

M.W. (Tris base /tris HCl) 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%

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

UREA ULTRAPURE FS-0114

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

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-7415D	Dulbecco's PBS Buffer (1X)	500ml
FSB-6201	TE Buffer (1X)	1 L
FSB-2052-100	PBS Buffer Tablets (200 ml/each)	100 tablets

DNA Extraction & Purification

GEL Extraction & PCR Clean UP Kit

MicroElute GEL Extraction and PCR Clean UP Kit

GEL EXTRACTION / PCR CLEAN UP KIT DE-001

Description

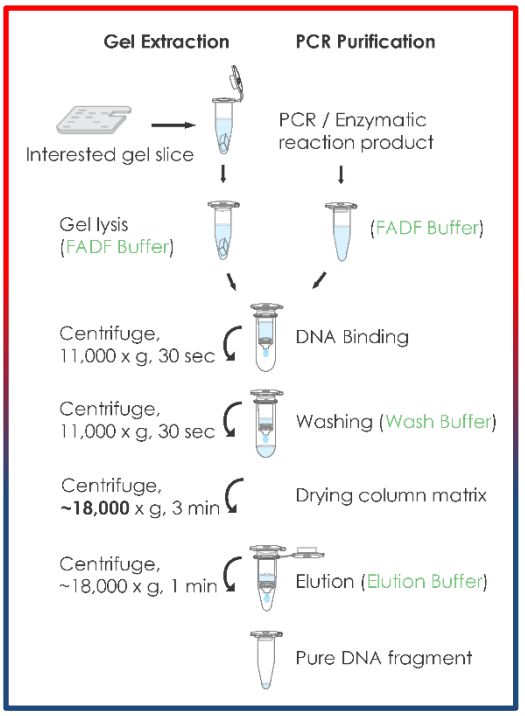
The DE-001 Gel Extraction & PCR Clean Up Kit is designed to recover or concentrate DNA Fragment (50bp- 10Kb) from agarose gel, PCR or other enzymatic reaction. The unique dual purpose application and high yield DNA column make this kit exceptional value.

Features

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

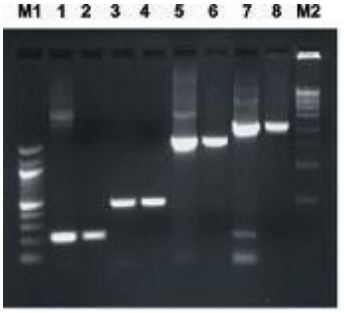
Applications

- 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



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	FSDF Buffer Wash Buffer (Conc.) Elution Buffer FSDF columns 2 ml Collection tubes	Store at room temperature for 1 year.
DE-002		300 preps		

MICROELUTE GEL EXTRACTION / PCR CLEAN UP KIT DE-020

Description

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 µl 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 µl of reaction solution

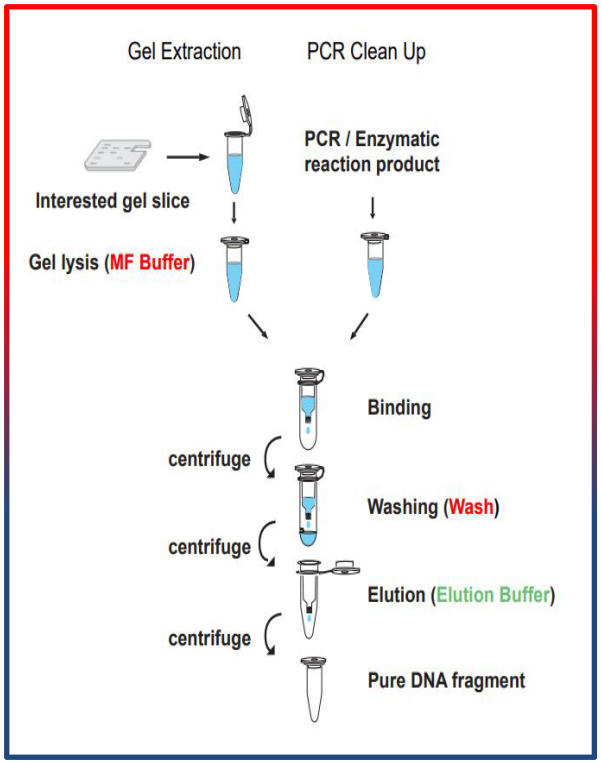
DNA size: 65 bp ~ 10 kbp

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.

