PrimeTaq™ Probe One-Step RT-qPCR Kit

User's Instruction

Description

PrimeTaq™ Probe One-Step RT-qPCR Kit is a high-quality premix based on probes for one-step Quantitative reverse transcription PCR (RT-qPCR), which is mainly used for specific ultra-high sensitivity quantitative detection of RNA. Since the probes used are generally TaqMan probes, this method is also often called TaqMan assays.

PrimeTaq™ Probe One-Step RT-qPCR Kit uses the extracted RNA as a template and uses qPCR primers to carry out reverse transcription and fluorescence quantitative PCR continuously in the same tube. It is easy to operate, which minimizes human errors, effectively reduces the risk of contamination, saves the operation time of the PCR experiment, and has large detection throughput.

Kit Contents

	100 preps
1. PrimeTaq™ Probe One-Step Enzyme Mix (10X)	200 µl
2. PrimeTaq™ Probe One-Step Reaction Buffer (2X)	1 ml
3. Nuclease-free Water	1 ml
4. Low ROX (50X)	40 µl
5. High ROX (50X)	40 µl

	500 preps
1. PrimeTaq™ Probe One-Step Enzyme Mix (10X)	1 ml
2. PrimeTaq™ Probe One-Step Reaction Buffer (2X)	5 ml
3. Nuclease-free Water	5 ml
4. Low ROX (50X)	200 µl
5. High ROX (50X)	200 µl

Detection Principle

Probe-based qPCR uses fluorescent and quencher-labeled DNA probes to target the sequence which will be amplified by PCR. Normally, quenching groups on the probe result in quenching of fluorescent groups due to the fluorescence resonance energy transfer

(FRET) in space. When the target sequence is amplified by PCR reaction, both primers and probes are annealed to the target gene. With the extension of primers, the 5' to 3' exonuclease activity of Taq enzyme will cause the probe bound to the target sequence to be degraded gradually from the 5' end. After the fluorescent group and quenching group of the probe are cleaved by Taq enzyme, the quenching group disappears, and the fluorescent group can be normally excited by the excitation light to produce fluorescence. After each PCR cycle, more fluorescent groups are released, and the fluorescence intensity is proportional to the number of newly synthesized target fragments, thus quantitative detection can be achieved. Probes are usually a fragment of linear DNA specific to the target sequence, labeled with fluorescent groups such as FAM or HEX at the 5' end and fluorescent quenching groups such as BHQ1, TAMRA or MGB at the 3' end.

ROX Normalization

PrimeTaq™ Probe One-Step RT-qPCR Kit provides Low ROX and High ROX, which are widely compatible with fluorescent quantitative PCR instrument without ROX and requiring Low ROX or High ROX as passive reference fye. The role of ROX is to calculate away variations caused by pipetting errors and sample evaporation. Different fluorescence quantitative PCR instruments have different requirements for ROX. Please check the following table for more details.

ROX Reference Dye	PCR Instruments
No need	• Roche, Bio-Rad, Eppendorf, and Illumina
Low ROX	• ABI 7500/7500 Fast/ViiA 7/QuantStudio 3/QuantStudio 5 • Stratagene MX3000P/MX3005P/MX4000P
High ROX	• ABI 5700/7000/7300/7700/7900/7900HT/7900HT fast/stepone/stepone plus

Features

- High specificity and sensitivity: specificity is not only dependent on PCR primers, but also specific binding and degradation of probes and target genes to generate fluorescent signals. The detection sensitivity and specificity are usually significantly higher than those of the methods using fluorescent dyes such as SYBR Green.

- Multiple detection: in a single reaction, different genes correspond to different probes and different probes correspond to different fluorescent markers, which can be used for multiple fluorescent quantitative PCR detection. PrimeTaq™ Probe One-Step RT-qPCR Kit can be used for the detection of 2-3 genes at the same time after optimization of primers and probes.

Protocol

Setting up One-Step RT-qPCR

1. Mix the reagents required for the One-Step RT-qPCR reaction and keep them on ice.
2. Set up One-Step RT-qPCR reaction as the following table (take 96-well plate, 20 μl per well as an example):

Reagent	Volume	Final Concentration
PrimeTaq™ Probe One-Step Reaction Buffer (2X)	10 μl	1 X
PrimeTaq™ Probe One-Step Enzyme Mix (10X)	2 μl	1 X
Forward and Reverse Primer Mix (3μM each)	2 μl	300 nM
Probe	0.5 μl	200 nM
Template RNA	2 μl	1 pg-2 μg
Without or Low/High ROX (50X)	0.4 μl	1 X
RNase-Free Water	Up to 20 μl	-

3. Gently mix the reaction. Collect all liquid to the bottom of the tube by a quick spin.
4. Transfer PCR tubes from ice to a PCR machine.

Thermocycling Conditions for One-Step RT-qPCR (Recommended)

1. Reverse transcription: 50℃ for 15-30 min
   a) Usually 15 min is enough. But if the fragment is long, you can extend to 30 min.
2. Initial denaturation: 95℃ for 2 min
3. 40 cycles:

a) 95℃ for 15 sec

b) 60℃ for 15-30 sec (for amplicons more than 350 bp or with high GC content, it is recommended to increase the extension time to 60 sec)

4. Melting curve analysis (optional): 95℃ for 15 sec, 60℃ for 15 sec, 95℃ for 15 sec

Storage

Store at -20℃ and protect from light. Avoid repeated freeze-thaw cycles.