

Catalogue

2023



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Taq DNA Polymerases

Ampliotherm DNA Polymerase

GOTAQ-Flexi DNA Polymerase Green G2

GOTAQ DNA Polymerase Green Premix G2

HOT-START Taq DNA Polymerase (GC rich Buffer)

GOTAQ-Flexi Hot Start DNA Polymerase Green G2

GOTAQ Green 2X Master Mix G2

Ampliotherm PCR 2X Master Mix

GOTAQ-Hot Start Green Master Mix G2

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

PFU DNA Polymerase

GC-Platinum pOWER TAQ 2X Master Mix (Blue Dye)

DESCRIPTION:

Ampliotherm DNA Polymerase is a thermostable 94 kDa DNA Polymerase purified from E.coli PVG-AI recombinant strain expressing *Thermus aquaticus* 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 |
|-----------------------------------|----------|
| Ampliotherm Taq DNA Polymerase | 250 U |
| 10X Reaction Buffer | 1 vial |
| 25mM MgCl ₂ 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 Ampliotherm 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 BUFFER | |
|---|--------------------------|
| 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 ul PCR Reaction | Volume | Final Conc. |
|------------------------------------|-------------|-------------|
| Ampliotherm 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 thermocycler 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.

Description

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

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

**Equivalent to GoTaq G2 Flexi DNA Polymerase*

| Description | FS-T-0531 |
|-------------------------------------|-----------|
| GOTAQ Flexi DNA Polymerase Green G2 | 500 U |
| 5X Green Buffer | 4 ml |
| 5X Colorless Buffer | 4 ml |
| 25mM MgCl ₂ Solution | 4 ml |

Storage Buffer

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

5X Green Buffer

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

5X Colorless Buffer

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

Unit Definition

1 unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid-insoluble material in 30 minutes at 75°C.

Storage Conditions

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

Quality Control

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

Recommended Protocol

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

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

General Cycling Conditions :

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

Notice:

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

Applications:

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

Description

GOTAQ DNA Polymerase Green Premix G2 is a thermally stable, processive, 5'-3' DNA polymerase. The 94 kDa protein possesses an inherent 5'-3' nick-translation moiety and lacks a 3'-5' proofreading function. GOTAQ DNA Polymerase Green Premix G2 is provided with 5X Green Buffer, 5X Colorless Buffer. The 5X Green Buffer contains loading dye for direct electrophoresis. And the 5X Colorless Buffer is used when direct fluorescence or absorbance readings are required without prior purification of the amplified DNA from the PCR. Both buffers contain MgCl₂ at a concentration of 7.5mM for a final concentration of 1.5mM in the 1X reaction.

**Equivalent to GoTaq G2 DNA Polymerase*

Kit Contents

| Contents | FS-T-0532 |
|---|-----------|
| GOTAQ DNAPolymerase Green Premix G2 (5U/μl) | 500 U |
| 5X Green Buffer | 4 ml |
| 5X Colorless Buffer | 4 ml |

Storage Buffer

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

5X Green Buffer

Proprietary formulation supplied at pH8.7 containing blue dye and yellow dye. The buffer contains Tris-HCl, KCl and 7.5mM magnesium. Vortex thoroughly after thawing and prior to use.

5X Colorless Buffer

Proprietary formulation supplied at pH8.7 contains Tris-HCl, KCl and 7.5mM magnesium. Vortex thoroughly after thawing and prior to use.

Unit Definition

1 unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid-insoluble material in 30 minutes at 75°C.

Storage Conditions

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

Quality Control

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

Recommended Protocol

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

| For 50μl PCR Reaction | Volum | Final |
|--|-------------|----------|
| GOTAQ DNA Polymerase Green PremixG2(5U/μl) | 0.25 μl | 1.25 U |
| 5X Green Buffer (or Colorless Buffer) | 10 μl | 1 X |
| dNTP mix (2.5 mM each) | 4 μl | 0.2 mM |
| Template DNA | X μl | < 500 ng |
| Forward Primer | X μl | 0.1~1 μM |
| Reverse Primer | X μl | 0.1~1 μM |
| Distilled water | up to 50 μl | |

General Cycling Conditions :

| Step | Temp (°C) | Time | Cycle |
|----------------------|-----------|--------------|---------|
| Initial Denaturation | 95 | 2 min. | 1 |
| Denature | 95 | 30 ~ 60 sec. | 25 ~ 40 |
| Anneal | 50~65 | 30 ~ 60 sec. | |
| Extend | 72 | 60 sec./kb | |
| Final Extension | 72 | 5 min. | 1 |

Notice:

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

Buffer Choice

We recommend using the 5X Green Buffer in any amplification reaction that will be visualized by agarose gel electrophoresis followed by ethidium bromide staining. The 5X Green Buffer is not recommended for any downstream applications using absorbance or fluorescence excitation, as the yellow and blue dyes in the reaction buffer may interfere with these applications. The Green and Colorless Buffers give approximately equivalent amplification yields. Balanced amplifications between the two buffers may require further optimization.

For application using absorbance or fluorescence, the 5X Colorless Buffer is recommended.

HOT START-TAQ DNA POLYMERASE (WITH GC RICH BUFFER) FS-T-2131-1

Description

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

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

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

Kit Contents

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

100 μl = 500 units

10X GC Rich Buffer:

Unit Definition

1 unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid-insoluble material in 30 minutes at 75°C.

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

Storage Conditions

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

Quality Control

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

Recommended Protocol

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

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

General Cycling Conditions :

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

*Subjected to the actual best annealing temperature

Notice:

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

Description

GoTaq Flexi Hot-Start DNA Polymerase Green G2 is an antibody mediated hot-start Taq DNA polymerase and quite suitable for high-specific hot-start PCR, real-time PCR and multiplex PCR. The GoTaq Flexi Hot-Start DNA Polymerase Green G2 is a thermally stable, processive, 5'→ 3' DNA polymerase. The 94 kDa protein possesses an inherent 5'→3' nick-translation moiety and lacks a 3'→5' proofreading function. GoTaq Flexi Hot-Start DNA Polymerase Green G2 is provided with 5X Green Buffer, 5X Colorless Buffer and MgCl₂ Solution. The 5X Green Buffer contains loading dye for direct electrophoresis. And the 5X Colorless Buffer is used when direct fluorescence or absorbance readings are required without prior purification of the amplified DNA from the PCR. Both buffers do not contain magnesium, allowing easy optimization in amplification reactions.

| Contents | FS-T-1731 |
|---|-----------|
| GoTaq Flexi Hot-Start DNA Polymerase Green G2 | 500 U |
| 5X Green Buffer | 4 ml |
| 5X Colorless Buffer | 4 ml |
| 25mM MgCl ₂ Solution | 4 ml |

Storage Buffer

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

5X Green Buffer

Proprietary formulation supplied at pH8.7 containing blue dye and yellow dye. The buffer contains Tris-HCl, KCl and buffer does not contain magnesium.

5X Colorless Buffer

Proprietary formulation supplied at pH8.7 containing Tris-HCl, KCl and buffer does not contain magnesium.

Unit Definition

1 unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid-insoluble material in 30 minutes at 75°C.

Storage Conditions

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

Quality Control

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

Recommended Protocol

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

| For 50ul PCR Reaction | Volume | Final Conc. |
|---|-------------|-------------|
| GoTaq Flexi Hot-Start DNA Polymerase Green G2 (5U/UI) | 0.25 ul | 1.25 U |
| 5X Green Buffer(or 5X Colorless Buffer) | 10 ul | 1 X |
| 25mM (MgCl ₂ Solution) | 2-8 ul | 1-4 mM |
| dNTP Mix(2,5mM) each | 4 ul | 0,2 mM |
| Template | To be det. | < 500 ng |
| Forward Primer | To be det. | 0.1~1 uM |
| Reverse Primer | To be det. | 0.1~1 uM |
| Distilled water | up to 50 ul | |

General Cycling Conditions :

| Step | Tem p (°C) | Time | Cycle |
|----------------------|------------|--------------|---------|
| Initial Denaturation | 95 | 3 min. | 1 |
| Denature | 95 | 15 ~ 60 sec. | 25 ~ 45 |
| Anneal | 50~65 | 15 ~ 60 sec. | |
| Extend | 72 | 60 sec./kb | |
| Final Extension | 72 | 5 min. | 1 |

Notice:

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

Buffer Choice

We recommend using the 5X Green Buffer in any amplification reaction that will be visualized by agarose gel electrophoresis followed by ethidium bromide staining. The 5X Green Buffer is not recommended for any downstream applications using absorbance or fluorescence excitation, as the yellow and blue dyes in the reaction buffer may interfere with these applications. The Green and Colorless Buffers give approximately equivalent amplification yields. Balanced amplifications between the two buffers may require further optimization.

For application using absorbance or fluorescence, the 5X Colorless Buffer is recommended.

| 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, MgCl₂ 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.

Description

Amplibiotherm PCR 2X Master Mix is an optimized premix containing DNA polymerase, dNTPs, MgCl₂, KCl and other stabilizers. This product is suitable for conventional PCR amplification. The template can be purified DNA, bacterial colonies/bacteria liquid, crude extract or cDNA, etc. This product can use complex genomic DNA as a template to amplify a target fragment of 5 kb in length or a simple template such as lambda DNA to amplify a target fragment of 10 kb in length. It is suitable for applications such as PCR reaction, colony PCR identification, vector construction and so on. etc.