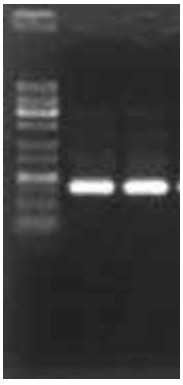


Fig.1: Agarose gel analysis of PCR product before and after purification with MicoElute Gel/PCR Purification.M: 1Kb DNA Ladder.
Lane 1: Before purification
Lane 2: after purification

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 DNA Extraction kit

Tissue Genomic DNA Extraction Kit

Mouse Tail Direct PCR Kit (Genotyping)

Mouse Tissue Direct PCR Kit

Mouse Tails Direct PCR Lysis Buffer

BLOOD & TISSUE GENOMIC DNA EXCTRATION KIT DE-047 & DE-049

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.**

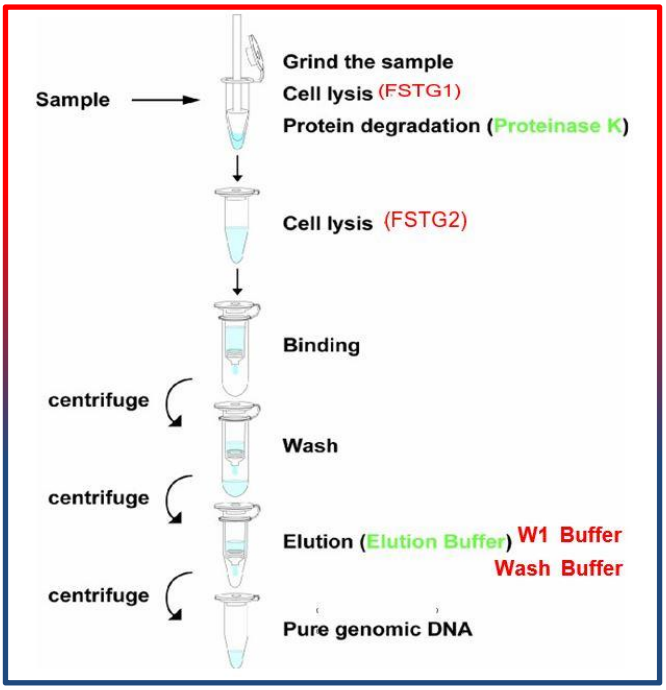
Features

-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
< 10^7 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	Blood & Tissue DNA Extraction	100 preps	Proteinase K (powder) FSTG1 Buffer FSTG2 Buffer W1 Buffer Wash Buffer (concentrated) FSTG Columns 2 mL Collection Tubes 1.5 Elution tubes Micropestles	Store at room temperature. Except Proteinase K, store at +4°C.
DE-049-200		200 preps		
DE-049-400		400 preps		

TISSUE GENOMIC DNA EXTRACTION KIT DE-012 -DE-013

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

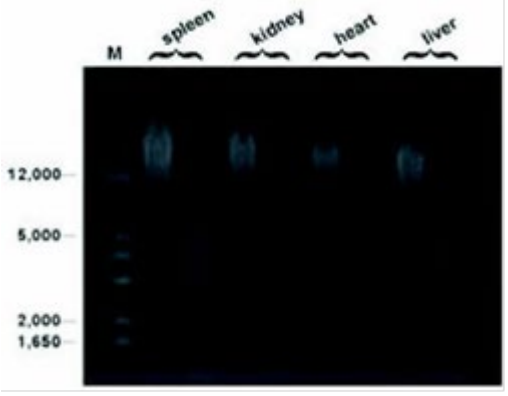
- **Principle:** mini spin column (silica matrix)
- **Operation time:** 30 ~ 60 minutes
- **Binding capacity:** up to 60 ug DNA/column
- **Typical yield:** 15 ~35 ug/ prep
- **Column applicability:** centrifugation and vacuum
- **Minimum elution volume:** 50 ul
- **Sample size**: < 25 mg animal tissue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

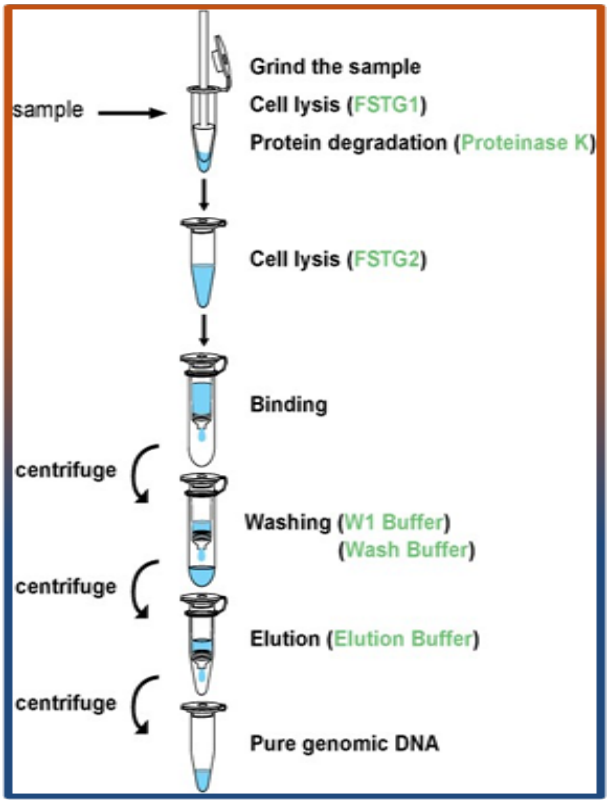
Applications

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 DE-070• DE-071

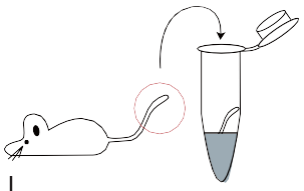
Description:

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

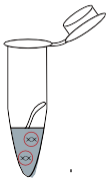
Buffer L should be stored at 4°C. Other reagents should be stored at -20°C. All reagents can be stored for 2 years.

