

One Step RT-PCR Probe Kit

Product Number: PCK55

Shipping and Storage

Store at -20°C, with a shelf life of 12 months.

Components

Component	100T	200T
2×qRT-PCR Probe Mix	1mL	2×1mL
Real Script OneStep Enzyme Mix	100μL	2×100μL
ROX Reference Dye	25μL	2×25μL
RNase free H ₂ O	1mL	2×1mL

Description

This product is a dedicated kit for one-step reverse transcription real-time fluorescence quantitative detection using the probe method. With extracted RNA as the template, reverse transcription and fluorescence quantitative detection are performed sequentially in the same reaction tube, ensuring simple operation while effectively preventing contamination and reducing pipetting errors. Based on high-efficiency reverse transcriptase, hot-start polymerase, and an optimized buffer system, it delivers excellent amplification performance for RNA templates with complex secondary structures and high GC content, making it highly suitable for detecting trace target genes such as RNA viruses. When using this product, simply add the template, primers, probes, ROX Reference Dye (used to correct fluorescence signal variations between wells, select based on the specific real-time PCR instrument), and water,

Adjust the working concentration to 1× to proceed with the reaction. It offers advantages such as rapid and simple operation, high sensitivity, strong specificity, and excellent stability, effectively minimizing human errors, saving PCR experimental time, and reducing contamination risks.

Application

Suitable for high-copy and low-copy gene detection; applicable to RNA templates with high GC content or complex secondary structures.

Protocol

1. Suggested reaction system (20μL):

Note: The 2 × qRT PCR Probe Mix should be thoroughly melted, inverted, and mixed before use (to avoid generating large amounts of bubbles due to violent vortices). Short term frequent use can be stored in a refrigerator at 4°C.

Prepare the reaction solution on ice according to the table below:

Components	Volume	Final Concentration
2×qRT-PCR Probe Mix	10μL	1 ×
Forward Primer (10μM)	0.5μL	0.25μM
Reverse Primer (10μM)	0.5μL	0.25μM
Probe (10μM)	0.5μL	0.25μM
Real Script OneStep Enzyme Mix	1μL	-
RNA template	XμL	1 ng-0.1μg
RNase free H ₂ O to final volume	20μL	Not applicable

Note: Please use the final concentration of 0.2-0.6μM as a reference for setting the primer concentration range. In the case of low amplification efficiency, the concentration of primers can be increased; When non-specific reactions occur, the primer concentration can be reduced to optimize the reaction system. The probe concentration can be adjusted between 50-300nM

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depending on the specific situation.

2. Real Time PCR amplification:

2.1. Mix and centrifuge the prepared reaction system.

2.2. Preheat the PCR instrument to 50°C, place the PCR tube in a fluorescence quantitative PCR thermal cycler, and perform the reaction according to the following procedure.

2.3. Two step amplification program:

Temperature	Time	Cycle
50°C	15-30min	1
94°C	2min	1
94°C	15sec	35-45
60°C	30sec	35-45

2.4. Three step amplification program:

Temperature	Time	Cycle
50°C	15-30min	1
94°C	2min	1
94°C	15sec	35-45
60°C	15sec	35-45
72°C	30sec	35-45

Note: The standard two-step PCR reaction procedure is preferred. If good experimental results cannot be obtained using this procedure, PCR conditions can be optimized, such as attempting to use a three-step method for amplification to enhance amplification efficiency.

3. Experimental result analysis, including amplification curve and melting curve analysis as needed.

Note

1. When multiple Real Time One Step qRT PCR reactions need to be performed simultaneously, a mixture of various reagents (Master Mix, including RNase free ddH₂O, Buffer, various enzymes, etc.) should be prepared first, and then divided into each reaction tube. This can make the volume of the taken reagent more accurate, reduce reagent loss, and avoid duplicate dispensing of the same reagent. At the same time, it can also reduce the errors generated between experimental operations or samples.
2. When using Real Script Enzyme Mix, carefully centrifuge and collect it to the bottom of the reaction tube before separation; Due to the high concentration and viscosity of glycerol in the enzyme storage solution, it should be slowly absorbed when aliquoting.
3. Fluorescent probes should be used when preparing the reaction system, and strong light should be avoided when storing or preparing the One Step qRT PCR reaction solution.
4. This product contains Rox Reference Dye, which can be used for fluorescence quantitative PCR instruments that require calibration of inter well differences with Rox dye.
5. To ensure a successful reaction, it is recommended to use high-quality RNA templates.
6. The optimal amount of RNA template required varies for different fragments, and it is recommended to adjust the template amount according to the reaction.
7. Only specific primers can be used, and Oligo (dT) and Random Primer cannot be used for one-step reverse transcription reactions.