Kit Contents

| Contents | FS-T-1041-10 | FS-T-1041-25 |
|--|--------------|--------------|
| Amplibiotherm PCR 2X Master Mix | 10 ml | 25 ml |

1 ml= 40 reactions (Volume of 50 µl)
 1 ml= 80 reactions (Volume of 25 µl)

Applications: Conventional PCR

Storage Conditions

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

Quality Control

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

Recommended Protocol

This standard protocol applies to a single reaction where only template, primers, and water need to be added to the Amplibiotherm PCR 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 | 25 µl | 50 µl | Final Conc. |
|---------------------------------|-------------|-------------|-------------|
| Amplibiotherm PCR 2X Master Mix | 12,5 µl | 25 µL | 1X |
| 10µM Forward Primer | 0.5 µL | 1 µL | 0.2 µM |
| 10µM Reverse Primer | 0.5 µl | 1 µL | 0.2 µM |
| Template DNA* | Variable | Variable | <300 ng |
| Water, RNase-Free | up to 25 µl | up to 50 µl | N/A |

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

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

Recommended PCR Program

| Step | Temp | Time | Cycles |
|------------------|---------|-----------|--------|
| Pre-denaturation | 98°C | 45 s | 1 |
| Denaturation | 98°C | 10 s | 30 |
| Annealing | 55-65°C | 30 s | |
| Extension | 72°C | 20-30s/kb | |
| Post-extension | 72°C | 5min | 1 |
| Hold | 4-12°C | ∞ | 1 |

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°C, 5-10 s

4. Annealing:

The annealing temperature of Amplibiotherm 2X PCR Mix is usually higher than other PCR polymerases. Generally, primers longer than 20 nt are annealed at (lower primer T_m+3)°C for 10-30 s; when the primers are shorter than 20 nt, an annealing temperature equivalent to the lower primer T_m. 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.

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

Description

GOTAQ Hot Start Green Master Mix G2 is **2X Ready-to-Use** Hot-start PCR pre-mixes are the innovation for convenience of your routine PCR. The GOTAQ Hot Start Green 2X Master Mix G2 is an optimized, **Ready-to-Use** PCR mixture of **GOTAQ Hot Start Green**, **PCR buffer**, **MgCl₂** 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 |
|--|--------------------|
| GOTAQ HS -PCR Green 2XMaster Mix G2 | 1 ml/100 reactions |

Applications

GOTAQ Hot Start Green 2X Master Mix G2 is suitable and tested for amplification of genomic targets ranging from 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 Hot Start Green 2X Master Mix G2 with primers and template DNA used in individual reactions. Thaw and mix all components thoroughly, spin down shortly and chill on ice.

Quality Control

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

**Equivalent to GoTaq G2 Hot Start Polymerase*

Recommended Protocol

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

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

Reaction Conditions

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

NOTE: In general, use greater than 0.5 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.

Description

Hot Start -PCR 2X Master Mix is ready-to-use Hot-start PCR pre-mixes are the innovation for convenience of your routine PCR. The Hot Start -PCR 2X Master Mix is an optimized, ready-to-use PCR mixture of Hot Start-Taq DNA Polymerase, PCR buffer, MgCl₂, dNTP's, except DNA template and primers. The optimized buffer formula maximizes the effect of the enzyme, achieving low mismatch rate and high amplification efficiency for complex templates. The original MasterMix formula makes the entire reaction system very stable and repeatable. This product already contains dyes, and the electrophoresis operation can be performed directly after the PCR procedure is completed. The amplified PCR product has an "A" base attached to the 3' end, so it can be used directly for T/A cloning. It is mainly suitable for the amplification of DNA templates with high GC content.

Kit Contents

| Description | FS-T-1141-1 | FS-T-1141-5 |
|--|-------------|-------------|
| Hot Start -PCR 2X Master Mix with GC-Rich Buffer | 5 ml | 25 ml |
| ddH ₂ O | 5x1 ml | 125x 1ml |

1 ml=40 reactions

Applications

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

MgCl₂ concentration: This product contains 3 mM MgCl₂, which is suitable for most PCR reactions.

Storage Conditions

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

Note

Do not contaminate the Hot Start -PCR 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.

Recommended Protocol

This standard protocol applies to a single reaction where only template, primers, and water need to be added to the Hot Start -PCR 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 | 25 ul reaction | 50 ul reaction |
|---|----------------|----------------|
| Hot Start PCR 2X Master Mix With GC_Rich Buffer | 12,5 µl | 25µl |
| 10µM Forward Primer | 1 µl | 2 µl |
| 10µM Reverse Primer | 1 µl | 2 µl |
| Template DNA | ≥ 1 µl | ≥ 1 µl |
| Water, RNase-Free | Up to 25 µl | up to 50 µl |

NOTE: Recommended amount of template per PCR reaction:

- **genomic DNA: 50-200 ng**
 - **plasmid DNA: 0.1-10 ng**
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 |
|------------------|-----------|------------|-------|
| Pre-denaturation | 94 | 5 min. | 1 |
| Denature | 94 | 30 sec. | 35 |
| Anneal | 50 -60 | 30 sec. | |
| Extension | 72 | 30 sec./kb | |
| Final Extension | 72 | 10 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. **Annealing temperature:** Please refer to the theoretical T_m value of the primer, and the annealing temperature can be set lower than the theoretical value of the primer by 2-5°C.
8. **Extension time:** Molecular identification is recommended at 30 sec/kb. Gene cloning is recommended at 60 sec/kb to ensure the highest amount of product.
9. After cycling, maintain the reactions at 4°C or store at -20°C until ready for analysis.

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 Polymerase (5 U/ul) | 1000 U |
| 10X Pfu Buffer (MgCl ₂) | 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.

10x Pfu Buffer with MgCl₂:

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

Concentration:5 u/ul

Quality control

Free of detectable, non-specific nucleases.

Storage Conditions

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

Application

- 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

General PCR reaction mixture for 50 ul Reaction :

On ice, prepare each of following master mixes, combine, and place in heated (to 94°C) thermal cycler:

- 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.

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

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 |

- 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.

- 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.

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

| Contents | FS-T-1641-5 | FS-T-1641-25 |
|--|-------------|--------------|
| GC-Platinum Power TAQ 2X Master Mix (blue dye) | 5 ml | 25 ml |

1 ml= 40 Reactions (50 µl volume)

1 ml= 80 Reactions (25 µl volume)

Applications

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

Storage Conditions

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

Note

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

Quality Control

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

Template:

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

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

Primers :

Oligonucleotide primers are typically 20-40 nucleotides in length with a GC content of 40-60%. Primers can be designed and analyzed using software such as Primer 3. The final concentration of each primer in the PCR reaction system should be in the range of 0.1-1 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 | 25 µl volume | 50 µl volume | Final Conc. |
|--|--------------|--------------|-------------|
| GC-Platinum Power TAQ 2X Master Mix (blue dye) | 12,5 µl | 25 µl | 1X |
| 10µM Forward Primer | 0.5 µl | 1.0 µl | 0.2 µM |
| 10µM Reverse Primer | 0.5 µl | 1.0 µl | 0.2 µM |
| Template DNA | variable | variable | 300 ng |
| Water, RNase-Free | up to 25 µl | up to 50 µl | NA |

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 2-6°C 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.

Real Time qPCR Master Mix

Sybr Green qPCR 2X Master Mix

Sybr Green Fast qPCR 2X Master Mix

2X Universal Sybr Green Fast qPCR Mix

EVAGreen qPCR 2X Master Mix

TAQMAN Probe Fast qPCR Master Mix

TAQMAN Probe qPCR 2X Master Mix (UDG)

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 |
| ddH ₂ O | 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 template is essential, recommending using diluted templates to reduce to increase aliquot accuracy, and the result is reproducible.
- 3) If templates are undiluted cDNA from standard reverse transcription reaction, the volume of template is no more than 10% of the reaction volume.

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

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

Running qPCR Reaction as follows:

| | | | | |
|----------------|---------------|----------|----------------------|----------------------------|
| Stage 1 | Pre-denature | 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 program 95°C/10 sec 60°C/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°C/10 sec. 56°C/30 sec 72°C/30 sec | Increase extending time 95°C/10 sec 56°C/30 sec 72°C/60 sec |
|--|--|

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

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.

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 |

Kit Contents

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

1 ml =100 reactions

Materials Required

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

Usage Notes

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 ₂ O | 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 R² 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 the T_m value in melt curve is usually in the range of 80 to 95°C.

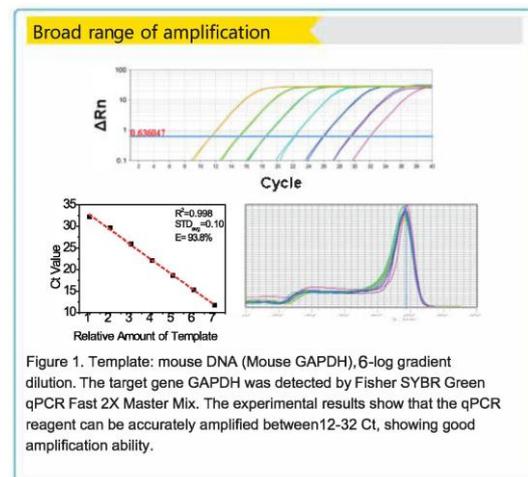


Figure 1. Template: mouse DNA (Mouse GAPDH), 6-log gradient dilution. The target gene GAPDH was detected by Fisher SYBR Green qPCR Fast 2X Master Mix. The experimental results show that the qPCR reagent can be accurately amplified between 12-32 Ct, showing good amplification ability.

| Description | FS-T-50215-5 | FS-T-50215-25 |
|---------------------------------------|---------------------|-----------------------|
| 2X Universal SYBR Green Fast qPCR Mix | 500 RXN 5 X 1 mL | 2,500 RXN 25 X 1mL |

1 ml= 100 reactions

Introduction

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. It contains Hot Start Taq, to avoid unexpected amplification Results. It is an optimized qPCR Mix you need to add 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.

Instruments

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

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 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

- (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 samples is suggested.

PROCEDURE:

1. Prepare the following reaction systems on ice

| Components | 20 uL Reaction |
|--|----------------|
| 2X Universal Sybr Green Fast qPCR Mix (No ROX) | 10 uL |
| Forward Primer (10 uM) | 0,4 uL |
| Reverse Primer (10 uM) | 0,4 uL |
| gDNA or cDNA (<50 ng) | 2 uL |
| RNase free ddH2O | To 20 uL |

- (1) Dissolve 2X Universal 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.

Description:

Eva Green qPCR 2x 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.

