FS™ Mix

#P2071, 1 ml

Contents:

2× *FS*™ Mix	1 ml
Nuclease-free water	1 ml

Store at -20°C

For research use only.

In total 2 vials.

Description

2× *FS*™ Mix is a premixed, ready-to-use solution containing hotstart *FS*™ Taq DNA Polymerase with antibody modification, dNTPs, Mg^{2+} and Reaction Buffer at optimal concentrations for efficient amplification of DNA templates by PCR. To prepare the final PCR, only need to add primers and template DNA. This premixed formulation saves time and reduces contamination due to the fewer pipetting steps required for PCR set-up. It also contributes to higher sensitivity by adding enhancer.

FS™ Taq DNA Polymerase is the latest generation Taq-based DNA polymerase developed by GDSBio. It possesses high amplification efficiency as Taq DNA Polymerase does, and fast elongation ability as KOD polymerase does, can be used in various kinds of PCR. The *FS*™ PCR Buffer is designed for *FS*™ Taq DNA Polymerase, can be used in fast amplification reaction. The elongation rate of *FS*™ Taq DNA Polymerase can be as fast as 3kb/min. It can shorten the amplification time by 2/3. It has 5' to 3' polymerase activity, but lacks of 3' to 5' exonuclease activity, that results in a 3'-dA overhangs PCR product.

Composition of the 2× FS™ Mix

0.3U/μl *FS*™ Taq DNA Polymerase, 2× *FS*™ Buffer, 0.4mM dNTPs, 3.2mM $MgSO_4$, 0.02% bromophenol blue.

FS™ Mix buffer is a proprietary formulation optimized for robust performance in PCR.

PRODUCT USE LIMITATION.

This product is developed, designed and sold exclusively *for research purposes and in vitro use only*. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Applications

- High throughput PCR
- Long and complex template PCR
- Routine PCR with high reproducibility
- Generation of PCR products for TA cloning

Features

- **Convenient**: only primers and template DNA are added when prepare final PCR
- **High yields** of PCR products with minimal optimization
- **High efficiency**: saving your time by simplifying the process
- **Reproducible**: lower contamination and pipetting error risk
- **Higher sensitivity and fast** compared to conventional Taq DNA Polymerase

Basic PCR Protocol

All solutions should be thawed on ice, gently vortex and briefly centrifuge.

1. Add the following components to a sterile microcentrifuge tube sitting on ice or at room temperature:

Reagent	Quantity	Final concentration
2× *FS*™ Mix	25 µl	1×
Forward Primer	variable	0.4-1 µM
Reverse Primer	variable	0.4-1 µM
Template DNA	variable	10pg-1µg
Water, nuclease-free	to 50 µl	–

Recommendation amounts of template DNA in a 50 µl reaction mix:

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

2. Mix contents in the 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.

3. Perform 25-35 cycles of PCR amplification as follows:

Initial Denaturation	94°C	3 minutes
25-35 Cycles	94°C 55-68°C 72°C	30 seconds 30 seconds 1-5 minutes
Final Extension	72°C	2 minutes

4. Incubate for an additional 2 min at 72°C and maintain the reaction at 4°C. The samples can be stored at -20°C until use.

5. Analyze the amplification products by agarose gel electrophoresis and visualize by nucleic acid dye staining. Use appropriate molecular weight standards.

Notes on cycling conditions

- *FS*™ Mix is for high specificity PCR applications.
- The half-life of enzyme is >40 minutes at 95°C.
- The error rate of *FS*™ Taq DNA Polymerase in PCR is $2.2x10^{-5}$ errors per nt per cycle.
- *FS*™ Taq DNA Polymerase accepts modified nucleotides (e.g. biotin-, digoxigenin-, fluorescent-labeled nucleotides) as substrates for the DNA synthesis.
- The number of PCR cycles depends on the amount of template DNA in the reaction mix and on the expected yield of the PCR product. 25-35 cycles are usually sufficient for the majority PCR reaction. Low amounts of starting template may require 40 cycles.

Guidelines for preventing contamination of PCR

During PCR more than 10 million copies of template DNA are generated. Therefore, care must be taken to avoid contamination with other templates and amplicons that may be present in the laboratory environment. General recommendations to lower the risk of contamination are as follows:

- Prepare your DNA sample, set up the PCR mixture, perform thermal cycling and analyze PCR products in separate areas.
- Set up PCR mixtures in a laminar flow cabinet equipped with an UV lamp.
- Wear fresh gloves for DNA purification and reaction set-up.
- Use reagent containers dedicated for PCR. Use positive displacement pipettes, or use pipette tips with aerosol filters to prepare DNA samples and perform PCR set-up.
- Always perform “no template control” (NTC) reactions to check for contamination.

Quality Control

The absence of endodeoxyribonucleases, exodeoxyribonucleases and ribonucleases is confirmed by appropriate quality tests. Functionally tested in amplification of a single-copy gene from human genomic DNA.

Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to a nicked DNA was observed after incubation of 25 µl *FS*™ Mix (2×) 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 25 µl *FS*™ Mix (2×) 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 25 µl *FS*™ Mix (2×) with 1µg E.coli [3H]-RNA (40000cpm/µg) for 4 hours at 37°C and 70°C.