Fast One Step Probe RT-qPCR Kit

Cat. No. : RK20413

Product components

Components	Component number	Size-1 100 RXN (25 μL/RXN)	Size-2 500 RXN (25 μL/RXN)
Fast One Step RT-qPCR Probe Buffer VI*	RM21485	400 μL	1 mL*2
Fast One Step Probe Enzyme Mix**	RM21484	100 μL	500 μL

***Note: Contains dNTP/dUTP Mix, and the addition of UDG prevents false positives caused by cross-contamination.**

****Note: Anti-DNA polymerase antibody was used, employing a hot-start system, containing RNase Inhibitor, and Heat-labile UDG.**

Product Description

The Fast One Step Probe RT-qPCR Kit is a reagent kit designed for rapid amplification quantitative RT-qPCR detection using the probe method. It includes reaction buffer, hot-start Taq polymerase, novel reverse transcriptase ABScript III Reverse Transcriptase, and heat-labile UDG. This kit utilizes RNA as a template and includes gene-specific primers, enabling consecutive reverse transcription and PCR reactions to take place in the same tube without the requirement for additional pipetting or tube opening steps. This streamlined process significantly enhances throughput. The kit utilizes dUTP/UDG to prevent contamination, with the heat-sensitive UDG rapidly degrading uracil-containing contaminants at room temperature. At 50℃ during reverse transcription, the heat-labile UDG quickly deactivates, ensuring it does not affect the efficiency and sensitivity of RT-qPCR.

Storage

-20℃

Notes

1. Fast One Step RT-qPCR Probe Buffer VI should be aliquoted before storage. Avoid repeated freeze-thaw cycles during use. Thoroughly thaw and mix before use, and store away from direct sunlight. When preparing multiple One Step RT-qPCR reactions simultaneously, prepare a mixture of all reagents and then aliquot into each reaction tube to minimize reagent loss.

2. Prior to use, gently mix the Fast One Step Probe Enzyme Mix to avoid excessive bubble formation from vigorous shaking. It is recommended to aliquot and store the enzyme mix. Before pipetting, briefly centrifuge to collect at the bottom of the tube, and return to -20℃ immediately after use.

3. Use uncontaminated pipette tips and microtubes for preparing and aliquoting reaction mixtures to minimize contamination.

4. To ensure successful reactions, high-quality RNA templates are recommended.

5. This kit is compatible only with specific primers and cannot be used with random primers or Oligo dT primers for reverse transcription.

6. For one-step RT-qPCR experiments, primer lengths for amplification are recommended to be between 70-200 bp for optimal results.

Operation Description

Experimental Preparation

1. RNase-free 1.5 mL EP tubes, RNase-free PCR tubes, pipettes and tips, icebox or ice.

2. PCR probes, primers, and templates.

3. Fluorescent quantitative PCR-specific tubes or plates.

Experimental Procedure

Users need to prepare their own reagents: RNA template, primers, probes.

E-mail:info@abclonal.com

Please follow the experimental procedures as specified in the user manual of the respective brand of fluorescent quantitative PCR instrument.

1. Recommended System

Components	25 μL
Fast One Step RT-qPCR Probe Buffer VI	4 μL
Fast One Step Probe Enzyme Mix	1 μL
Upstream/Downstream Primers, Probe	Y μL*
Template	X μL
Nuclease-free H2O	to 25 μL

Note: 1) The amount of primers/probe needs to be titrated for the desired concentration for fast programs, which may differ from standard reactions. The optimal concentration range for primers and probes in the FAM channel is 0.16-0.32 μM, and for the VIC/ROX/TAMARA channels, the concentration range is 0.32-0.48 μM.

2) For reaction volumes of 30 μL and 50 μL, amplification can be carried out on machines such as ABI StepOne Plus, ABI 7500, and Bio-rad CFX-96 according to the corresponding proportions.

3) For the recommended 30 μL reaction volume on Chinese domestic instrument AGS8830, adjust the volume of Fast One Step Probe Enzyme Mix to 2 μL.

2. Recommended One Step RT-qPCR Reaction Program

Step	Temp	Time	Cycles
Reverse Transcription	50 ℃	2 min	1
Pre-denaturation	95 ℃	2 s	1
Cycle Reaction	95 ℃	10 s	
	60 ℃	5-20 s*	41

***Note: Please adjust the extension time according to the minimum time required for data collection by the Real-Time PCR instrument you are using. Set to 31 s when using ABI 7300 and 7500. The extension time for other instruments needs to be tested according to the instrument's specifications.**

Note: This product is also compatible with the conventional amplification program: Reverse Transcription at 50°C for 5 min, Pre-denaturation at 95°C for 3 min, Denaturation at 95°C for 15 s, and Extension at 60°C for 30 s.

3. After the reaction, confirm the Real-Time PCR amplification curve and proceed with standard curve construction, etc.

E-mail:info@abclonal.com