Eva Green qPCR 2x 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 |
|--|-----------|
| Eva Green qPCR Master Mix Plus Low-ROX | 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. |
|------------------------------|-----------------------------|-------------------|
| Eva Green qPCR 2x 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: EVA Green qPCR 2X 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 EVA Green qPCR 2X 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 T_m at 55 °C, run both RT step and extension step at 55 °C. For accurate quantitation of transcript levels, a no-RT control is recommended to check for possible genomic DNA contamination.

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

4. ROX reference dye: For certain instruments, ROX is necessary for accurate Ct determination from well to well. Refer to Table 1 (See Page 5-6) for the recommended 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 10x ROX 1:10 with H₂O to obtain 1x ROX; add 1 to 2 uL of 1x 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 System Cepheid: SmartCycler 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 under fast cycling conditions.

| Cycling Step | Temp. | Holding Time | N. of Cycles |
|---|------------------------------|--------------------------|--------------|
| Enzyme Activation | 95 °C | 2 min | 1 |
| Denaturation Annealing & Extension | 95 °C 60 °C | 15s 60s | 45 |

B. Two-step fast cycling protocol

This cycling protocol should be applicable to most amplifications where the primer T_m's are designed to be 60°C. 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 °C 60 °C | 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 °C 50~60°C 72 °C | 10 s 10s Note 6) 10s (Note 7) | 45 |

Description

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. The 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 |
|------------------------------------|------------|---------|
| *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: SmartCycler
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 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 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.

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

Reaction Conditions

| | 20ul reaction | Final conc. |
|-----------------------------------|----------------|-----------------|
| TaqMan 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.

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

Description

TaqMan Probe qPCR 2X Master Mix with UDG 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 need

Kit Contents

| Contents | CAT. N° | Size |
|--|------------|--------|
| TaqMan Probe qPCR 2X Master Mix with UDG | FS-T-50217 | 500 RX |
| Rox Dye I (high Rox) 50X | | 1 Vial |
| Rox Dye II (low Rox) 50X | | 1 Vial |

1ml = 100 Reactions

ROX dye - Real Time Machines:

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

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

No ROX Dye – Real Time Machines

BioRad: iCycler, MyiQ, MiQ 2, iQ5, CFX-96, CFX-384, MJ Opticon, option2, Chromo4, MiniOpticon **Roche:** LightCycler 480, LightCycler 2.0

Eppendorf: Mastercycle realplex - **illumina:** Eco RealTime PCR System Cepheid: SmartCyler

Applications

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

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

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

Note

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

Additional Material Required but not Supplied

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

-qPCR primers and probes

-DNA or cDNA templates

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

Precautions

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

2. The TaqMan 2X qPCR Probe Master Mix with UDG contains glycerin. Mix gently before use without generating air bubbles. Spin briefly to collect all the contents at the bottom.

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

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

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

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

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

Important points before set up:

1. A final primer concentration of 0.2 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.

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

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

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

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

6. Triplicates are recommended as technical replicates in real-time PCR reactions.

Set up: Prepare the reaction mix.

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

Reaction Conditions

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

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

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

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

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

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

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

PCR Conditions

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

*To ensure signal acquisition after extension, the extension temperature should be based on the Tm value of the primer probe. Line adjustment.

** It is recommended that the shortest pre-denaturation time should not be shorter than 30s, and the longest should not exceed 10 min; the shortest denaturation time during the cyclic reaction is not less than 5s, and the longest is not more than 15s; the cyclic reaction; the shortest extension time in the application is not less than 10s, and the longest can be based on the primer probes and signals used by yourself. The set needs to be adjusted by itself.

Reverse Transcription Enzymes & Kits

One Step Sybr Green 2X RT PCR Mix

cDNA Synthesis Kit (Plus gDNA Eraser)

M-MLV Reverse Transcriptase

RNase INHIBITOR (40= U/ul)

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 |
|-----------------------|-----------------|---------------------|
| 2xOne Step RT-PCR Mix | 1 ml (100 rxns) | 5 x 1 ml (500 rxns) |
| RT-PCR Enzyme Mix | 150 µl | 750 µl |
| 50x 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 to the 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 |
| 2xOne 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. | / |

Introduction

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

The kit contains a mixture with both oligo(dT)18 and pd(N)9 random hexamer primers.

It is suitable for the synthesis of cDNA up to 13 kb.

The gDNA Eraser included in the kit, can quickly and completely remove genomic DNA.

Suitable for reverse transcription of various RNAs such as mRNA, lncRNA and circRNA.

The kit can also be used for gene-specific reverse transcription, such as miRNA reverse transcription.

Kit Components

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

*(it includes Oligo dT and Random Primer)

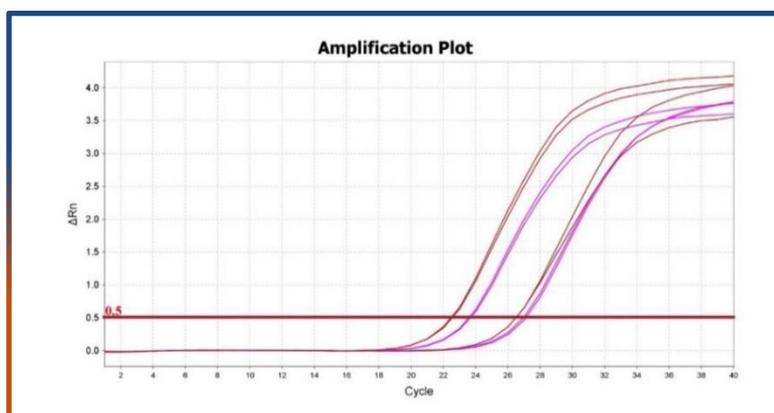
Highlights

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

Applications

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

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



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

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.

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

Kit Components

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

Kit application

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

RNase INHIBITOR (40 U/ul)

FS-RT-1152-1

Introduction

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

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

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

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

Application

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.

Nucleotides & DNA Ladders

dNTP SET (High Concentration)

dNTP SET Mix

1 Kb DNA Ladder (RTU)

100 bp DNA Ladder (RTU)

50 bp DNA Ladder (RTU)

6X Loading Dye

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.

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

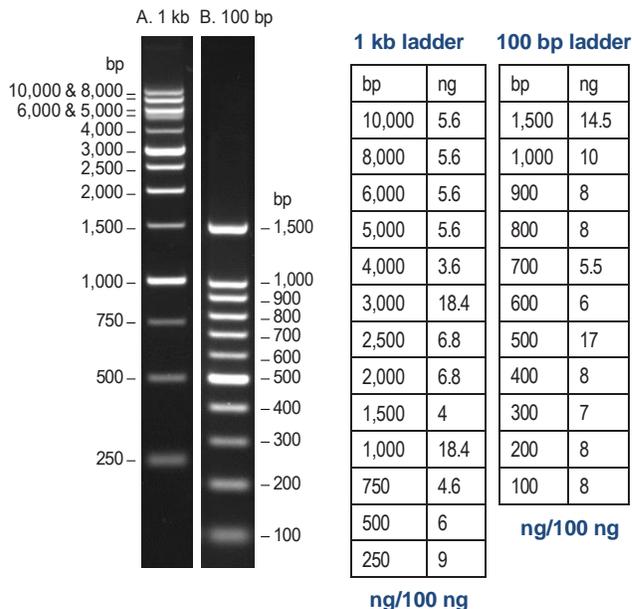


Figure 1. 100 ng of 1 kb DNA Ladder or 100 bp DNA Ladder were run on a 1% agarose/TBE gel containing 1X GelRed Nucleic Acid Gel Stain in 1X TBE at 5 V/cm for 90 minutes. Gels were imaged using a UVP GelDoc-It imaging system with ethidium bromide filter. Fragment sizes in base pairs (bp) are shown next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

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 UI for Ready-to-Load Ladder.

Concentration:

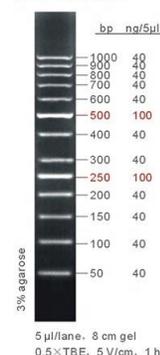
500 µg/ml in 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA for markers without loading dye; 83 ng/ul for Ready-to-Load Ladders.

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 |

50bp ladder plus M1051/M1052



dNTP Set (High Concentration)

FS-013-1 **4 X 250 ul**

Description

Ready-to-Use dNTP Solutions are in Sodium Salt for use in DNA polymerization Reactions all DNA labeling and Sequencing processes

Purity: ≥ 99 % (HPLC)

Form: clear aqueous solution, pH 8.5 ±0.1 (4 °C)

Concentration: 100-110 mM

pH: 8.5 ±0.1

Quality Control Specifications:

18 kb long range PCR (template dilution series): suitable; contamination with bacterial and human DNA: not detectable; activity of DNase, Protease or Phosphatase: not detectable

dNTP Set MIX

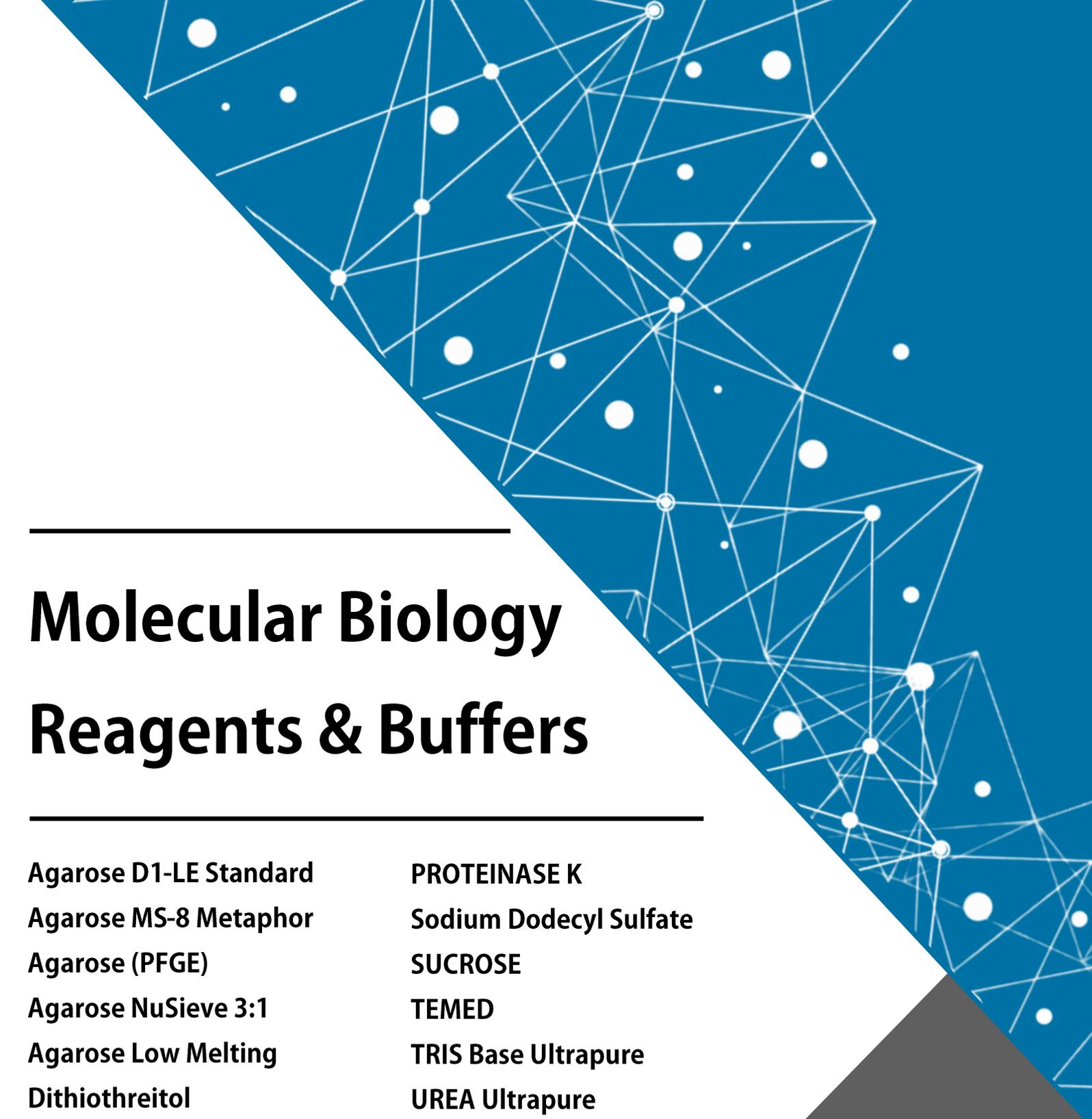
Description: Contains all 4 dNTPs in a pre-mixed solution, ready for immediate use. For procedures requiring unlabeled mixtures of all 4 dNTPs such as PCR or cDNA synthesis.

Purity: 97-99% by HPLC, PCR reaction is successful for the template up to 10 kb length indicating high purity and absence of deleterious terminators

| | |
|-----------------|--|
| FS-013-2 | <p>DNA Polymerization Mix 10</p> <p>10 mM each dNTP, each 5 μmol volume 500 μl</p> |
| FS-013-4 | <p>DNA Polymerization Mix 20</p> <p>20 mM each dNTP, each 10 μmol volume 500 μl</p> |

Storage Conditions: store at -20 °C

Stability: 24 months



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

Biological Buffers

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 where high 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: C₁₀H₁₆N₂O₈ 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: C₃H₈O₃
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 titration); 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 (SDS) FS-0109 **Ultrapure**

| | |
|--|---------------------------------|
| Formula : C ₁₂ H ₂₅ NaO ₄ S | Formula weight : 288.38 |
| CAS # 151-21-3 | Form : White crystalline flakes |
| Moisture : ≤1 % | A(280nm,3%1cm):≤0.1 |
| Insolubles : ≤0.003% | A(230nm,3%1cm):≤0.2 |
| Chloride (Cl) : ≤0.01 | Assay : ≥99% (titrimetric) |
| Phosphate (PO ₄) : ≤1 ppm | Assay(C1 2) : ≥98% (GC) |
| Copper (Cu) : ≤5 ppm | Storage : Ambient |
| Iron (Fe) : ≤1 ppm | Sizes: 100g , 500g |
| Lead (Pb) : ≤5 ppm | |

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, colorless 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-T1503 **equivalent to TRIZMA BASE (Sigma)**

| | |
|--|-------------------------|
| M.W. (Tris base /tris HCl) 121.1 / 157.6 | |
| Purity: >99.8% | Magnesium:< 0.0001% |
| Moisture: <1.0% | Heavy Metals:<0.0001% |
| A ₂₈₀ (1.0M,water) <0.05% | DNase, RNase, protease: |
| Insolubles: < 0.005% | none detected |
| Arsenic: <0.0005% | Storage : Rt |
| Copper: <0.0001% | Sizes: 1 kg , 5 kg |
| Iron: <0.0001% | |

UREA ULTRAPURE FS-0114

| | |
|---|------------------------------|
| Formula : NH ₂ -CO-NH ₂ | Copper (Cu) : ≤0.5 ppm |
| Formula weight : 60.06 | Iron (Fe) : ≤0.5 ppm |
| CAS #57-1 3-6 | Lead (Pb) :≤0.5 ppm |
| Form:White crystalline powder | Chloride (Cl) :≤0.0005% |
| Identity : By IR | Cyanate : None detected |
| Assay : ≥99.5% | Conductivity : ≤1 5 µmho/cm |
| Melting point : 132 - 135°C | DNase (endo) : None detected |
| Insolubles : Negligible | Rnase : None detected |
| Turbidity : ≤2NTU | Protease : Non detected |
| A(260nm,6M, 1 cm) : ≤0.055 | Storage : RT |
| A(280nm,6M, 1 cm) : ≤0.044 | 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 reliable 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° | Description | Size |
|---------------------|----------------------------------|-------------|
| FSB-6002-10 | TAE Buffer (10X) | 1 L |
| FSB-6000-10 | TBE Buffer (10X) | 1 L |
| FSB-6300-10 | Tris Glycine 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

PCR Clean UP Mini Kit

Description

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

Features

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

Applications

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

Specification:

Principle: spin column (silica matrix)

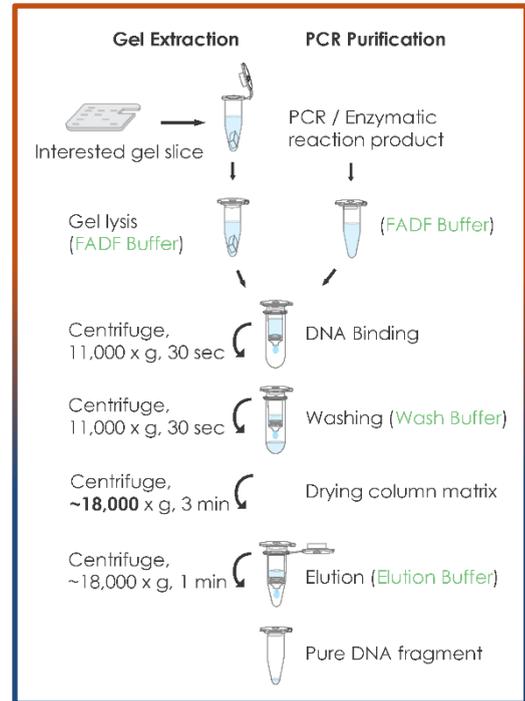
DNA Binding capacity of spin column: 20 µg

Sample size: up to 300 mg of agarose gel
up to 100 µl of reaction solution

Recovery: 70% ~ 85% for Gel extraction
90% ~ 95% for PCR clean-up

Operation time: 10 ~ 20 min

Elution volume: 40 µl



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

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 directly for 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 applications such as sequencing, ligation, labeling, amplification 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 unincorporated 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.

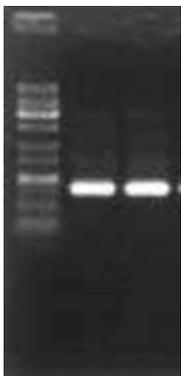
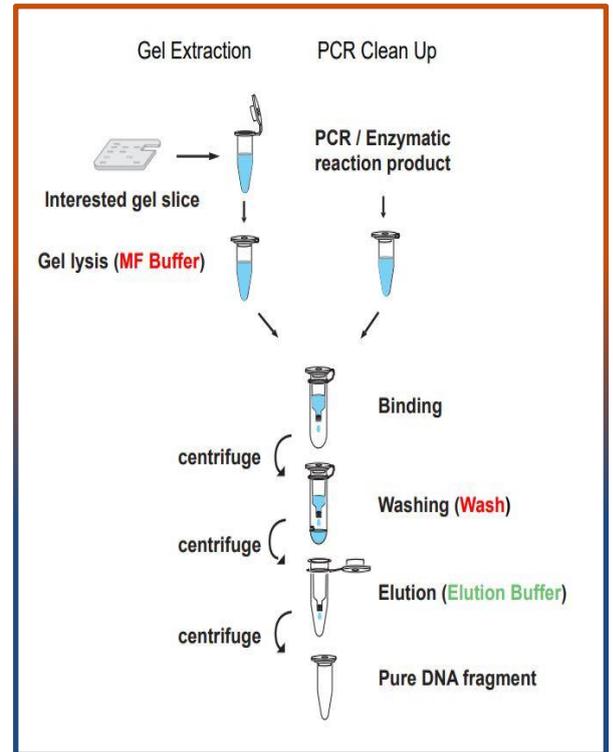


Fig.1: Agarose gel analysis of PCR product before and after purification with MicoElute Gel/PCR Purification.M: 1Kb DNA Ladder.
Lane 1: Before purification
Lane 2: after purification

Ordering Information

| CAT.N° | Product Name | Size | Kit Components | Storage |
|--------|--|-----------|---|---------------------------|
| DE-019 | MicroElute Gel Extraction/ PCR Clean UP Kit | 50 preps | GEL Lysis Buffer PCR Binding Buffer Wash Buffer conc. | Store at RT for 1 year |
| DE-020 | | 200 preps | Elution Buffer FAPC-2 Columns 2 ml Collection Tube | |

PCR Clean-up Mini Kit provides spin columns, buffers, and collection tubes for silica-membrane-based purification of PCR products >100 bp. DNA of up to 10 kb is purified using a simple and fast bind-wash-elute procedure and an elution volume of 40 µl.

