FastTrack Direct PCR Kit® for Cultured Cell

 # G45313A **100 Reactions**

Store the FastTrack 1X PCR Mix and control primer at -20oC and FastTrack lysis buffer at 4oC after receiving.

Introduction

Fastrack Direct PCR Kit® employs a specially engineered high-fidelity DNA polymerase that is resistant to many PCR inhibitors found in blood, other biofluids and crude cell lysates. The kit is optimized to amplify target sequences directly from cultured cells, thereby eliminating prior DNA purification. Both freshly collected or stored cultured cell samples are suitable for this kit. Recommended for end point PCR, the kit ensures high yield and is time saving as DNA isolation from samples can be avoided prior to PCR.

Kit Components

Components	Amount
FastTrack Cultured cell Lysis Buffer	6 ml
FastTrack Cultured cell 1X PCR Mix (FastTrack DNA Polymerase, Reaction buffer, dNTP(10uM)	1.25 ml x 2
Control Primer (Forward), 10uM	10µl
Control Primer (Reverse), 10uM	10µl

Guidelines for sample handling

- Pellet down 104 to 106 cells and wash with 1X PBS, 2 – 3 times.
- Resuspend in 1X PBS solution.
- Collect 25ul of this cell suspension in a PCR tube, add 50ul of FastTrack Cultured Cell 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 FastTrack Cultured cell 1X PCR mix in a flat capped PCR tube
- Add 0.25ul of each Primers (from 10uM stock) to the master mix.
- Use 0.75 µl of FastTrack Cultured cell lysate (from step 6) as template for the PCR reaction.
- While setting up the PCR cycle initial denaturation should be for 5 minutes at 950C followed by denaturation at 950C 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 Cultured cell lysate used in the actual experiment to ensure that the PCR conditions are optimal.
- Tm for control primer is 58^{O}C and it amplifies a 300bp fragment
- 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.**

Troubleshooting

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