Protocol



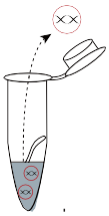
Add protease mixture to the tissue tub

Tissue Digestion



Incubate at 55°C for 15 min to release genomic DNA

Protease Inactivation



After digestion, incubate at 95°C for 5 min to inactivate the proteases

PCR

After centrifuge, 12,000 rpm for 10 min, the supernatant of digested solution can be used as the template for PCR

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[a]	2 mL	5 mL

a. 2x PCR Master Mix includes more powerful DNA polymerase, dNTPs, Mg^{2+}, and DNA Loading Dye.

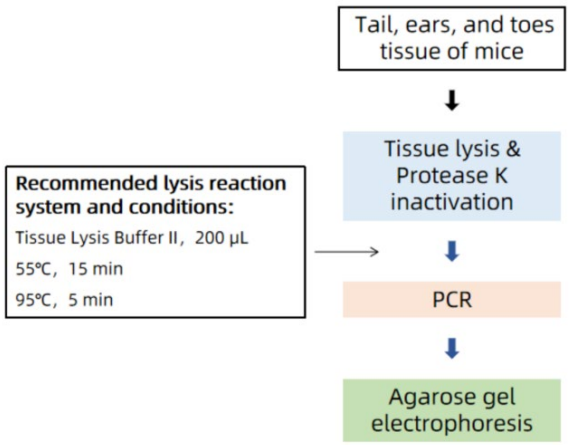
MOUSE TISSUE DIRECT PCR KIT DE-736
(Genotyping)

The Mouse Tissue Direct PCR Kit is specially designed for the rapid genotyping of mouse, which contains Tissue Lysis Buffer II and HS 2X PCR Mix for Mouse Genotyping for DNA release and PCR amplification. This kit can be used for the rapid extraction of genomic DNA from mouse tails, ears, toes, and other tissues. The extracted genomic DNA can be used directly as template for PCR amplification, which greatly shortens the experimental time. Tissue Lysis Buffer II in this kit already contains proteinase K, which does not require additional preparation.
Hot Start 2X PCR Mix for Mouse Genotyping in this kit only needs to add primers and templates to perform amplification, and the reagent contains loading buffer, PCR products can be directly loaded for electrophoresis.

Tissue Lysis Buffer II in this kit already contains proteinase K, which does not require additional preparation, and Tissue Lysis Buffer II has been tested to be stable storage for 4 weeks at room temperature or 4°C, thus avoiding repeated freeze-thaw cycles.
HS 2X PCR Mix for Mouse Genotyping in this kit contains high-performance DNA Polymerase, dNTP,and an optimized buffer system. It only needs to add primers and templates to perform amplification, thereby reducing pipetting operations, significantly controlling cross-contamination among samples, and improving detection throughput and reproducibility of results.The amplification system contains protective agents that keep HS 2X PCR Mix for Mouse Genotyping stable in activity after repeated freezing and thawing. HS 2X PCR Mix for Mouse Genotyping contains loading buffer, PCR products can be directly loaded for electrophoresis after the reaction.

PCR Product End: blunt end

Experiment workflow



Operation Description Standard Protocol

1. Take a clean 1.5 mL EP tube and add mouse tail to the EP tube (1-2 mm or 3-5 mg is recommended for mouse tail tip and toes, and 1-5 mm 2 or 3-5 mg for mouse ears).

2. Add 200 μL of Tissue Lysis Buffer II to the EP tube to completely submerge the tissue. Note: Tissue Lysis Buffer II must be fully dissolved till becoming clear before use.

The recommended lysis reaction conditions are as follows:

Step	Temp	Time
Digestion	55℃	15 min
Inactivation	95℃	5 min

Note:
To ensure the efficiency of DNA release, be sure to immerse all tissues in the lysis buffer. After the incubation, the tissue block may not be completely digested, which is normal and does not affect the use.

Components	DE-736-100	DE-736-500
Hot Start 2xPCR Mix For Mouse Genotyping	100 RXN (25 μL/Rxn)	500 RXN (25 μl/Rxn)
Tissue Lysis Buffer II	20 ml	100 ml

***Note**：Tissue Lysis Buffer II contains proteinase K, which can be stored at 4°C/Room temperature for less than 4 weeks, -20°C is recommended if stored for more than 4 weeks.