- For purification of PCR products or reaction mixtures (concentration and desalination of reaction mixtures)

Specification:

Principle: spin column (silica matrix)

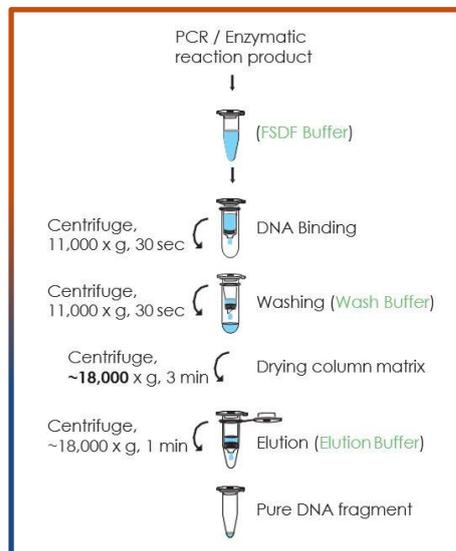
DNA Binding capacity of spinlumn: up to 20 µg

Sample size: up to 100 µl of reaction solution

Recovery: 95% for PCR clean-up

Operation time: 10 ~ 20 min

Elution volume: 40 µl



| CAT.N° | Product Name | Size | Kit components | Store at |
|----------|-----------------------|-----------|---|--------------------------------------|
| DE-017 | PCR Clean Up Mini kit | 50 preps | FSDF Buffer * W ash Buffer (concentrate) Elution Buffer FSDF Column 2 ml Collection Tube | Store at room temperature for 1 year |
| DE-017-1 | | 100 preps | | |
| DE-018 | | 300 preps | | |

*FSDF Buffer contains pH indicator allowing easy determination of the optimal pH for DNA binding to the silica membrane

Blood & Tissue DNA Extraction & Purification

Blood & Tissue Genomic DNA Extraction Kit

Tissue Genomic DNA Extraction Kit

Tissue Genomic DNA MicroElute Kit

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, octopus and insects.**

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 debris 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

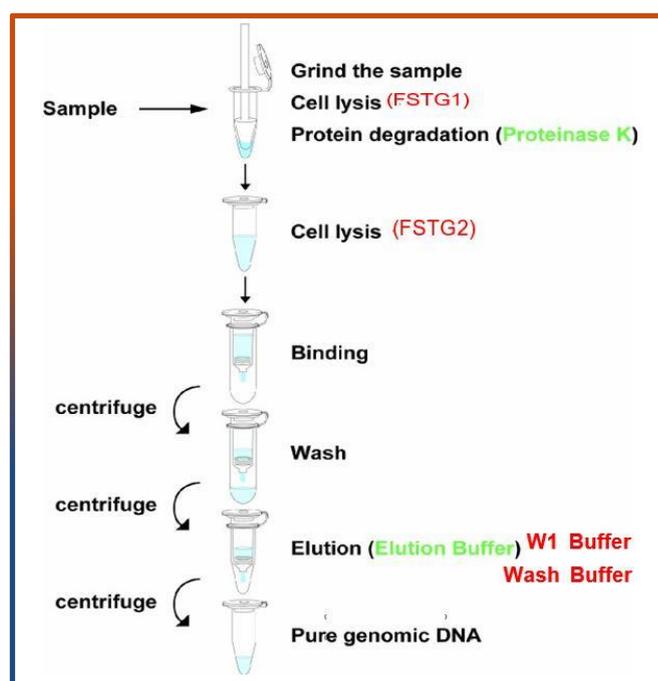
< 25 mg animal tissue

1.2 cm mouse tail

< 10⁷ cultured cells

DNA Yield: 4~8 µg/ 200 µl (whole blood)

DNA Yield: 15 ~35 µg/ prep (tissues)



| Cat. No. | Product Name | Size | Kit Contents | Store at |
|----------|-----------------------------------|-----------|--|--|
| DE-047 | Blood & Tissue DNA Extraction Kit | 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 | | 300 preps | | |

TISSUE GENOMIC DNA EXTRACTION KIT

DE-011

The Tissue Genomic DNA Extraction Kit is designed for rapid extraction of pure, small-scale genomic DNA from several types of tissues, fixed tissues (fresh, frozen, paraffin), animal Culture cells, yeast or bacteria, Whole Blood (not frozen), dried blood spots and amniotic fluid, sea urchins, marine mollusks, octopus and insects.

Features

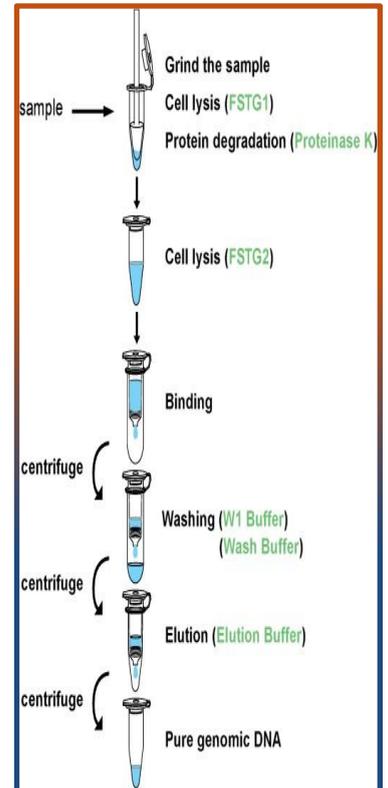
- Rapid isolation cellular DNA from animal tissue, bacteria, yeast etc.
- Purified DNA is ready for downstream application such as PCR, Southern blotting.
- Centrifugation-based method.
- Efficiently remove cellular debris and inhibitors
- No phenol/chloroform extraction and ethanol precipitation.

Applications

- PCR
- Southern Blotting
- Medicolegal Analysis

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

| Cat. No. | Product Name | Size | Store at |
|----------|--------------------------------|-----------|---|
| DE-011 | Tissue DNA Extraction Mini Kit | 50 preps | Store at room temperature for 1 year. (Except Proteinase K Powder: store at -20 °C.) |
| DE-012 | | 100 preps | |
| DE-013 | | 300 preps | |



TISSUE GENOMIC DNA EXTRACTION MICROELUTE KIT

DE-076

The Tissue Genomic DNA Extraction MicroElute Kit simplifies purification of genomic DNA with a fast spin-column procedure. Optimized protocols are included for use with small amounts of tissues, (paraffin formalin) fixed tissues.

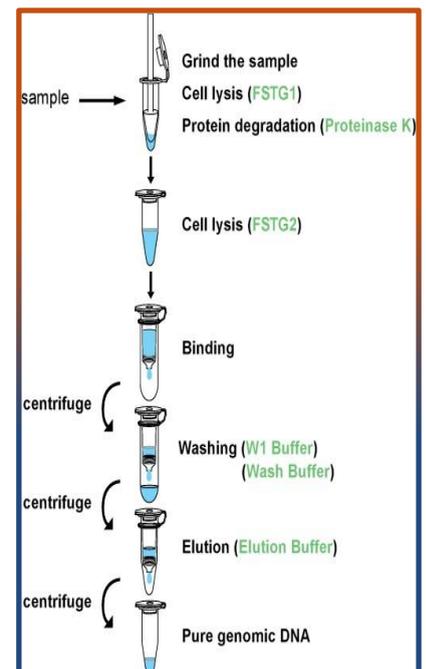
For purification of genomic DNA from small tissue samples (animal Culture cells, yeast or bacteria, whole Blood, dried blood spots and amniotic fluid)

Features and Specifications

Simple, Pure and Fast:

- Quick and easy to use
- Extract gDNA from 25mg of paraffin-embedded tissues, formalin-fixed tissues
- No phenol-chloroform extraction or ethanol precipitation required
- Minimum elution volume: 10 µl
- High recovery: up to 5 µl DNA recovery

| Cat. No. | Product Name | Size | Store at |
|----------|--|-----------|-------------------------------------|
| DE-076 | Tissue Genomic DNA Extraction MicroElute | 50 preps | Store at RT Proteinase K: at 4°C |
| DE-077 | | 100 preps | |



