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2×One Step SYBR Green RT-PCR Mix Instructions

Composition

Cat. No.	7405100	7405500
2×One Step SYBR Green RT-PCR Mix	1 ml	1 ml×5
RT-PCR Enzyme Mix	40 µl	200 µl
50×ROX Reference Dye	40 µl	200 µl
RNase Free Water	1.5 ml	1.5 ml×5
Instructions	1	1

Storage

It can be stored at -20℃ for more than 2 years.

Technical Support

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Introduction

This product is a special reagent for Real Time PCR using SYBR Green I chimeric fluorescence method. Using this product for Real Time RT-PCR reaction can be carried out continuously in the same reaction tube, simple operation, and can effectively prevent contamination. Because the reaction system can detect the amplified products in real time, the detection sensitivity is greatly improved, and the electrophoresis step after PCR reaction is omitted, which is very suitable for the detection of trace RNA.

This product uses reverse transcriptase suitable for Real Time RT-PCR and Taq enzyme with high amplification efficiency and high amplification specificity and can perform stable Real Time One Step RT-PCR reaction.

Equipment And Reagents to Be Supplied by Users

1. PCR primers.
2. RNA template.
3. Single tube, 8-strip tube, or 96-well PCR tube (plate) for fluorescent quantitative PCR.
4. Pipettes and RNase-free tips with filter element.
5. Real Time PCR amplification instrument (authorized instrument).

Cautions

1. When several Real Time One Step RT-PCR reactions are required at the same time, a mixture of various reagents should be prepared first (Master Mix: Including RNase Free Water, Mix, various enzymes, etc.), and then divide it into each reaction tube. This can make the reagent volume taken more accurate, reduce reagent loss, and avoid repeated divide of the same reagent. At the same time, it can also reduce the error between experimental operations or experimental samples.
2. When using the RT-PCR Enzyme Mix, it should be lightly mixed to avoid foaming; Spin down the reaction tube before separating; Due to the high concentration of glycerin in the enzyme preservation solution and its high viscosity, it should be slowly pipetted when separating.
3. If there are any precipitates in 2×One Step SYBR Green RT-PCR Mix after thawing, please mix thoroughly until all the precipitates dissolved.
4. When preparing and dividing the reaction liquid, please be sure to use new tips, centrifuge tube, etc., to avoid contamination as far as possible.
5. This product can only use specific reverse transcription primers and cannot use Random Primer and Oligo dT Primer for reverse transcription reaction.

Protocol

1. Prepare the RT-PCR reaction solution according to the following table (Prepare the reaction solution on ice).

Reagent	Dosage	Final Concentration
2×One Step SYBR Green RT-PCR Mix	25 µl	1×
RT-PCR Enzyme Mix	1 µl	
Forward primer (10 µm) *1	1 µl	0.20 µM
Reverse primer (10 µm)	1 µl	0.20 µM
50×ROX Reference Dye*2	1 µl	
RNA template*3	-	
RNase Free Water*4	To 50 µl	

*1 Good results are usually obtained with a final primer concentration of 0.2 µm. When the reaction performance is poor, the primer concentration can be adjusted in the range of 0.1~1.0 µM.

*2 Used to correct the fluorescence signal error generated between holes. Use Applied Biosystems 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, Applied Biosystems StepOne™, StepOnePlus™ and other fluorescent quantitative PCR instruments requiring high concentration of ROX Reference Dye, the addition of 50 × ROX Reference Dye was 1/50 of the PCR reaction system; Applied Biosystems 750, 7500 Fast, ViiA™7, Stratagene MX4000™, MX3005P™, MX3000P™ and other fluorescent quantitative PCR instruments requiring low concentration of ROX Reference Dye, the addition of 50 × ROX Reference Dye was 1/250 of the PCR reaction system. The following fluorescent quantitative PCR instruments do not need to add ROX Reference Dye: 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, etc.

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

*4 Determine the volume of the reaction system according to the requirements of different instruments.

2. Perform the RT-PCR reaction in Real Time One Step

Spin down the PCR reaction tube and then put into the fluorescence quantitative PCR instrument for Real Time PCR reaction. It is recommended to use the standard PCR reaction protocol as shown in the following table. If good experimental results are not obtained by using this protocol, then optimize the PCR conditions.

Number Of Cycles	Steps	Temperature	Time	Content
1	1	50 ℃	5 min	Reverse transcription
	2	95 ℃	10 sec	
40	3	95 ℃	5 sec	PCR reaction
	4	60 ℃	30~35 sec	
1	5	95 ℃	15 sec	Dissociation Protocol
	6	60 ℃	1 min	
	7	95 ℃	15 sec	

3. Analysis of results

The amplification curve and melt curve of RT-PCR were confirmed in Real Time One Step after the reaction, and the standard curve was made when quantitative RT-PCR was performed. For analytical methods, refer to the operating manual of the instrument.