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 single-tube systems 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 and Purification

Plasmid DNA Extraction Mini Prep Kit

Plasmid DNA Endofree Extraction Midi Prep Kit

Plasmid DNA Endofree Extraction Maxi Prep Kit

PLASMID DNA EXTRACTION MINI PREP KIT DE-034

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

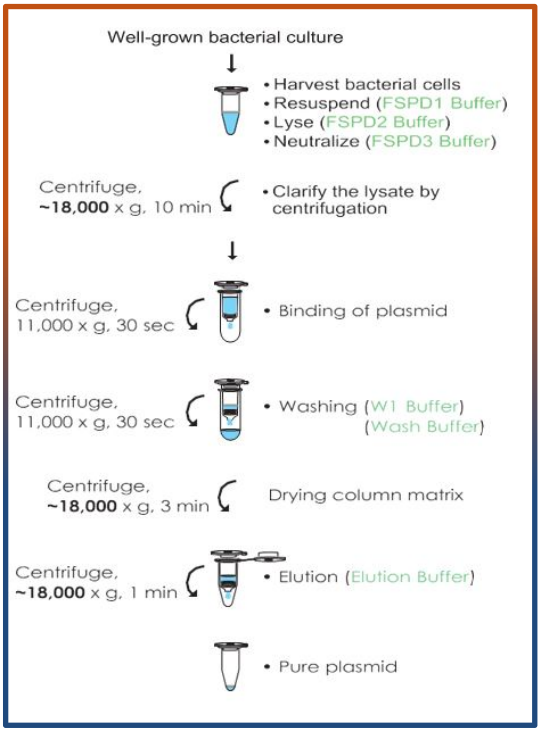
Specifications

Principle: mini spin column (silica matrix)
Sample size: 1 ~ 5 ml
Size of plasmid or construct:< 15 kb
Operation time: < 25 minutesTypical
Yield: 20 ~ 30 µg of high copy plasmid 3
~ 10 µg of low copy plasmid
Binding 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	100 preps.	Store at RT for 1 year Store FSPD1 Buffer with RNase A included at +4°C. Store the RNAse A vial at -20°C for 1 year.
DE-035	Plasmid DNA Extraction Mini Prep	300 preps.	

PLASMID DNA ENDOFREE EXTRACTION MIDI PREP KIT DE-50103

The Plasmid DNA Endofree Extraction Midi Kit is designed for rapid and efficient extraction of high quality plasmid DNA from Bacterial cultures with Spin Columns, the kit uses a modified SDS-alkaline lysis method to lyse cells.
The special P4 Buffer and the Filtration Columns 1 can effectively remove impurities such as endotoxin, protein and other impurities.
The kit is suitable for extracting plasmid DNA from 5-15 mL bacterial cultures, and the whole experimental procedure of plasmid DNA extraction could be finished within 1 h. Plasmid DNA prepared is suitable for a variety of downstream applications including restriction enzyme digestion, PCR, sequencing, ligation, transformation and cell transfection.

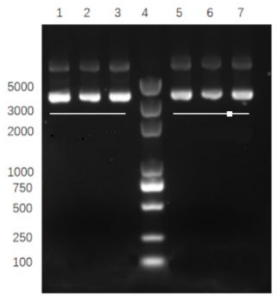
Specifications:

High yield: Up to 70 μg plasmid DNA can be quickly extracted from 5 mL-15 mL bacterial cultures within 1 h.

High purity: Optimized Buffer is used to reduce endotoxin, protein, SDS and other residues.

Wide application: Plasmid DNA prepared is suitable for a variety of downstream applications including restriction enzyme digestion, PCR, sequencing, ligation, transformation and cell transfection.

Components	DE-50103 50 preps
BL2 Buffer	270 ml
P1 Buffer	30 ml
P2 Buffer	30 ml
P4 Buffer	30 ml
DW Buffer	30 ml
DW2 Buffer	12 ml
EB2 Buffer	15 ml
RNase A* (10 mg/mL)	300ml
Spin Column 5	50 pk
Filtration Columns 1	50 pk
Collection tubes (2ml)	2x50 pk



FMB vs Competitor
Plasmid DNA extracted from Bacterial Culture cells

Important Notes:

* RNase A vial is for adding to each P1 Buffer individually.

1.Buffer P2 may precipitate at low temperature, it can be placed in a water bath at 37°C for 10 minutes to dissolve precipitate.
2.Prior to the first use, the total volume of RNase A supplied with the kit should be added to Buffer P1 (final concentration 100 μg/mL) and stored at 2-8°C for 6 months.

Precautions:

1. Prior to the first use, add 48 mL of absolute ethanol (self-prepared by the user) to Buffer DW2 and mix thoroughly.
2. Add all the RNase A to Buffer P1 and store at 2-8°. Keep it at room temperature before use.
3. Check Buffer P2 and Buffer P4 before use to see if there is any precipitate formed. If necessary, dissolve the precipitate by warming at 37°C for several minutes.
4. Unless otherwise specified, all centrifugation steps should be completed at room temperature, using a benchtop centrifuge at a speed greater than or equal to 12,000 rpm (~13,400 x g).
5. The final amount of extracted plasmid is determined by bacterial culture concentration and plasmid copy number. If the culture contains low-copy plasmids or large plasmids (>10 kb), the amount of bacterial culture should be appropriately increased. For these larger extraction volumes, the amount of solution P1, P2, P4 should be increased in proportion, while all other steps remain the same.
6. Reagent bottle should be tightly capped immediately after use to avoid volatilization, oxidation and pH changes caused by prolonged exposure to air.

