Hangzhou Simgen Biotechnology Co., Ltd.
5th floor, Building No. 4, No. 8 Xiyuan 1st Road, Hangzhou, P. R. China.
Pc: 310030 Tel: +86-571-87381295 Fax: +86-571-87381295

2×One Step Probe RT-qPCR Mix Instructions

Composition

Cat. No.	7406100	7406500
2×One Step Probe RT-qPCR Mix*1	1 ml	1 ml×5
RT-Taq enzyme Mix*2	80 µl	400 µl
50×ROX Reference Dye*3	40 µl	200 µl
RNase-free Water	1.5 ml	1.5 ml×5
Instructions	1	1

*1 Contains dNTP Mix, Mg^{2+}, etc.

*2 Contains reverse transcriptase, Taq DNA polymerase, Taq DNA polymerase antibody, RNase inhibitor, etc.

*3 For correcting the fluorescent signal error generated between wells.

Storage

This kit can be stored at -20°C and protected from light for up to two years.

Technical Support

R&D Department, Hangzhou Simgen Biotechnology Co., Ltd. E-mail: technical@simgen.cn, Tel: 400-0099-857.

Introduction

This product is a two-fold concentration of master mix designed for Real Time RT-PCR using the probe method. Real-Time RT-PCR reactions with this product allow for continuous reverse transcription and qPCR amplification in the same reaction tube, which is simple to operate and can effectively prevent contamination. This reaction system can greatly improve the detection sensitivity due to the real-time monitoring of the amplified products, and omit the electrophoresis step after the PCR reaction, which is very suitable for the detection of RNA viruses.

This product uses high-efficiency reverse transcriptase and high-specificity, hot-start Taq DNA polymerase, which can perform stable and efficient Real Time One Step RT-PCR reactions. For q PCR machines that use ROX as the correction dye, this product is also specially provide ROX dye to correct the fluorescence signal error generated between the wells of the quantitative PCR instrument.

Equipment and Reagents to Be Supplied by User

1. PCR primers, probes and RNA template.
2. Single-tube, 8-tube strip, or 96-well PCR tube (plate) suitable for real-time PCR.
3. Micropipettes and clean tips with filters.
4. Real-time PCR instrument (authorized instrument).

Precautions

1. When several RT-PCR reactions are required at the same time, prepare a mixture of various reagents (Master Mix: include RNase-free Water, Buffer, Enzymes, etc.) and then aliquot into each reaction tube. This allows for more accurate reagent volumes, reduces reagent loss, and avoids duplicate dispensing of the same reagent. At the same time, it can also reduce the errors generated by experimental operations or between experimental samples.
2. When using the RT-Taq enzyme Mix, it should be mixed gently to avoid foaming. Spin down the mix to the bottom of the reaction tube before dispensing. Due to the high concentration of glycerol in the enzyme preservation solution, the viscosity is high, and it should be slowly absorbed when dispensing.
3. When preparing and dispensing the reaction solution, please be sure to use new pipette tips with filter, centrifuge tubes, etc., to avoid contamination as much as possible.
4. This product can only use specific reverse transcription primers and cannot use Random Primer and Oligo dT Primer for reverse transcription reactions.

Procedure

1. Prepare an RT-qPCR reaction mixture for the number of reactions needed according to the following Table. (Keep the components and reaction mixture on ice at all times).

Component	Usage	Final Concentration
2×One Step Probe RT-qPCR Mix	25.0 µl	1×
RT-Taq DNA enzyme Mix	2.0 µl	
Forward Primer (10 µM) *1	1.0 µl	0.2 µM
Reverse Primer (10 µM)*1	1.0 µl	0.2 µM
Probe (10 µM)	1.0 µl	0.2 µM
50×ROX Reference Dye*2	1.0 µl	1×
RNA template*3	—	
RNase-free Water	Add to 50.0 µl*4	

*1 In general, a final concentration of 0.2 µM for primers and probes will give good results. When the reaction performance is poor, the concentration of primers and probes can be adjusted in the range of 0.1~1.0 µM.

*2 * For correct the fluorescence signal error generated between wells. When using qPCR instruments such as Applied Biosystems 5700，7000，7300，7700，7900，7900HT，7900HT Fast；Applied Biosystems StepOne™，StepOnePlus™, the dosage of the 50×ROX Reference Dye is 1/50 of the PCR system volume. When using qPCR instruments such as Applied Biosystems 7500，7500 Fast，ViiA™7，Stratagene MX4000™，MX3005P™，MX3000P™, the dosage of the 50×ROX Reference Dye is 1/250 of the PCR system volume. When using qPCR instruments such as Bio-Rad CFX96™，CFX384™，iCycler iQ™，iQ™5，MyiQ™，MiniOpticon™，Opticon®，Opticon 2，Chromo4™，Cepheid SmartCycler®，Eppendorf Mastercycler®ep realplex，realplex 2，Illumina Eco qPCR，Qiagen/Corbett Rotor-Gene®Q，Rotor-Gene® 3000，Rotor-Gene® 6000，Roche Applied Science LightCycler™ 480，Thermo Scientific PikoReal Cycler, there is no need to add ROX.

*3 It is recommended to use 20 pg~200 ng of total RNA as a template in a 50 µl reaction system.

*4 The volume of the reaction system is determined according to the requirements of each instrument.

2. Perform a Real-Time One Step RT-PCR reaction

Spin down to allow the reaction solution to the bottom of the PCR tube, and then place the PCR tube in a real-time PCR instrument for real-time PCR reaction. It is recommended to use the standard PCR reaction program shown in the chart below, and to optimize the PCR conditions if the results are not good with this procedure.

Steps	Number of Cycles	Temperature (℃)	Reaction Time
1 Reverse Transcription	1	50	5 min
2 Denaturation	1	95	10 sec
3 PCR Reaction	40	95	5 sec
		60	30~35 sec

3. Analysis of experimental results

After the reaction is completed, the amplification plot of Real Time One Step RT-PCR is confirmed, and a standard curve is created for RT-PCR quantification. The analytical method refers to the operator's manual of the instrument.