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FastKing One Step RT-PCR Kit

Cat. no. SLPCR227

Kit Contents

Contents	50 μl × 50 rxn
2×FastKing One Step RT-PCR MasterMix	1.25 ml
25×RT-PCR Enzyme Mix	100 μl
Forward Primer (10 μM)	2 × 1 ml
Reverse Primer (10 μM)	1

Storage

Store at -30~-15°C for up to one year.

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Introduction

FastKing One Step RT-PCR Kit allows both reverse transcription and gene amplification to take place in a single tube, which avoids cross contamination between samples and improves the sensitivity of detection. The 25×RT-PCR Enzyme Mix contains King reverse transcriptase, which is a high efficient reverse transcriptase expressed by engineering bacteria; a further-modified hot start Taq DNA polymerase, which provides high efficiency and accuracy for the amplify reaction; and RNase inhibitor. With a special modified hydrophobic motif, King RTase gets a significant affinity for RNA and facilitates transcription through of RNA templates, especially for templates with high GC content or complex secondary structures. The 2×FastKing One Step RT-PCR MasterMix contains appropriate ion concentration, dNTPs and PCR enhancer. It could stabilize both RTase and polymerases and keep their efficiency within the whole reaction process.

Materials required but not supplied

1. RNA templates
2. gene-specific PCR primers

Protocol

1. Thoroughly thaw the template RNA, gene-specific PCR primers, 2×FastKing One Step RT-PCR Master Mix and RNase-Free ddH_2O, centrifuge briefly and place them on ice.
2. Prepare a reaction solution according to the following table on ice:

Contents	Volume/Reaction
2×FastKing One Step RT-PCR MasterMix	1.25 ml
25×RT-PCR Enzyme Mix	2 μl
Forward Primer (10 μM)	2 × 1 ml
Reverse Primer (10 μM)	2 × 1 ml
Template RNA	10 ng-1 μg total RNA
RNase-Free ddH_2O	Up to 50 μl

Notes: If setting up more than one RT-PCR reaction, mix all components one time and divide into each tube.

3. Set up thermal cycler conditions according the following table.

Steps	Reaction	Time	Temperature
1	Reverse transcription	30 min	42°C
2	Initial denaturation	3 min	95°C
3	Denaturation	30 sec	94°C
4	Annealing	30 sec	55-65°C
5	Extension	1 kb/min	72°C
6	35-40 cycles from step 3 to step 5		
7	Final extension	5 min	72°C

Notes: To avoid unspecific amplification, start the RT-PCR program while PCR tubes are still on ice. Wait until the thermal cycler has reached 42°C. Then place the PCR tubes in the thermal cycler. The annealing temperature depends on different primers.

4. Analyze the PCR products using agarose gel electrophoresis.

Important Notes

1. RNA template could be total RNA or mRNA. TRNzol or RNAprep pure kits can be used to purify high-quality total RNA.
2. RNase contaminations should be avoided in one-step RT-PCR. Some measures can be taken as below:
   1) Wear a disposable gloves and respirator to avoid the RNase contaminations from skin and saliva
   2) Operate the RNA related experiments in an RNase-free environment using RNase-free apparatus and consumable items.
   3) Consumable items related with RT-PCR should be incubated in 0.1% DEPC solution at 37°C for 12 hours and sterilized for 30 min before use.
3. 25×RT-PCR Enzyme Mix should be centrifuged briefly before use. Pipet slowly and store at -30~-15°C immediately after use.
4. Completely mix the 2×FastKing One Step RT-PCR MasterMix and centrifuge the reagent to the botiom of the tube before use.
5. Only use gene-specific primers and use specific primers according to specific requirements of experiments.