Cat.#	Description	Size	Store at:
DE-50103	Plasmid DNA Endofree Extraction Midi Prep	10 preps	RNAse A-20°C for long term

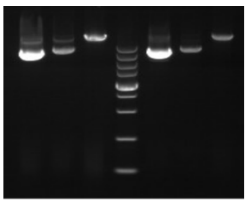
PLASMID DNA ENDOFREE EXTRACTION MAXI PREP KIT DE-30103

The Plasmid DNA Endofree Extraction Maxi Prep kit is designed for rapid and efficient extraction of high quality plasmid DNA from Bacterial cultures with Spin Columns, the kit uses a modified SDS-alkaline lysis method to lyse cells. The unique endotoxin remover can effectively remove endotoxins. The silicone matrix membrane in the spin column selectively binds plasmid DNA at high salt and low pH conditions, allowing for impurities and other bacterial components to be removed by the addition of Buffer PR and Buffer WB.

The purified plasmid DNA is eluted from the silicone matrix membrane using low-salt and high-pH Buffer EB.

The yield and quality of extracted plasmids are dependent upon the species and culture conditions of host bacteria, cell lysis, plasmid copy number, plasmid stability, and use of antibiotics.

Components	DE-30103 10 preps
Buffer ER	25 mL
Buffer P1	75 mL
Spin Column 3	10 pk
Buffer P2	75 mL
Buffer N3	75 mL
Buffer PR*	65 mL
Buffer WB**	2 × 25 mL
Buffer EB	20 mL
RNase A (10 mg/mL)	750 µL
Spin Column 3	10 pk
Collection Tube 50 mL	4 × 5 pk



FMB Vs Competitor Plasmid DNA extracted from Bacterial Culture cells.

***Note: Add 38 mL of absolute ethanol to Buffer PR prior to initial use.**

****Note: Add 100 mL of absolute ethanol to each Buffer WB bottle prior to initial use. Final reagent volume in each Buffer WB bottle is 125 mL.**

Specifications

- **High yield:** 0.5-2 mg of pure high-copy plasmid DNA can be rapidly extracted from 150-300 mL of Luria-Bertani culture medium, with an extraction rate of 80-90%;
- **Low endotoxin content**: endotoxin <0.1 EU/µg DNA, excellent cell transfection effect;
- **Stable performance:** minimal difference in adsorption capacity between columns, and satisfied repeatability.

Storage

1. This kit is stable for 12 months when stored at room temperature.
2. RNase A, Buffer ER and Buffer P1 can be transported at room temperature for a short time, but for long-term storage, RNase A and Buffer ER should be kept at -20℃; Buffer P1 should be kept at 4℃.
3. Minor precipitation of reagents does not affect experimental results. If necessary, reagents can be placed in a water bath at 37°C for 10 minutes to dissolve precipitate.

Highlights

1. The unique Deproteinization Buffer (Buffer PR) efficiently removes residual nucleases, including those from host strains with abundant nucleases such as JM series and HB101, effectively preventing plasmid degradation by nucleases.
2. Neither toxic reagents (i.e. phenol and chloroform) nor ethanol precipitation are required.
3. Final products have minimal endotoxin content (< 0.1 EU/µg DNA).
4. It is a fast and convenient high-quality plasmid DNA extraction method. From 150-300 mL of LB medium, 0.5-2 mg of pure high-copy plasmid DNA can be rapidly extracted with an extraction efficiency of 80-90%.

Scope of Application

The plasmid DNA extracted by this kit can be directly used in cell transfection experiments along with various molecular biological experiments such as enzymatic digestion, transformation, PCR, in vitro transcription, and sequencing.

Cat.#	Description	Size	Store at:
DE-30103	Plasmid DNA Endofree Extraction Maxi Prep	10 preps	RNAse A-20°C for long term

RNA Extraction & Purification

Tissue Total RNA Etraction Mini Kit

TRIZOL Ultrapure

RNA-ZOL Direct Clean Up-Plus Kit

RNA Stabilization Reagent

Viral Nucleic acid (DNA& RNA) Extraction Kit

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 ~ 60 minutes
Binding capacity: up to 100 µg total RNA/ column
Column applicability: centrifugation and vaccum
Minimum elution volume: 40 µl

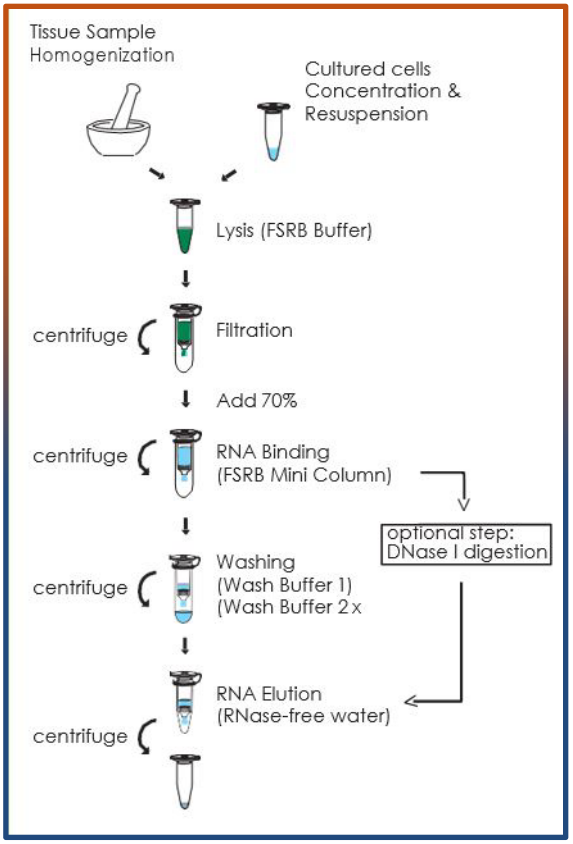
Applications
- Nothern blotting hybridizations
- Primer extension
- RT-PCR
- RNase protection assays
- Differential display
- As starting material for purification of mRNA for cDNA synthesis

