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2×Probe qPCR Mix Instructions

Composition

Cat. No.	7206100	7206500
2×Probe qPCR Mix	1 ml	1 ml×5
50×ROX Reference Dye*	40 µl	200 µl
ddH_2O	1 ml	1 ml×5
Instructions	1	1

* For correct the fluorescence signal error generated between wells.

Apply Applied Biosystems 5700, 7000, 7300, 7700, 7900, 7900 HT, 7900HT Fast, Applied Biosystems StepOne™, StepOnePlus™ and other qPCR instruments need to add a high concentration of ROX Reference Dye, the addition of 50× ROX Reference Dye is 1/50 of the PCR reaction system volume. Applied Biosystems 7500, 7500 Fast, ViiA™7, Stratagene MX4000™, MX3005P™, MX3000P™ and other qPCR instruments with low concentration ROX Reference Dye need to be added, the addition of 50×ROX Reference Dye was 1/250 of the PCR reaction system volume. The following qPCR 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 LightCyclerTM480, Thermo Scientific PikoReal Cycler, et al.

Storage

This kit can be stored for more than two years below -20°C.

Technical Support

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Introduction

2×Probe qPCR Mix is a two-fold concentration of master mix designed for probe-based real-time PCR. The product uses a hot-start DNA polymerase with anti-Taq antibody, which can be combined with the most suitable Buffer for Real Time PCR, which can greatly improve the amplification efficiency of PCR and perform high-sensitivity Real Time PCR amplification reactions. The product is separately equipped with ROX dye, which is suitable for use in mainstream qPCR instruments such as Applied Biosystems, Bio-Rad, Eppendorf, Roche, etc. This product is suitable for fast Real Time PCR amplification reactions and can obtain a good standard curve in a wide range of quantification regions, and accurately quantify and detect target genes, with good reproducibility and high confidence.

Equipment and Reagents to Be Supplied by User

1. Primers and probes, DNA or cDNA as the template.
2. Single-tube, 8-tube strip, or 96-well PCR tube (plate) suitable for real-time PCR.
3. Micropipettes and clean tips.
4. Real-time PCR instrument (authorized instrument).

Precautions

1. 2× Probe qPCR Mix stored at -20°C can be slowly dissolved in the hand before use, gently invert until the pellet is completely gone. Do not vortex to mix.
2. When preparing PCR reactions, reagents should be kept on ice and should be protected from bright light.
3. Avoid repeated freeze-thaw cycles of this product, as repeated freeze-thaw may degrade the performance of the product.
4. When preparing reactions, use clean tips (filtered tips are recommended) and centrifuge tubes to minimize contamination.

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Protocol

◆ Protocol for Applied Bio systems 7300/7500/7500 Fast Real-Time PCR Instrument and Step One Plus™ Real-Time PCR Instrument

1. Prepare a 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	Usage	Final concentration
2×Probe qPCR Mix	10.0 μl	25.0 μl	1×
PCR Forward Primer（10 μM）	0.4 μl	1.0 μl	0.2 μM*1
PCR Reverse Primer（10 μM）	0.4 μl	1.0 μl	0.2 μM*1
Probe (10 μM)	0.4 μl	1.0 μl	0.2 μM*1
50×ROX Reference Dye*2	0.4 μl	1.0 μl	1×
DNA template*3	2.0 μl	5.0 μl	
ddH_2O	6.4 μl	16.0 μl	
Total	20.0 μl*4	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 When using 7500 Real-Time PCR System and 7500 Fast Real-Time PCR System, the dosage of the 50×ROX Reference Dye is 1/250 of the PCR reaction system volume. When using ABI PRISM 7300 Real-Time PCR System and Step One PlusTM, the dosage of the 50×ROX Reference Dye is 1/50 of the PCR reaction system volume.

*3 In a 20 μl reaction system, the amount of DNA template added is typically less than 100 ng. Because different types of DNA templates contain different copy numbers of target genes, serial dilutions can be performed if necessary to determine the optimal amount of DNA template to add. If you want to use this product for the second step PCR amplification reaction of the two-step RT-PCR amplification reaction, do not add more than 10% of the total volume of the PCR reaction system when the RT reaction product is used as a DNA template in the first step.

*4 The reaction mixture is prepared according to the recommended system of each instrument.

2. Perform a Real-Time PCR reaction

The following two-step PCR reaction is recommended, and if does not yield good results, the PCR conditions should be optimized. If the amplification performance of the two-step PCR reaction is poor due to the use of primers with low Tm values, a three-step PCR amplification reaction can be tried.

1) Program The Two-Step PCR Amplification Reaction for Applied Biosystems 7300/7500 And Step One Plus™ Instrument.

Steps	Number of Cycles	Temperature (℃)	Reaction Time (Sec)
1 Initial Denaturation	1	95	30
2 PCR reaction	40	95	5
		60	30~34 * (Fluorescence Acquisition)

* Please set the time to 30 sec when using Step OnePlus™ instrument. When using the Applied Biosystems 7300 instrument, please set it to 31 sec. When using the Applied Biosystems 7500 instrument, set it to 34 sec.

2) Program The Two-Step PCR Amplification Reaction for 7500 Fast Real-Time Instrument.

Steps	Number of Cycles	Temperature (℃)	Reaction Time (Sec)
1 Initial Denaturation	1	95	30
2 PCR reaction	40	95	3
		60	30 (Fluorescence Acquisition)

3. Analysis of experimental results

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

◆ Protocol for LightCycler®/LightCycler®480 Real Time PCR Instrument

1. Prepare a 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×Probe qPCR Mix	10.0 µl	1×
PCR Forward Primer（10 µM）	0.4 µl	0.2 µM*1
PCR Reverse Primer（10 µM）	0.4 µl	0.2 µM*1
Probe (10 µM)	0.4 µl	0.2 µM*1
DNA template*2	2.0 µl	
ddH_2O	6.8 µl	
Total	20.0 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 In a 20 µl reaction system, the amount of DNA template added is typically less than 100 ng. Because different types of DNA templates contain different copy numbers of target genes, serial dilutions can be performed if necessary to determine the optimal amount of DNA template to add. If you want to use this product for the second step PCR amplification reaction of the two-step RT-PCR amplification reaction, do not add more than 10% of the total volume of the PCR reaction system when the RT reaction product is used as a DNA template in the first step.

2. Perform a Real-Time PCR reaction

For capillary PCR reaction, spin down the PCR reaction mixture to the bottom of the tube, and then put the tube into the LightCycler® for Real Time PCR reaction. The following two-step PCR reaction is recommended, and if does not yield good results, the PCR conditions should be optimized. If the amplification performance of the two-step PCR reaction is poor due to the use of primers with low Tm values, a three-step PCR amplification reaction can be tried.

1) Program The Two-Step PCR Amplification Reaction for LightCycler® Instrument.

Steps	Number of Cycles	Temperature (℃)	Reaction Time (Sec)	Heating Rate (℃/sec)
1 Initial Denaturation	1	95	30	20
2 PCR reaction	40	95	5	20
		60	20	20

2) Program The Two-Step PCR Amplification Reaction for LightCycler®480 Instrument.

Steps	Number of Cycles	Temperature (℃)	Reaction Time (Sec)	Heating Rate (℃/sec)
1 Initial Denaturation	1	95	30	4.4
2 PCR reaction	40	95	5	4.4
		60	30	2.2

Analysis Mode: quantitative analysis (40 cycles). Acquisition Mode : Single

3. Analysis of experimental results

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