TIANGEN®

FastKing One Step RT-qPCR Kit (Probe)

For real-time RT-PCR using sequence-specific, hydrolysis probes

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Cat. no. 4992292/4992293

Kit Contents

Contents	4992292 50 µl × 50 rxn	4992293 50 µl × 200 rxn
2×FastKing One Step Probe RT-qPCR MasterMix	1.25 ml	4 × 1.25 ml
25×FastKing Enzyme Mix	100 µl	400 µl
50×ROX Reference Dye	250 µl	1 ml
RNase-Free ddH_2O	2 × 1 ml	5 × 1 ml
Hand Book	1	1

Storage

FastKing One Step RT-qPCR Kit could be stored at -20°C for 1 year.

The kit can be used with the following devices:

1. PRISM 7000/7700/7900HT, 7300/7500 Real-Time PCR System, 7500 Fast Real-Time PCR System, Viia 7 (Applied Biosystems)
2. OPTICONTM / CFX96 (BIORAD)
3. Light Cycler 480 (Roche)
4. Smart Cycler® System (Cepheid)
5. Mx3000P/Mx3005P (Stratagene)
6. Other Real Time PCR thermal cycler

Introduction

FastKing One Step RT-qPCR Kit provides rapid real-time quantification using probe method (TaqMan®, Molecular Beacon, etc). The 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×FastKing 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 accuricy for the amplify reaction; and RNase inhibitor. With a special modified hydrophobic motif, King RTase gets a significant affinity for RNA and facilitates transcripion through of RNA templates, especially for templates with high GC content or complex secondary structures. The 2×FastKing One Step Probe RT-qPCR MasterMix contains appropriate ion concentration, dNTPs and PCR enhancer. It could stabilize both polymerasesand keep their efficiency within whole reaction process.

Materials required but not supplied

1. Primers and probes
2. Templates
3. Disposable gloves and other laboratory supplies

Protocol

1. Fully melt RNA template, primers, 2×FastKing One Step Probe RT-qPCR MasterMix, 50×ROX Reference Dye and RNase-Free ddH_2O. Centrifuge transiently and put all of them on ice.
2. Prepare a reaction solution accord to the following table (All the steps should be operated on ice).

Contents	Volume /Reaction
2×FastKing One Step Probe RT-qPCR MasterMix	25 μl
25×FastKing Enzyme Mix	2 μl
Forward Primer (10 μM)	1.25 μl*1
Reverse Primer (10 μM)	1.25 μl*1
fluorescence probe (10 μM)	1.0 μl*2
RNA Template	10 pg-1 μg total RNA
50×ROX Reference Dye*3	-
RNase-Free ddH_2O	To 50 μl

*1 A final primer concentration of 0.25 μM is optimal for most applications. However, for individual determination of optimal primer concentration, a primer titration from 0.05-0.9 μM can be performed. Increase the concentration of the primers will increase the amplification efficiency, and reduce the the concentration of the primers could reduce the nonspecific amplification.

*2 The probe concentration is differed from RT-qPCR instruments, probe types and fluorophore types. We recommend checking the instructions of instruments and probes throughly before use. A final probe concentration of 0.20 μM is optimal for most applications. However,for individual determination of optimal primer concentration, a probe titration from 0.1-0.5 μM can be performed.

*3 The optimal concentration of ROX Reference Dye for commonly used Real-Time PCR instruments is as below:

Instrument	Final Concentration
ABI PRISM 7000/7300/7700/7900HT/Step One etc. volume)	5× (e.g. 5 µl ROX/ 50 µl
ABI 7500, 7500 Fast, Viia 7; Stratagene Mx3000P, Mx3005P and Mx4000 etc.	1× (e.g. 1 µl ROX/ 50 µl volume)
Instruments of Roche, Bio-Rad and Eppendorf etc.	No need

3.Real-Time One Step quantitative RT-PCR

The PCR reaction tubes are centrifuged transiently and put into the fluorescence quantitative PCR instrument for Real Time PCR reaction. The following table shows the recommended standard PCR program. PCR condition should be further optimized if experimental result is not ideal by this program.

Cycle	Temperature	Time	Contents
1×	50°C	30 min	Reverse transcription
1×	95°C	3 min	Initial denaturation
40×	95°C	15 sec	Denaturation
	60°C	30 sec	Annealing and extension. Collect the fluorecent signal.

4. Result analysis

After reaction, confirm amplification curves and CT value, draw the standard curve, calculate and analysis the results.

Important Notes

1. The RNA template should be total RNA or mRNA. We recommend using TIANGEN TRNzol reagent or RNAprep kit to extract high-quality RNA template.
2. To avoid RNase contamination, the operator must: i. Put on disposable gloves and breathing mask. ii. Use RNase-free material such as reagents, pipette tips, microtubes and instruments. iii. Do the experiments in certain area that especially for RNA operation.
3. 25×FastKing Enzyme Mix should be centrifuged transiently before use and slowly pipetted. Put it back to -20°C soon after use.
4. Completely mix the 2×FastKing One Step Probe RT-qPCR MasterMix and centrifuge the reagent to the bottom of the tube before use.
5. Use specific reverse transcription primer only. Random Primer and Oligo dT Primer can not be used in reverse transcription reaction.
6. To perform several Real Time One Step RT-qPCR at the same time, a mixture of all reagents should be firstly prepared and then divided into each reaction tube. It reduces reagent loss, avoids repeatedly adding the same reagents, and reduces the error by adjusting the volume of each components.