Sample Size: Animal cells: from 1 x 10^6 up to 5 x 10^6 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 10^6 Cells)	NIH/3T3 HeLa COS-7 LMH	1 X 10^6 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 10 30 50 35 15 45
Bacteria	E.coli B. subtilis	1 x 10^9 Cells	60 40
Yeast (up to 5 x10^7 cells)	S. cerevisiae	1 x 10^7 Cells	25

Brief procedure:



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.

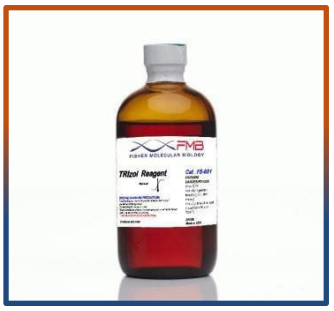
Cat. No.	Product Name	Size	Store at
RE-006	Tissue & Cells Total RNA Mini Kit	100 preps	At Room Temperature at 15-25°C for 1 year

TRIZOL ULTRAPURE-RNA / DNA / PROTEIN ISOLATION REAGENT FS-881

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 guanidine 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
FS-881	TRIZOL DNA/RNA Protein Isolation Reagent	100 ml
		200 ml



RNA-ZOL DIRECT CLEAN-UP PLUS KIT RE-040

The RNA-Zol Direct Clean Up Plus kit provides a streamlined method for the purification of up to 100 µg (per column) of high-quality 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 Kit (Tri-Zol included)	50 preps	Store at 15°C for 1 years
RE-041		200 preps	
RE-042	RNA-Zol Direct Clean-Up Plus Kit (Tri-Zol not included)	50 preps	
RE-043		200 preps	

RNA LATER STABILIZATION REAGENT FS-883

RNA*later* 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 Later .
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 Later
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℃ 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℃ 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

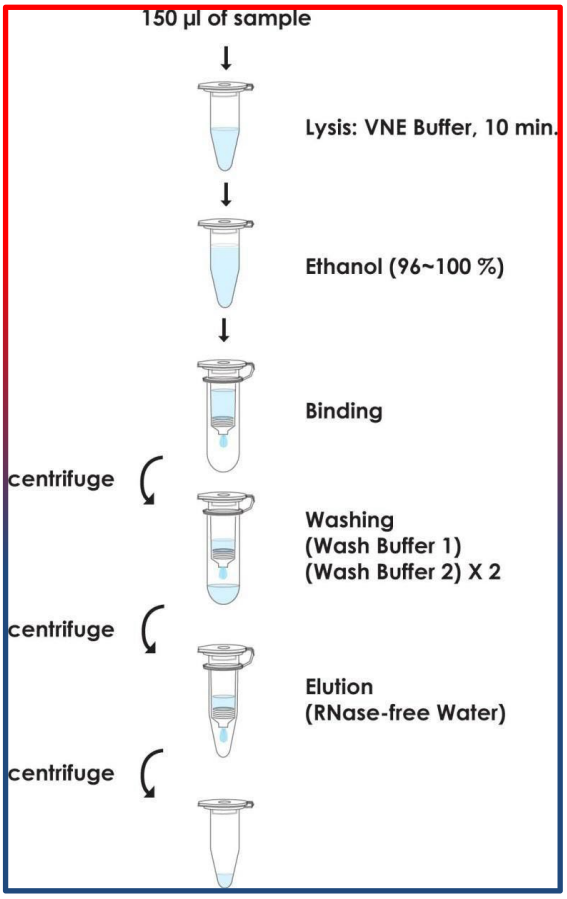
Recovery rate: 80-90%

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

Environmental DNA/RNA Extraction Kits

Plant Genomic DNA Extraction Mini Kit

Soil DNA Isolation Mini Kit

Stool DNA Isolation Mini Kit

Viral Nucleic acid (DNA& RNA) Extraction Kit

Fungi/Yeast Genomic DNA Extraction Mini Kit

Plant Total RNA Purification Mini Kit

PLANT GENOMIC DNA EXTRACTION MINI KIT DE-021

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

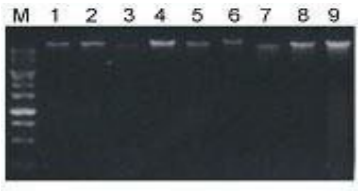
About 30-60 minutes depending upon the sample types.

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 °C). Stable for 1 year at room temperature at 15-25°C.