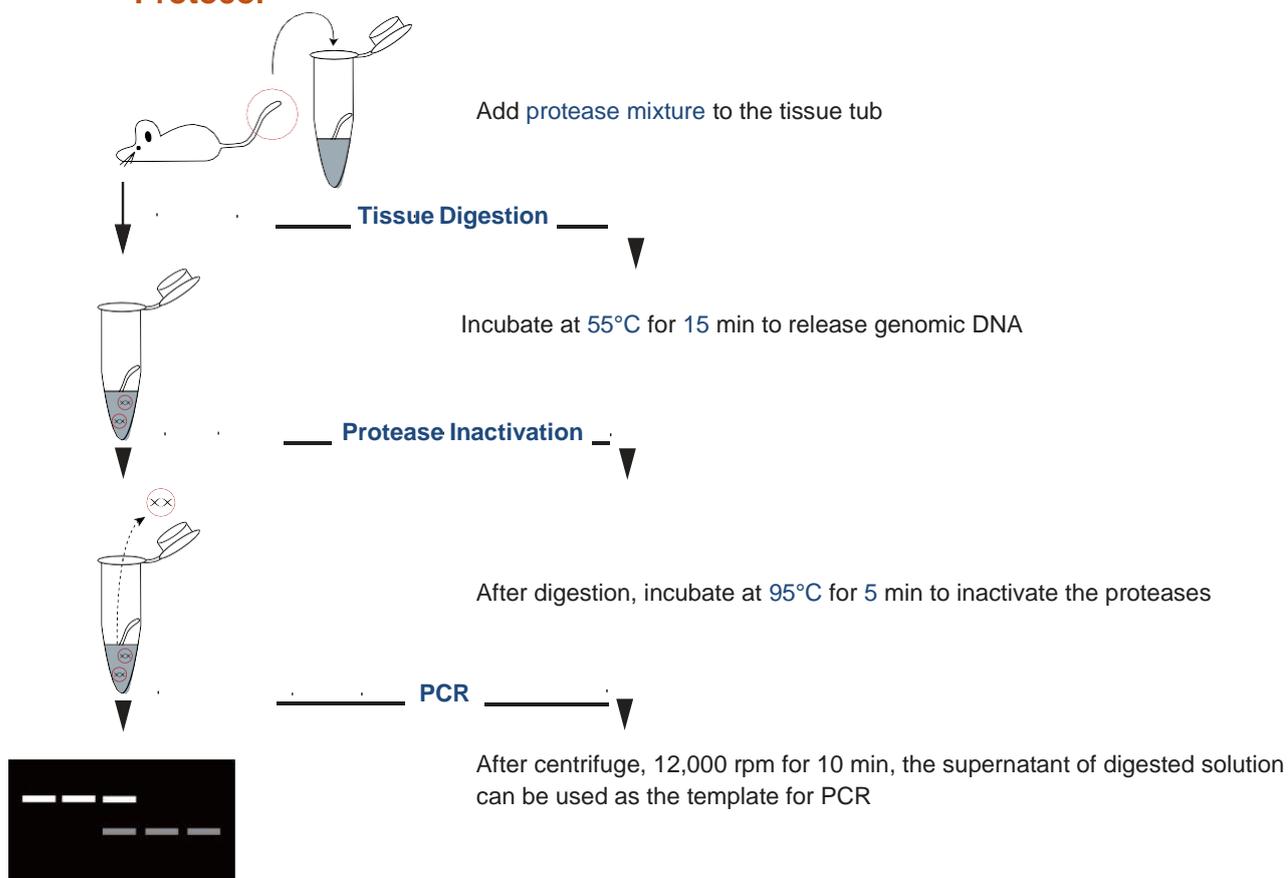
Description:

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



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, Mg²⁺, and DNA Loading Dye.

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 & Purification

Plasmid DNA Extraction Mini Prep Kit

Plasmid DNA Extraction Midi Prep Kit

Plasmid DNA Extraction Maxi Filter Kit

The Plasmid DNA Extraction Kit provides a rapid, phenol-free method for the extraction of high-purity plasmid DNA from bacterial cultures such as E. coli, which bacteria is pelleted, 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 / washing / 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

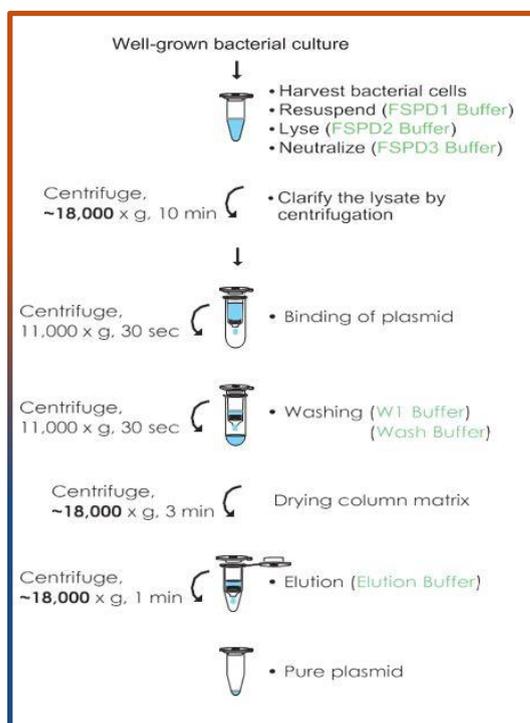
Specifications

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 vacuum

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. | |

The Plasmid DNA Extraction Midi Kit is designed for efficient extraction of high quality plasmid DNA from bacterial culture. This kit provide the alkaline lysis reagents and the columns packed with anion-exchanger resin. After the cells lysis , the plasmid DNA is bound to the resin inside the column by a gravity-flow procedure, and the contaminants can be remove with wash buffer. After using this convenient kit, the purified plasmid DNA is suitable for downstream application such as: transfection, in vitro transcription and translation, and all enzymatic modification.

Specifications: bacterial culture

Sample Size: up to 60 ml of high-copy number plasmid

up to 120 ml of low-copy number plasmid

Plasmid or construct Range: 3kbp-150 kbp

Binding Capacity: 650 µg / Midi column

Features:

- **Time saving:** Complete the process in less than 2 hours.

Applications:

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

- **Purity:** equity to that obtained by 2x CsCl-gradient centrifugation .

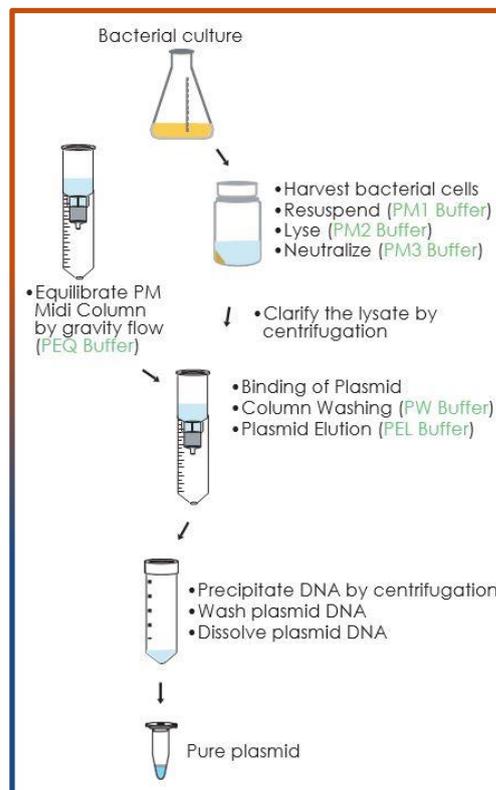
Safe: Eliminates the use of phenol, chloroform, ethidium bromide.

- Transfection
- Microinjection
- Sequencing
- PCR

- Restriction enzyme digestion

Operation Time :Less than 2 hours

Operation Format: Gravity-Flow



Ordering Information

| Cat. N° | Product Name | Size | Store at: |
|----------|--|----------|---|
| DE-050 | Plasmid Extraction Midi Prep Kit | 25 preps | At Room Temperature RNase A at -20°C |
| DE-051 | Plasmid Extraction Midi Prep Kit | 50 preps | |
| DE-051EF | Plasmid Extraction Midi Prep Kit Endotoxin Free | 25 preps | |

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

Specifications: bacterial culture

Sample Size:

- 60 - 240 ml of high copy plasmid
- 200 -480 ml of low copy plasmid

Binding Capacity: up to 1.5 mg of DNA/Maxi Column

Additional Requirements:

- 50 ml centrifuge tube
- Isopropanol
- 70% ethanol

Features

Time saving: 1,5 hour

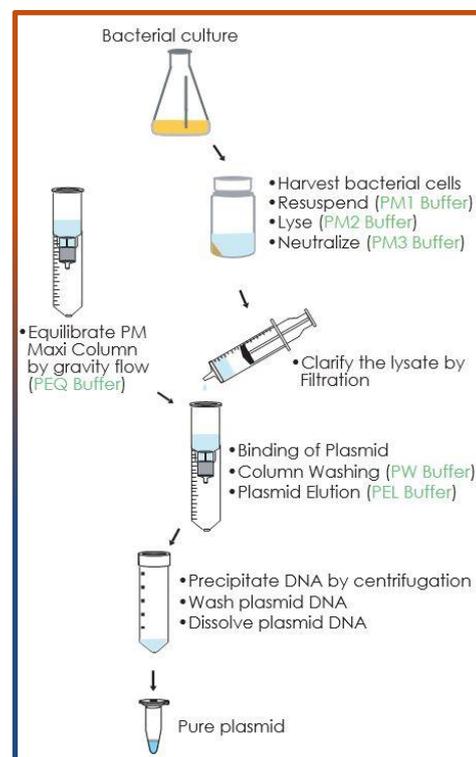
DNA Yield : up to 1,500µg/column

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

Applications

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

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



Procedure

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

Ordering Information

| Cat. N° | Product Name | Size | Store at: |
|----------|--|----------|---|
| DE-055 | Plasmid Extraction Maxi Filter Kit | 10 preps | At Room Temperature RNase A: at-20°C |
| DE-055-1 | Plasmid Extraction Maxi Filter Kit | 20 preps | |
| DE-055EF | Plasmid Extraction Maxi Filter Kit Endotoxin Free | 10 preps | |

