AccurSTART U+ One Step RT-qPCR Probe Kit (FOR FAST)

Q231

Version 24.1

Product Description

AccurSTART U+ One Step RT-qPCR Probe Kit (FOR FAST) is a probe-based RT-qPCR fast detection kit suitable for single-plex or multi-plex quantitative PCR using RNA as a template (such as RNA virus). This product uses a new generation of antibody-modified Taq DNA Polymerase and one-step dedicated Reverse Transcriptase, with an optimized buffer for rapid amplification, which has faster amplification speed, higher amplification efficiency and specificity. It supports balanced amplification in both single-plex and multi-plex of low and high concentration samples in a short time. In addition, the dUTP/UDG anti-contamination system is introduced in it, which can work at room temperature to eliminate the influence of amplification product contamination on qPCR and ensure the accuracy of results.

Components

	Components	Q231-01 200 rxns	Q231-02 1,000 rxns	Q231-03 10,000 rxns
☐	RNase-free ddH_2O	3 × 1 ml	15 ml	150 ml
■	5 × One Step U+ Mix[a]	800 µl	4 × 1 ml	40 ml
■	One Step U+ Enzyme Mix[b]	200 µl	1 ml	10 ml
	50 × ROX Reference Dye 1[c]	80 µl	400 µl	4 × 1 ml
	50 × ROX Reference Dye 2[c]	80 µl	400 µl	4 × 1 ml

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

b. It contains Reverse Transcriptase, RNase inhibitor, Heat-labile UDG, and *Taq* DNA Polymerase.

c. It is used to correct the error of fluorescence signals between wells. Use 50 × ROX Reference Dye 1 for ABI 7900HT/7300 Real-Time PCR System and StepOnePlus; Use 50 × ROX Reference Dye 2 for ABI 7500, 7500 Fast Real-Time PCR System, Stratagene Mx3000P. Don't use ROX for Roche and Bio-Rad Real-Time PCR instruments.

Storage

Store at -30 ~ -15°C and ship at ≤0°C.

Applications

It is applicable for detection of various RNA nucleic acids of animals, plants and microorganisms (viruses, etc.).

Notes

1. One Step U+ Enzyme Mix contains high concentration of glycerol. Please centrifuge briefly and mix gently before use.
2. To avoid contamination, please use RNase-free tips and EP tubes.

Experiment Process (Using ABI QuantStudio 3 as a test machine)

1. Prepare the reaction solution in an RNase-free PCR tube as follows:

Components	Volume	
RNase-free ddH_2O	to 20 μl	☐
5 × One Step U⁺ Mix	4 μl	■
One Step U⁺ Enzyme Mix	1 μl	■
50 × ROX Reference Dye 2	0.4 μl	
Primer Forward (10 μM)	0.4 μl	
Primer Reverse (10 μM)	0.4 μl	
TaqMan Probe (10 μM)	0.2 μl	
Template RNA	Total RNA: 1 pg - 1 μg	

For each component, the recommended volume can be adjusted as follows:

▲ Generally, the final concentration of primer should be 0.2 μM. If necessary, it can be adjusted in the range of 0.1 - 1.0 μM.

▲ The final concentration of TaqMan Probe can be adjusted between 50 - 250 nM.

▲ The accuracy of template volumes have significant impacts on the qPCR results, due to the high sensitivity of qPCR. Therefore, to improve the experimental repeatability, it is recommended to dilute the template (e.g. diluted to 2 - 5 μl/sample) to the reaction system.

▲ The size of the amplified products should be within the range of 80 - 200 bp.

2. Reaction program

Fast program

Select "**Fast**" mode under "Run mode" on the "Experiment Properties" interface.

Stage 1	Reverse Transcription	Rep: 1	55°C	2 min	3.19°C/sec
Stage 2	Initial Denaturation	Rep: 1	95°C	2 sec	3.19°C/sec
Stage 3	Cycles	Reps: 40	95°C	1 sec	3.19°C/sec
			60°C	10 sec	2.45°C/sec

▲ Please adjust the reaction time and temperature of each step according to the actual used Real-Time PCR instrument and self requirement.

Standard program

Stage 1	Reverse Transcription	Rep: 1	55°C	15 min	1.6°C/sec
Stage 2	Initial Denaturation	Rep: 1	95°C	30 sec	1.6°C/sec
Stage 3	Cycles	Reps: 45	95°C	10 sec	1.6°C/sec
			60°C	30 sec	1.6°C/sec

3. Confirm the amplification curve of Real-Time PCR and draw a standard curve, etc.