The Quality of DNA After Purification



7: Populus tremula (Aspen)

8: Flammulina velutipes

9: Oxalis comiculats (Fourleaf clover)

DNA Yield

Sample	DNA yield (ug)	
	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

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

SOIL DNA ISOLATION MINI KIT DE-025 & DE-026

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)

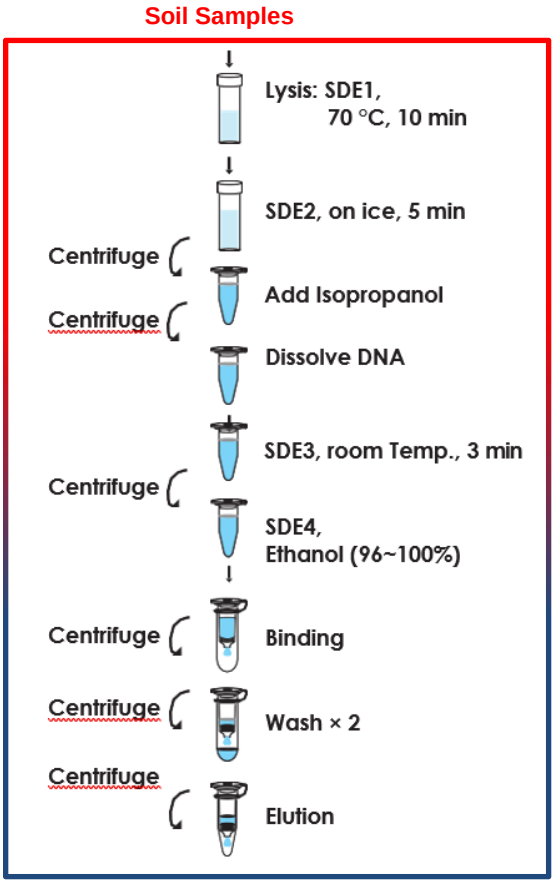
Sample: **0,25 -0,5 g**

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°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 DE-023 & DE-024

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)

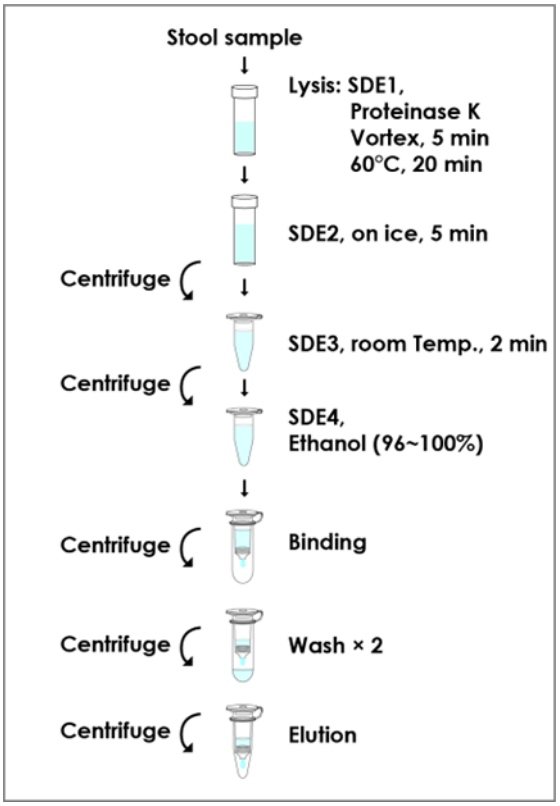
Sample: 50~200 mg

Operation time: < 60 min

Elution volume: 50~200 µl

Important Notes

1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. 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°C. If DNA is isolated from gram positive bacteria, prepare a heating block or a water bath to 95°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.



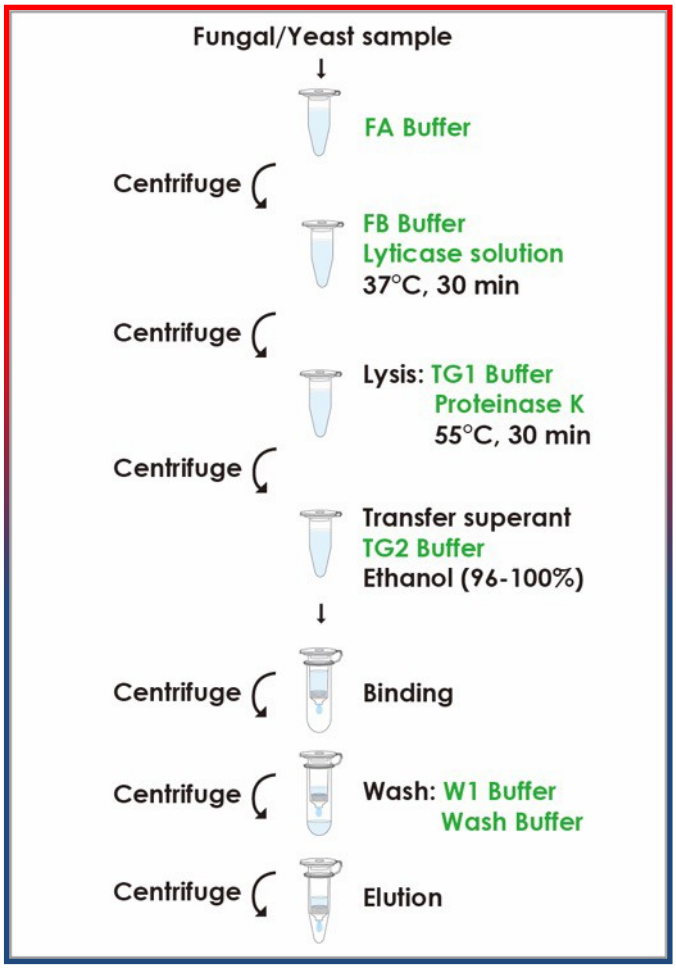
Cat. N°	Product Name	Size
DE-023	Stool DNA Isolation kit	50 Reactions
DE-024		100 Reactions