RNA Extraction & Purification

Blood / Tissue Total RNA Extraction Mini Kit

Tissue Total RNA Extraction Mini Kit

Trizol Ultrapure

RNA-ZOL Direct Clean-UP Plus Kit

RNA Stabilization Reagent

Viral Nucleic acid (DNA&RNA) Extraction Kit

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

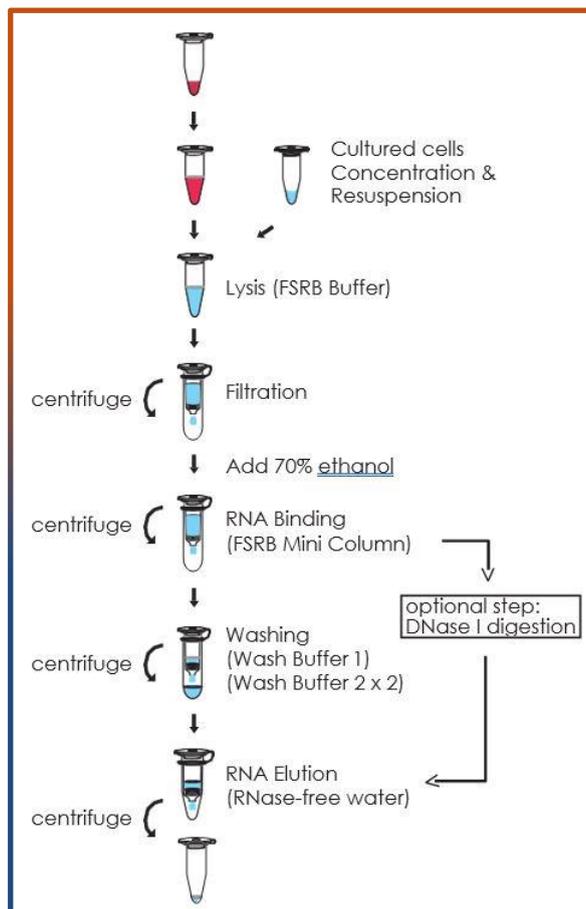
This method first lyses cells by using a chaotropic salt , then binds RNA to silica-based membranes, washes RNA with ethanol-contained wash buffer and then elutes purified RNA by RNase-free ddH₂O. It takes 30 min for an entire procedure, and the purified RNA is ready for RT-PCR, northern blotting, primer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

Sample amount and yield:

Handling time: about 30 minutes

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

Brief procedure:



Ordering Informations

| Cat. N° | Product Name | Size | Store at: |
|---------|---------------------------------------|-----------|---|
| RE-003 | Blood/Tissues RNA Extraction Mini Kit | 50 preps | At Room Temperature RNase A at -20°C |
| RE-004 | Blood/Tissues RNA Extraction Mini Kit | 100 preps | |
| RE-004B | Blood/Tissues RNA Extraction Mini Kit | 300 preps | |

The Tissue 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

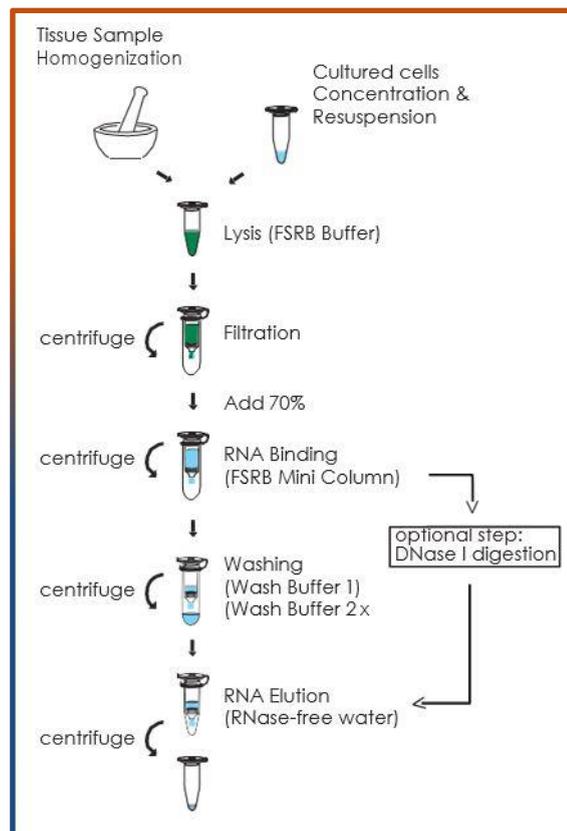
- Northern blotting hybridizations
- Primer extension
- RT-PCR
- RNase protection assays
- Differential display
- As starting material for purification of mRNA for cDNA synthesis

Sample Size: Animal cells: from 1×10^6 up to 5×10^6 cells
 Animal Tissues: (Mouse/Rat) from 10 mg up to 30 mg
 Bacteria: 1ml or up to 1×10^9 cells
 Yeast: (up to 5×10^7)

Yield of Purification

| Sample | Recommended amount of sample used | Yield (ug) |
|---|-----------------------------------|------------|
| Animal Cells (up to 5×10^6 Cells) | NIH/3T3 | 10 |
| | HeLa | 15 |
| | COS-7 | 30 |
| | LMH | 12 |
| Animal Tissue (mouse/rat) (Up to 30 mg) | Embryo | 25 |
| | Heart | 10 |
| | Brain | 10 |
| | Kidney | 30 |
| | Liver | 50 |
| | Spleen | 35 |
| Bacteria | E. coli | 60 |
| | B. subtilis | 40 |
| Yeast (up to 5×10^7 cells) | S. cerevisiae | 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

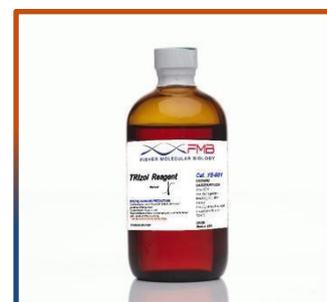
Tissue Total RNA Mini Kit can be stored at room temperature (15-25°C). Stable for 1 year at room temperature at 15-25°C.

| Cat. No. | Product Name | Size | Store at |
|----------|---------------------------|-----------|---|
| RE-005 | TISSUE TOTAL RNA MINI-KIT | 50 preps | At Room Temperature at 15-25°C for 1 year |
| RE-006 | | 100 preps | |

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 small 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 Chomczynski 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

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 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 culture) 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°C and used for cDNA synthesis.

Protocol for Whole Blood

1. Collect fresh human blood in an anticoagulant-treat collection tube.
2. Transfer up to 300 ul fresh blood to a 1.5ml microcentrifuge tube (not provided). If the sample is more than 300 ul (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 ul of RBC Lysis Buffer.
6. Store 100ul of RBC Lysis Buffer with 1 ml of RNA Stabilization Solution at –20°C until RNA isolation.
7. When processing, thaw and homogenize tissue in RNA Stabilization Solution.
8. Transfer 0.8 ml of the homogenate mix into a 2 ml tube and add 0.8 ml of the acid-phenol, pH 5.2, and 320 ul of chloroform.
9. Vortex the mixture vigorously by mixing 4 times, 30 sec for each.
10. Centrifuge at 12,000 rpm for 2 min.
11. Transfer the upper aqueous phase (containing RNA) to a fresh 2 ml tube, taking care not to disturb the interface (containing DNA/protein).
12. Precipitate the RNA by adding an equal volume (0.8 ml) of isopropanol and 80 ul of 3 M NaAc at –20°C for 30 min
13. Centrifuge at 12,000 rpm for 15 min and discard the supernatant.
14. Wash the RNA pellet by using 200 ul of 70% ethanol and gentle inverting the tube for several times.
15. After a brief spin and careful removing the supernatant, let the RNA pellet to air dry for about 5-10 min.
16. Dissolve the RNA pellet in 20 ul DEPC-treated TE.
17. Store the samples at –20°C and used for cDNA synthesis.

| Cat. No. | Product Name | Samples | Size | Store at |
|----------|---|-----------------------------------|--------|---------------|
| FS-883 | RNA LATER STABILIZATION REAGENT | Tissues Cell Cultures | 100 ML | Store at +4°C |
| FS-884 | RNA LATER STABILIZATION REAGENT + LYSYS BUFFER | Tissues Cell Cultures Blood | 100 ML | |

Viral Nucleic Acid Extraction Mini Kit I is designed for extraction of Viral DNA or RNA from cell free fluids 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

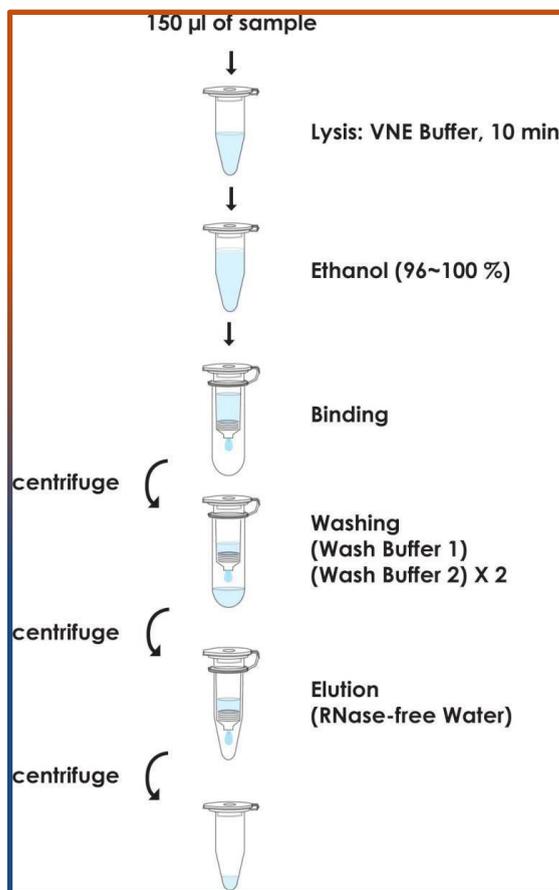
Binding capacity: 60 ug RNA/column

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-001 | Viral Nucleic Acid (DNA/RNA) Extraction Kit I | 50 preps | Store at room temperature for 1 year |
| DR-002 | | 100 preps | |

Enviromental DNA/RNA Extraction Kits

Plant Genomic DNA Extraction Mini Kit

Soil & Stool DNA Isolation Mini Kit

Plant Total RNA Purification Mini Kit

Fungi/Yeast Genomic DNA Extraction Mini Kit

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

Features

- **High Purity:** DNA is immediately suitable for a variety of applications, including amplification, digestion, PCR etc.
- **High Speed:** Using a column type extraction system to allow a more rapid, more convenient methods compared to the conventional methods. 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 not necessary to treat the sample with harmful organic solvents such as phenol and chloroform.

Applications

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

Time Required

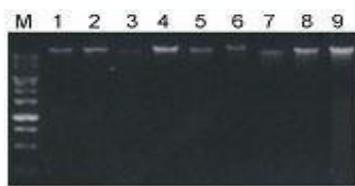
About 30-60 minutes depending upon the sample types.

