One Step RT-qPCR Probe Kit v2

REF：EG22109S

Storage Condition

Store at -20℃

Components

Component	Amount
RTQ Enzyme Mix	250 μl
2×RTQ Reaction Buffer	3×1 ml

Note: This product does not contain the ROX reference dye. If needed, it is recommended to use ROX Reference Dye (REF: CP18103).

Description

One Step RT-qPCR Probe Kit v2 is a kit for multiple reverse transcription - fluorescent quantitative PCR reactions using RNA as a template. During the experiment, reverse transcription and quantitative PCR are performed in the same reaction tube, which simplifies the experimental operation and reduces the risk of contamination.

The kit contains thermo-stable Reverse Transcriptase to efficiently synthesize the first-strand cDNA, and contain hot-start Ab-Taq DNA Polymerase to quantitative amplification, making it suitable for probe-based qPCR. The product already includes all components for reverse transcription - fluorescent quantitative PCR, excluding primers and sample RNA, including RNase inhibitors, RTase, and Ab-Taq. It can reduce operation steps, shorten sample addition time, and lower the risk of contamination.

Equipment selection

Does not require Rox reference:

Bio-Rad、Roche、Eppendorf、Takara、Qiagen、HongShi、TianLong、Bioer、YaRui;

Requires addition of High Rox:

ABI 5700, 7000, 7300, 7700, 7900HT Fast, StepOne™, StepOne Plus™;

Requires addition of Low Rox:

ABI 7500, 7500 Fast, ViiA™7, QuantStudio™ 3 and 5, Stratagene MX3000P™, MX3005P™, MX4000P™.

Protocol

1. Notes

① It should be thaw out at room temperature before use, mixed well and centrifuged briefly.

② Bubbles should be avoided in the reaction solution.

③ The sample RNA should be fresh, and the RNase contamination should be strictly prevented during the extraction process.

2. Prepare the following mixture in a qPCR tube

Reagent	Amount	Final concentration
RTQ Enzyme Mix	1 μl	/
2×RTQ Reaction Buffer	10 μl	/
Forward primer (10 μM)[a]	0.4 μl	0.2 μM
Reverse primer (10 μM)[a]	0.4 μl	0.2 μM
TaqMan probe (5 μM)[b]	1 μl	0.25 μM
RNA template[c]	1 pg~1 μg	/
Nuclease-Free Water	To 20 μl	

a. Commonly used primer concentration: 0.2 μM final. Adjust between 0.1~1 μM. The primer length should be set at 18~25 bp, with a GC content of 40%~60%.

b. The final concentration of the probe is recommended to be 0.25 μM. Adjusted between 0.1~1 μM.

c. Suggested template volume: 1~5 μl. qPCR has extremely high sensitivity, and it is recommended to dilute the template to control the Ct value between 20~35.

d. Please prepare it inside the clean bench and use nuclease-free pipette tips and reaction tubes; it is recommended to use pipette tips with filters. Avoid cross-contamination and aerosol contamination.

3. Program (Adjust according to the instrument)

Step	Temperature	Time	
Reverse Transcription	50℃	10 min	
Pre-denaturation	97℃	1 min	
Denaturation	97℃	5 s	40~45 Cycles
Annealing & Extension	58℃	30 s	

Notice

1. Please use RNase-free consumables during the experiment.

2. For your safety and health, please wear lab coats and disposable gloves for operation.

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FAQ & Troubleshooting

Problem	Possible Reason	Solution
Disordered or missing amplification curves	Incorrect instrument settings	Adjust settings according to the instrument manual
	Improper primer or template concentration	Adjust primer and template concentrations
	Inappropriate PCR reaction conditions	Reduce annealing temperature, extend extension time, etc. For target fragments with high GC content, consider extending the denaturation time appropriately
	Primers or templates with complex secondary structures	Redesign primers
	Poor sample purity	Further purify the sample
Poor reproducibility of quantitative values	The instrument settings are incorrect	Adjust settings according to the instrument manual
	Poor sample purity	Further purify the sample
	Improper primer concentration	Try increasing the primer concentration appropriately
	Inappropriate PCR reaction conditions	Try reducing the annealing temperature, extending the extension time, etc
	Inappropriate primer design	Redesign primers, Reduce the complex secondary structure of the target fragment
	Experimental operational errors	Strictly follow the operating procedures to ensure accurate volumes of each component in the reaction system
Signal from blank control	Contamination has occurred	Change water, primers, pipette tips, and PCR tubes one by one to find and eliminate the contaminant source. Open a fresh tube of Mix if necessary.

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