TIANGEN®

FastKing One Step RT-qPCR Kit (SYBR)

For real-time RT-qPCR using SYBR Green I

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FastKing One Step RT-qPCR Kit (SYBR)

Cat. no. GFP313

Kit Contents

Contents	GFP313-01 50 µl × 50 rxn
2× FastKing RT-qPCR Buffer (SYBR Green)	1.25 ml
25× RT-PCR Enzyme Mix	100 µl
50× ROX Reference Dye	250 µl
RNase-Free ddH_2O	1 ml
Handbook	1

Storage

This kit should be stored at -30~-15°C for one year and protected from light.

Compatible Real-Time Instruments

ABI PRISM 7000/7700/7900HT, 7300/7500, 7500 Fast, ViiA 7, QuantStudioTM, StepOneTM/ StepOne PlusTM, 12K Flex (Applied Biosystems)

OPTICONTM, CFX series (BIORAD)

Smart Cycler® System (Cepheid)

Mx3000 P/Mx3005P (Stratagene)

Line-Gene (Bioer)

Roche series

And others

Introduction

FastKing One Step RT-qPCR Kit is designed for Real-Time One Step RT-qPCR by using SYBR Green I. The kit can be used in real-time one step RT-qPCR of RNA targets, with reverse transcription and PCR taking place sequentially in the same tube. Therefore the operation is simple, and it minimizes the risk of contamination.

The 25× RT-PCR Enzyme Mix in this kit is a premix of TIANGEN's novel reverse transcriptase (FastKing RTase), novel antibody modified hot start *Taq* DNA polymerase and RNase Inhibitor. The FastKing RTase is a new type of molecular modified reverse transcriptase. With the specifically added hydrophobic motif, which has stronger RNA affinity and thermal stability. The FastKing RTase has improved reverse transcription efficiency and the ability to extend the RNA template with complex secondary structure. The new type of hot start *Taq* DNA polymerase with excellent performance is used in the PCR process, making the PCR reaction after reverse transcription have higher amplification efficiency and specificity. In addition, the 2× FastKing RT-qPCR Buffer (SYBR Green) in this product is a new reaction system specially optimized for the above two key enzymes, including necessary ionic components, dNTPs, SYBR Green dye, one step stabilizer and enhancer, which can ensure that FastKing RTase and the new hot start *Taq* DNA polymerase can play the most effective role in the whole one-step reaction process.

This kit provides good standard curves in a wide range of quantitative area. It can accurately and quantitatively detect a variety of high and low abundance target genes with good repeatability and high reliability.

Product Feature

1. Increased reaction efficiency: The high-quality reverse transcriptase and *Taq* polymerse ensure the high reaction efficiency.
2. Easy and fast operation: The dual component form makes the procedure easy and fast.
3. Capable of reading through complex templates: Suitable for RNA templates with high GC content and complex secondary structure.
4. High compatibility: Compatible for RNA templates from different sample source or with impurities.

Reagents and Materials not Supplied

1. Specific primers
2. Template RNA
3. Disposal gloves and other consumables

Application

The RT-qPCR can be used to detect gene expression and RNA virus content in cells and tissues.

Protocol

1. Fully thaw the template RNA, specific primers, 2× FastKing RT-qPCR Buffer (SYBR Green), 50× ROX Reference Dye and RNase-Free ddH_2O, and place on ice after short-spinning.
2. Prepare a reaction mix on ice according to the following table.

Components	Volume/Reaction
2× FastKing RT-qPCR Buffer (SYBR Green)	25 μl
25× RT-PCR Enzyme Mix	2 μl
Forward primer	1.25 μl[*1]
Reverse primer	1.25 μl[*1]
Template RNA	10 pg-1 μg total RNA
50× ROX Reference Dye[*2]	According to the instrument
RNase-Free ddH_2O	Up to 50 μl
Total Volume	50 μl

[*1] Primer with the final concentration of 0.25 μM can obtain good amplification results in most systems. When the amplification efficiency is not high, the primer concentration in the PCR reaction system can be increased. When non-specific amplification occurs, the primer concentration in the PCR reaction system can be appropriately reduced. If the concentration of primer needs to be further optimized, it can be adjusted in the range of 0.05 ~ 0.90 μM.

[*2] The optimum ROX Reference Dye concentrations for several common instruments are shown in the following table:

Instrument	Final concentration
ABI 5700/7000/7300/7700/7900HT/Step One™/StepOne Plus™	5× (e.g. 5 μl ROX /50 μl system)
ABI 7500, 7500 Fast, ViiA 7, QuantStudio™, 12K Flex, Agilent Mx3000P, Mx3005P, Mx4000	1× (e.g. 1 μl ROX /50 μl system)
Roche, Bio-Rad, Eppendorf instruments, etc.	No need

3. Run the Real Time One Step RT-qPCR reaction

   Centrifuge briefly before starting real-time PCR. It is recommended to run the standard PCR reaction program in the following table. If the program can't generate optimal results, it is recommended to perform further optimization.

Temperature	Time	Cycle No.	Comments
50°C	30 min	1	Reverse transcription
95°C	3 min	1	Initial denaturation
95°C	15 sec	40	PCR cycling
60°C	30 sec		Please collect fluorescent signal at this step
Melting/Dissociation Curve Stage			

4. Result analysis

   After the reaction, confirm the amplification curve, melting curve, CT value and standard curve of the Real Time One Step RT-qPCR, and analyze the RT-qPCR quantitative results.

Important Notes

1. Total RNA or mRNA can be used as RNA template. It is recommended to use TRNzol, RNAprep Pure or RNA Easy Fast series produced by TIANGEN company to prepare high quality total RNA.
2. RNase contamination should be avoided during the one-step RT qPCR experiment:
   1) Since RNase is found on the surface of human skin and saliva, disposable gloves and masks should be worn in the experiment;
   2) Special instruments and consumables should be used in the one-

step RT qPCR experiment, and it is suggested that RNA should be operated in special areas;

3) One step RT-qPCR experiment related consumables should be treated with 0.1% DEPC (diethyl pyrocarbonate) solution at 37°C for 12 hours and autoclaved for 30 min.

3. The 25× RT-PCR Enzyme Mix should be centrifuged for a short time before pipetting. The action should be slow, and it should be put back to -30~-15°C as soon as possible after use.
4. 2× FastKing RT-qPCR Buffer (SYBR Green) should be fully mixed and centrifuged before use.