FS™ Kit

Catalog No: P3051 (5x1ml)

Contents: 2X FSTM Reaction Mix 5x1ml
FSTM Taq DNA Polymerase (2.5U/µl) 160µl

Store at -20°C
In total 6 vials.

Description

FS™ Kit contains a *FS*™ Taq DNA polymerase and a 2X premixed, ready-to-use *FS*™ reaction Mix. The *FS*™ reaction mix is supplied as a 2X concentrated solution, that contains dNTPs and all other PCR components, except DNA template and primers. To prepare the final PCR reaction system, mix *FS*™ Taq DNA polymerase and the *FS*™ reaction mix, then add primer and template. The reaction Mix has higher amplification sensitivity due to optimized components and enhancer.

FS™ Taq DNA Polymerase is the latest generation Taq-based DNA polymerase. It possesses high amplification efficiency as Taq polymerase does, and fast elongation ability as KOD polymerase does, can be use in various kinds of PCR. The *FS*™ PCR Buffer is designed for *FS*™Taq DNA polymerase, can be used in fast amplification reaction. *FS*™ Taq DNA polymerase have an elongation rate 2x higher than regular Taq DNA polymerase, and can shorten the amplification time by half. It has 5' to 3' polymerase activity but lack of 3' to 5' exonuclease activity, that results in a 3'-dA overhangs PCR product.

<u>PRODUCT USE LIMITATION.</u>

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.

Unit Definition

One unit is defined as the amount of the enzyme required to catalyze the incorporation of 10 nmole of dNTPs into an acid-insoluble form in 30 minutes at 70°C using hering sperm DNA as substrate.

Storage Buffer

20 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 100 mM KCl, 3.2mM $MgCl_2$, 0.1% Triton X-100, 0.1 % TW 20, 0.2mg/ml BSA, 50 % Glycerol

Composition of the 2x FS™ Reaction Mix

2X *FS™ PCR* buffer, 0.4mM dNTPs, 3.2 mM $MgSO_4$, 0.02% bromophenol blue.

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

Features

- **High efficiency**: saving your time by simplifying the process
- **High yields** of PCR products with minimal optimization.

miscalculation and contamination

- **Reproducible** -lower contamination and pipetting error risk.
- **Flexible:** the amount of polymerase is flexible and

controllable.

Applications

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

Basic PCR Protocol

All solutions should be thawed on ice, gently vortexed and briefly centrifuged.

1. Add in a thin walled PCR tube on ice:

For a total 50µl reaction volume

Component of sample	Volume	Final concentration
2X *FS*™ Reaction Mix	25 µl	1X
Forward Primer	variable	0.1-1 µM
Reverse Primer	variable	0.1-1 µM
Template DNA	variable	10 pg-1 µg
FS™ Taq DNA Polymerase (2.5U/µl)	0.5-1 µl	1.25-2.5U/50µl
Water, nuclease-free	to 50 µl	–

Recommendations with Template DNA in a 50µl reaction volume

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

2. Gently vortex the sample and briefly centrifuge to collect all drops to the bottom of the tube. Overlay the sample with mineral oil or add an appropriate amount of wax.

This step may be omitted if the thermal cycler is equipped with a heated lid.

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4. Preform PCR using the following thermal cycling conditions.

Initial Denaturation	94°C	3 minutes
25-35 Cycles	94°C 55-68°C 72°C	30 seconds 30 seconds 10-60 seconds
Final Extension	72°C	2 minutes

5. 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.

6. Analyze the amplification products by agarose gel electrophoresis and visualize by ethidium bromide staining. Use appropriate molecular weight standards.

Notes on cycling conditions

- *FS*™ Taq DNA Polymerase 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; the accuracy (an inverse of the error rate) an average number of correct nucleotides incorporated before making an error, is $4.5x10^{-4}$ (determined according to the modified method described in).
- *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 reaction

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 10U *FS*™ Taq 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 *FS*™ Taq 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 *FS*™ Taq DNA Polymerase with 1µg E.coli [3H]-RNA (40000cpm/µg) for 4 hours at 37°C and 70°C.