FastTrack Direct PCR Kit® for Tissue

#G45314A **100 Reactions**

Storage: Please refer to Table 1

Introduction

FastTrack Direct PCR Kit® for Tissue is designed to perform PCR directly from tissue, without prior DNA purification. Freshly collected or stored tissue samples, all are suitable samples for this kit. The kit employs a specially engineered high-fidelity DNA polymerase enzyme that exhibits high resistance to many PCR inhibitors found in blood and other biofluids. The kit ensures high yield and is time saving as DNA isolation from samples can be avoided prior to PCR. The kit is recommended for end-point PCR.

Kit Components

Components	Amount	Storage
FastTrack Tissue Lysis Buffer	5ml	4°C
FastTrack Tissue 1X PCR Mix	1.25ml x 10	-20°C
Control Primer (F), 10uM	10µl	-20°C
Control Primer (R), 10uM	10µl	-20°C

Guidelines for sample handling

- When working with tissue, it is recommended to start with 10mg sample.
- Collect the tissue samples in a PCR tube, add 30ul FastTrack Lysis Buffer, vortex well and incubate them at 95^{O}C for 15 minutes.
- After incubation centrifuge the PCR tubes at 15000rpm for 5 minutes. Collect the clear supernatant in a separate microfuge tube.
- Use 0.75 ul of this clear lysate as template for the PCR reaction.

Guidelines for PCR setup

- Add 23.5ul 1X FastTrack Tissue Direct PCR mix in a Flat Capped PCR tube.
- Add 0.25ul of each Primer (from 10mM stock) to the master mix.
- Use 0.75 µl of FastTrack lysate as template for the PCR reaction.
- While setting up the PCR cycle keep the initial denaturation time to be 5 minutes at 95^{O}C followed by denaturation at 95^{O}C for 10 -15s and annealing at the desired Tm. For extension, use 1min for 1Kb amplicon size.

Guidelines for Control reactions

We recommend setting up a separate control PCR reaction (use provided primer as working stock) with the FastTrack lysate used in the actual experiment to ensure that the PCR conditions are optimal. Tm for control primer is 560C and it amplifies a 125bp fragment from a highly conserved region of mice genomic DNA. It is recommended to add a no-template control to all PCR assays

Important Notes

- Carefully mix and spin down all tubes before opening to ensure homogeneity and improve recovery. The PCR setup can be performed at room temperature.
- **Always add the Tissue lysate last to the reaction.**
- Always ensure that the volume of lysate that is being added to the PCR reaction is 4% of the total reaction volume**. Do not add more lysate to the reaction mix.**

Troubleshooting

In case of non-specific amplification, increase the annealing temperature, reduce the total number of cycles or design a new set of primers