FUNGI /YEAST GENOMIC DNA EXTRACTION MINI KIT DE-046

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 x10^6 cell culture fungal/yeast cells
Operation time:~ 60 minutes
Binding capacity:60 μg/ column
Column applicability: centrifugation and vaccum



Ordering Information

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℃.

PLANT TOTAL RNA PURIFICATION MINI KIT RE-007

Description

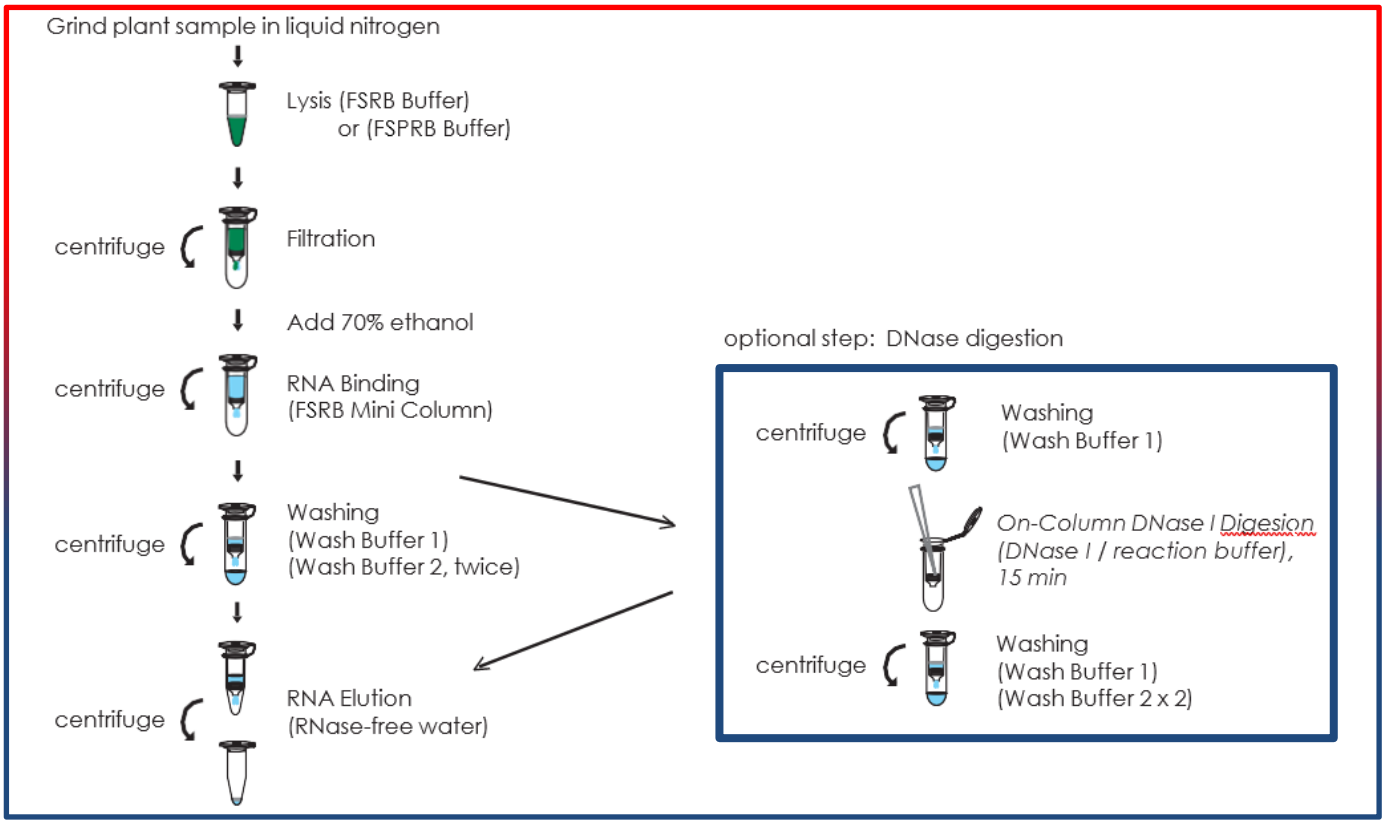
The Plant Total RNA Purification Mini Kit is designed for purification of total RNA from plant tissues and cells using the modified salt precipitation procedure and RNase inhibitors without the use of hazardous solvents such as phenol. Plant RNA is quickly and efficiently isolated and is immediately 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 $1x10^7$ 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



Ordering Information

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.