One-Step RT-qPCR Kit

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Product information

Product Name	Cat. No.	Size
One-Step RT-qPCR Kit	HMD4001-01	100 rxn
	HMD4001-02	1000 rxn
	HMD4001-03	10000 rxn

Product description

One-Step RT-qPCR Kit is a multiplex fluorescence quantitative PCR kit with RNA as template. During the reaction process, reverse transcription and quantitative PCR are completed in one tube, no additional opening operation is required, which reduces the risk of contamination.The One-Step RT-qPCR Kit uses reverse transcriptase to efficiently synthesize the first strand cDNA, and selects the best ratio of Hot Start Taq DNA polymerase (antibody modification) and RNase inhibitor, with additions to inhibit non-specific amplification and increase multiple The enhancer buffer system for quantitative amplification efficiency, with a detection sensitivity of up to 0.1 pg, can provide stable and reliable amplification, and can perform up to four-fold reactions.

Product components

Conponent	2×One-Step Buffer	Enzyme Mix
HMD4001-01	1 mL	0.15 mL
HMD4001-02	12.5 mL	1.5 mL
HMD4001-03	15×10 mL	10×1.5 mL

Note:

a. 2 × One-Step Buffer includes dNTP Mix and Mg2+.

b. Enzyme Mix mainly contains reverse transcriptase, Hot Start Taq DNA polymerase (antibody modification) and RNase inhibitor.

c. ROX: You need to choose the calibration according to the model of the testing instrument.

Transportation and storage methods

≤0℃ for transportation; -25～-15℃ for storage.

Precautions

Please use RNase free pipette tips, EP tubes, etc. during the preparation of the experiment.

Reaction system

Component	Volume	Amount
2×One-Step Buffer	10 μL	1×
Enzyme Mix	1.5 μL	-
Primer Mix	x	0.1 -1 μM
TaqMan Probe Mix	y	50-300 nM
Template RNA	Total RNA 1 pg-1 ug	-
RNase Free H2O	to 20 μL	—

Note:

a. The primer concentration is usually 0.2 μM. When the amplification performance is poor, the primer concentration can be adjusted within the range of 0.1-1.0 μM according to the situation.

b. Probes containing multiple fluorescent signals can be used, and the final concentration of each probe with different signals can be adjusted between 50-300 nM.

c. The sensitivity of qPCR is extremely high. It is recommended to dilute the template and add it to the reaction system. It is appropriate to control the Ct value between 20-35.

d. It is recommended to use 20 μl or 50 μl for the reaction system.

e. The length of the amplified product is selected in the range of 80-250 bp.

f. Please fully dissolve and absorb before use to avoid excessive bubbles caused by violent shaking.

Amplification program

Step	Temperature	Time
Reverse Transcription	50℃	10 min
Pre-denaturation	95℃	5 min
Cycles 40×	95℃	10 s
	50-68℃	30 s

Note:

a. For templates with complex secondary structure or high GC content, it is recommended to increase the reverse transcription temperature to 55 ° C, which is beneficial to increase the amplification efficiency.

b. The amplification reaction temperature can be adjusted approximately 5℃ below average Tm of primers and probe.

c. If RT-PCR products longer than 1kb, an additional extension step with 68 ℃ is suggested to added in cycle step. The extension time is calculated based on the size of the amplified fragment, generally 1 kb/min.