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 |
| DE-022 | | 100 preps | Store RNase A at -20°C |

The Soil & Stool DNA Isolation Kit is designed for isolation of high quality total DNA from 50~200 mg of soil, sludge , sediment samples or stool sample. The Soil & Stool DNA Isolation Mini Kit operates through our high-quality beads-beating disruption method and is perfect for use with diverse soil and stool samples of up to 200 mg. Our 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. During entire procedure is not required the phenol-chloroform, extractions and can be finished within 60 minutes.

The purified DNA is ready for PCR and other downstream applications.

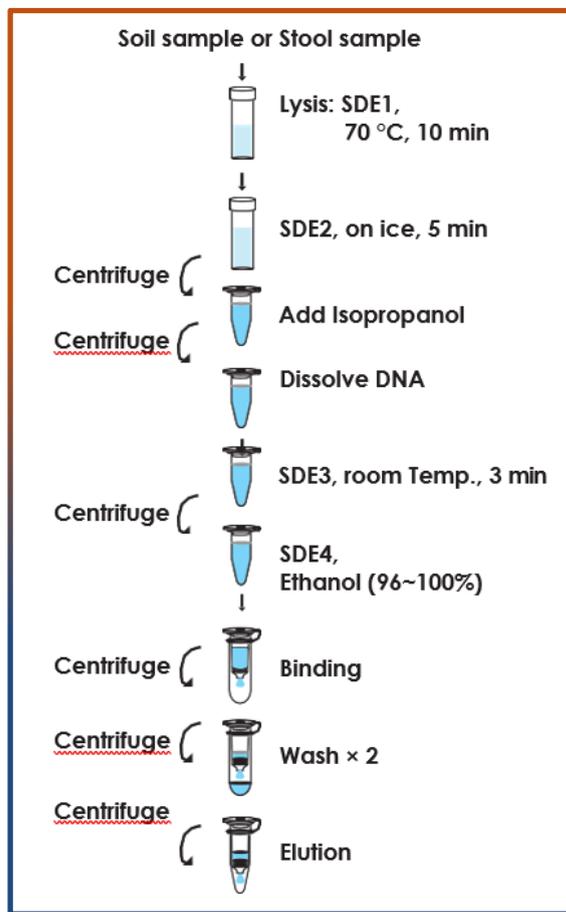
Specifications:

Principle: Spin Column (silica membrane)

Sample: from 50 mg up to 200 mg of soil or stool sample

Operation time: < 60 min

Elution volume: 50~200 µl



| Cat. N° | Product Name | Size |
|---------|--------------------------------|---------------|
| DE-095 | Soil & Stool DNA Isolation kit | 50 Reactions |
| DE-096 | | 100 Reactions |

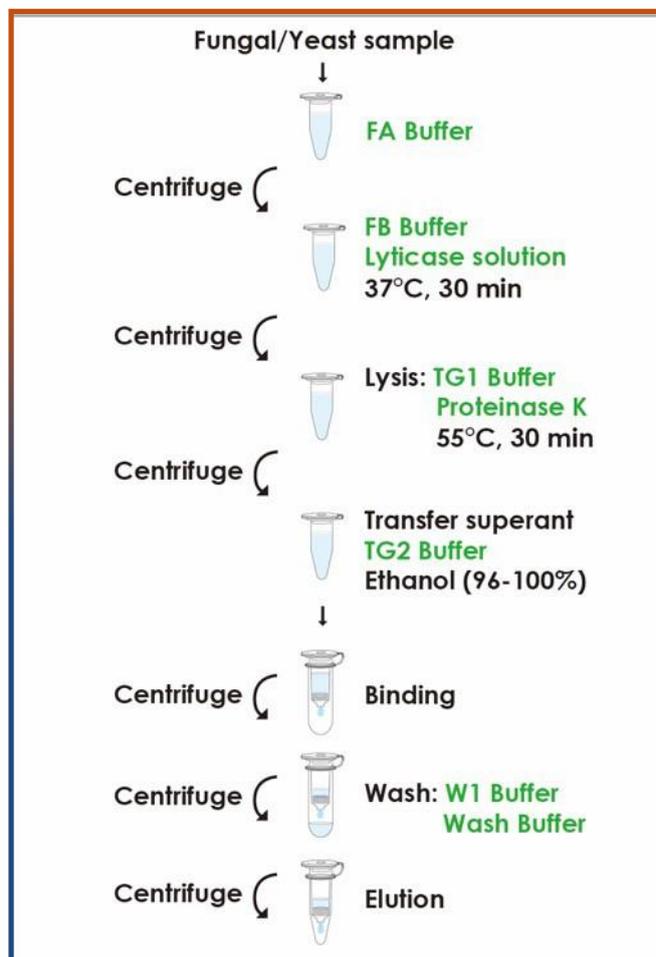
Description

The Fungi/Yeast Genomic DNA Extraction Mini Kit is designed for the purification of DNA from fungus and yeast cells. The enzyme treatment (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 x10⁶ cell culture fungal/yeast cell
 Maximum sample size: up to 50 mg (wet weight)
 Operation time:~ 60 minutes
 Binding capacity:60 µg/ column
 Column applicability: centrifugation and vacuum

Tested Samples

Saccharomyces cerevisiae 2-8 µg/5 x 10⁷ cells
 Auricularia auricula-judae 0.5-2 µg/50 mg wet weight
 Fusarium sp. 1-2 µg /50 mg wet weight



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°C. |

Description

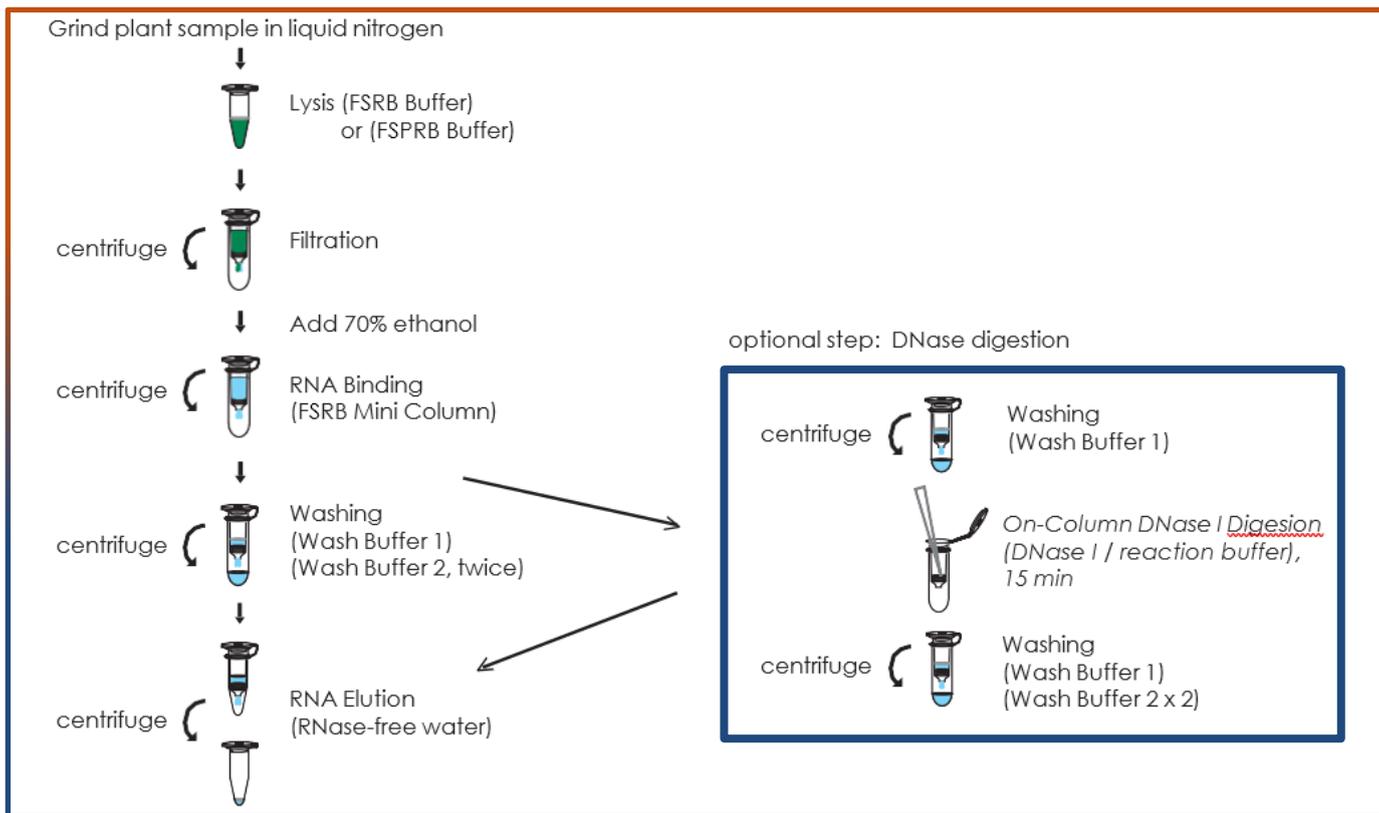
The Plant Total RNA Purification Mini Kit is designed for purification of total RNA from plant tissues and cells using the modified salt precipitation procedure and RNase inhibitors without the use of hazardous solvents such as phenol. Plant RNA is quickly and efficiently isolated and is immediately 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 recommend RE-015 (50 preps) and RE-016 (100 preps).

Specification:

- Principle: spin column (silica membrane)
- Sample: up to 100 mg plant tissues or 1×10^7 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

- Northern 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 RE-009 | Plant Total RNA Purification Mini Kit | 50 preps 100 preps 300 preps | Store at room temperature at 4-8°C for 1 year. |

Nucleic Acid Stains & ETB Destroyer

Green Gel Safe Nucleic Acid Stain

Clearsight Nucleic Acid Stain

Green Gel Plus Nucleic Acid Stain

Sybr Safe Nucleic Acid Stain

Eurosafe Green

Midogreen

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 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 | CLEAR SIGHT 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 | MIDOGREEN | 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 |