FastTrack Direct PCR Kit® for Blood

#G45312A **100 Reactions**

Store the FastTrack 1X PCR Mix and control primer at -20^{O}C and

FastTrack lysis buffer at 4^{O}C after receiving.

Introduction

FastTrack Direct PCR Kit® for Blood is designed to perform PCR directly from blood, clotted blood etc. without prior DNA purification. Freshly collected or old samples, all are suitable 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
Fastract Lysis Buffer	5ml	4°C
Fastract 1X PCR Mix	1.25ml x 2	-20°C
Control Primer (F), 10uM	10ml	-20°C
Control Primer (R), 10uM	10ml	-20°C

Guidelines for sample handling

- When working with clotted blood, use a cotton swap to mix the clot directly, and for blood add 10ul in the FastTrack Lysis Buffer.
- Collect samples in a PCR tube, add 30ul FastTrack Lysis Buffer, vortex well and incubate them at 95^{0}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 1 ul of this clear lysate as template for the PCR reaction.

Guidelines for PCR setup

- Add 23.5ul 1X FastTrack Direct PCR mix in a Flat Capped PCR tube.
- Add 0.25ul of each Primer (from 10mM stock) to the master mix.
- Use 1 µl of FastTrack lysate as template for the PCR reaction.
- Volume make upto 25 ul with nuclease free water.
- While setting up the PCR cycle keep the initial denaturation time to be 5 minutes at 95^{0}C followed by denaturation at 95^{0}C for 10 - 15 s 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. T_m for control primer is 58^{0}C and it amplifies a 221bp fragment from a highly conserved region of human mitochondrial DNA. Set up control reaction as given in the following

Cycle step	3-step protocol		Cycles
	Temp.	Time	
Initial denaturation	95^{O}C	3 min	1
Denaturation	95^{O}C	15 sec	35
Annealing	58^{O}C	15 sec	
Extension	72^{O}C	30 sec	
Final extension	72^{O}C	7 min	1
Store	4^{O}C	Hold	

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