One-step Probe RT-qPCR Kit V2

Cat. No.: V5009，V5010

Components

Component	V5009 (200 rxn, 30 μl/rxn)	V5010 (5000 rxn, 30 μl/rxn)
2X RT-qPCR Mix [a]	760 μl × 4	38.4 ml × 2
One-step Enzyme Mix V2 [b]	308 μl	7.7 ml

a. Contains dNTP/dUTP Mix, Mg^{2+}.

b. Contains Reverse Transcriptase, RNase inhibitor, Heat-labile UDG and hotstart Taq DNAPolymerase.

Storage

This reagent should be kept at -20°C.

Description

The One-step Probe RT-qPCR Kit V2 is designed for quantitative PCR detection using RNA as a template, such as viral RNA. Using gene-specific primers (GSP), reverse transcription and qPCR reactions are performed in a single tube, requiring no additional tube opening/pipetting, significantly increasing detection throughput and reducing the risk of contamination. This Kit introduced dUTP/UDG system. Heat-labile UDG can rapidly degrade the pollutants containing U at room temperature. When reverse transcription is performed at 55°C, heat-labile UDG will be inactivated rapidly without affecting the efficiency and sensitivity of RT-qPCR. Thermostable reverse transcriptase can maintain stable activity at 55°C. The hot-start antibody-modified fast DNA polymerase and optimized buffer system ensure the extremely high sensitivity and specificity of the One-Step Probe RT-qPCR Kit V2. The kit is available as a convenient Master Mix. 2X RT-qPCR Mix contains an optimized buffer system and dNTP/dUTP Mix, which is suitable for high-specificity detection systems with fluorescent labeled probes such as TaqMan®.

Protocol

1. Preparation of Reaction System

Add the following reagents to the proper thermal cycler reaction tube or plate on ice:

Component	Volume	Final concentration
2X RT-qPCR Mix	15 μl	1X
One-step Enzyme Mix V2	1.5 μl	-
Forward Primer (10 μM)	0.6 μl	0.2 μM
Reverse Primer (10 μM)	0.6 μl	0.2 μM
Probe (10 μM)	0.3 μl	0.1 μM
Template RNA	1 pg-1 μg	1 pg-1 μg/30 μl
RNase-free ddH_2O	To 30 μl	–

The amount of each component in the reaction system can be adjusted according to the following principles:

• The optimal range for primers is 0.1~1.0 μM. In general, the primers with a final concentration of 0.2 μM work well.

• The optimal range for probes is 50-250 nM.

• qPCR is highly sensitive, and the accuracy of the amount of template added to the reaction system will have a great impact on the final quantitative results. It is recommended to add the template to the reaction system after dilution (such as dilution to 2-5 μl/ sample), which can effectively improve the repeatability of the experiment.

• The length of the amplification product should be in the range of 80-200 bp.

2. Preform RT-qPCR using the following thermal cycling condition

Standard reaction system (maximum amplification sensitivity):

Step	Stage	Cycle No.	Temperature	Time
Reverse transcription	1	1	55°C [a]	15 min
Initial denaturation	2	1	95°C	30 sec
Circular reaction	3	45	95°C	10 sec
			60°C [b]	30 sec

Fast reaction system (for most applications) [c]:

Step	Stage	Cycle No.	Temperature	Time
Reverse transcription	1	1	55°C [a]	5 min
Initial denaturation	2	1	95°C	30 sec
Circular reaction	3	45	95°C	5 sec
			60°C [b]	20 sec

a. For templates with complex secondary structures or high GC regions, increasing the reverse transcription temperature to 55ºC is conducive to improving amplification efficiency and sensitivity.

b. The extension time should be adjusted according to the minimum time limit of data collection required by the Real Time PCR instrument you use: at least 30 seconds with ABI 7700 and 7900HT; at least 31 seconds when using ABI 7000 and 7300; use ABI 7500 for at least 34 seconds.

c. Whether the Real Time PCR instrument actually used supports rapid amplification cycle or not, please conduct preliminary experiment to confirm the initial attempt.

3. Analyze the results

Data analysis varies depending on the instrument used. Please refer to your instrument user guide for information.

Product Use Limitations

This product is sold exclusively for research purposes and *in vitro* use. Neither the product, nor any individual components, was tested for use in diagnostic applications or for drug development, nor is it suitable for administration to humans or animals. Please refer to the MSDS, available upon request.