

FSTM Taq Mix Direct for Tissue

Catalog No: P4011 (5x1ml)

Contents:	2X FS TM Mix T	5x1ml
	Extraction solution	40ml
	Neutralization solution	5ml
	Nuclease-free water	5x1ml

Store at -20°C
In total 12 vials.

Description

FSTM 2X Taq Mix Direct for Tissue is a premixed, ready-to-use solution containing FSTM Taq DNA Polymerase, dNTPs, and all other PCR components, except DNA template and primers. FSTM Taq Mix T is specific for tissue direct amplification. It contributes to fast, specific, sensitive and reproducible PCR by reducing the risk of pipetting errors, miscalculation and contamination. The FSTM Mix (2X) T can be used with conventional PCR machines. This product cut off the process of complicated and waste time DNA extraction. Minimize the cross contamination in the reaction.

FSTM 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 FSTM PCR Buffer is designed for FSTMTaq DNA polymerase, can be used in fast amplification reaction. FSTM 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.

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.

BKL ZONE. Calle Finlandia, parcela 108, nave 5. Polígono Industrial Tecnocórdoba.
14014 Córdoba, España.

Web: b-kl.eu
e-mail: b-kl@b-kl.eu

Applications

- Amplification for Tissue
- High throughput PCR.
- Long and Complex template PCR

Feature

- **Convenient** –*Direct PCR for Tissue.*
- **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.

Composition of the 2xFSTM Mix T

0.3U/ul FSTM Taq DNA polymerase, 2X FSTM buffer T, 0.4mM dNTPs, 3.2 mM MgSO₄, 0.02% bromophenol blue.

FSTM Mix T buffer is a proprietary formulation optimized for robust performance in PCR

Basic PCR Protocol

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

1. Tissue sample process.

- Place 3-5mg tissue to a new 1.5ml microcentrifuge tube. Add 180ul Extraction solution and vortex thoroughly. Incubate the mixture at 95°C for 10min.
- Add 20ul Neutralization solution to the mixture and vortex thoroughly. Centrifuge for 2min at 5000rpm. Take 0.5-2ul of the flow-through for PCR reaction.

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

For a total 50µl reaction volume

Component of sample	Volume	Final concentration
FS TM Mix T (2X)	25 µl	1X
Forward Primer	variable	0.1-1 µM

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Reverse Primer	variable	0.1-1 μ M
Template	variable	10 pg-1 μ g
Water, nuclease-free	to 50 μ l	–

Recommendation for Tissue Template in a 50 μ l reaction volume is 0.01-1 μ g tissue.

3. Gently vortex the sample and briefly centrifuge to collect all drops to the bottom of the tube.

4. 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.

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

Initial Denaturation	94°C	4 minutes
25-35 Cycles	94°C	30 seconds
	55-68°C	30 seconds
	72°C	20 seconds
Final Extension	72°C	2 minutes

6. 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.

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

Notes on cycling conditions

- 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

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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 25µl *FS*TM Mix T (2X) with 1µg pBR322 DNA in 50µl for 4 hours at 37°C and 70°C.

Exodeoxyribonuclease Assay

No detectable degradation of lambda DNA-HindIII fragments was observed after incubation of 25µl *FS*TM Mix T (2X) with 1µg digested DNA in 50µl 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 25µl *FS*TM Mix T (2X) with 1µg E.coli [3H]-RNA (40000cpm/µg) in 50µl for 4 hours at 37°C and 70°C.

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