AccurSTART U+ One Step RT-qPCR Probe Kit (Glycerol-free)

QL227

Version 24.1

Product Description

AccurSTART U+ One Step RT-qPCR Probe Kit (Glycerol-free) is a glycerol-free reagent using RNA as a template (such as RNA virus), which is suitable for the development and design of lyophilized products. This product integrates the excellent performance of one-step dedicated Reverse Transcriptase and hot-start Champagne Taq DNA Polymerase with optimized buffer. Therefore, it has superior amplification efficiency, specificity and amplification balance for templates with different concentrations, and is compatible with different lyophilization processes. 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	QL227-01 200 rxns	QL227-02 1,000 rxns	QL227-03 5,000 rxns
☐	RNase-free ddH_2O	3 × 1 ml	15 ml	75 ml
■	5 × One Step U+ Mix[a]	800 µl	4 × 1 ml	20 ml
■	One Step U+ Enzyme Mix (Glycerol-free)[b]	200 µl	2 × 500 µl	5 ml
	50 × ROX Reference Dye 1[c]	80 µl	400 µl	2 × 1 ml
	50 × ROX Reference Dye 2[c]	80 µl	400 µl	2 × 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, and 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, microorganisms (viruses, etc.).

Notes

1. 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. Mix the following components in an RNase-free centrifuge tube:

Components	Volume	
RNase-free ddH_2O	to 20 μl	☐
5 × One Step U⁺ Mix	4 μl	■
One Step U⁺ Enzyme Mix (Glycerol-free)	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	

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

▲ When the mix is used for lyophilization, a lyoprotectant must be added, otherwise the lyophilization will be affected.

▲ Generally, a good result can be obtained when the final concentration of primer in the reaction system is 0.2 μM. If the result is not as expected, the primer concentration can be adjusted between 0.1 - 1.0 μM.

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

▲ Due to the high sensitivity of qPCR, the accuracy of template volume has a significant impact on qPCR results. In order to effectively improve the repeatability of the experiment, it is recommended to dilute the template (e.g., dilute to 2 - 5 μl/sample).

▲ The size of the amplification product should be within the range of 80 - 200 bp.

2. Reaction Program

Standard program

Stage 1	Reverse Transcription	Rep: 1	50°C[a]	15 min
Stage 2	Initial Denaturation	Rep: 1	95°C	30 sec
Stage 3	Cycles	Reps: 45	95°C	10 sec
			60°C	30 sec

Fast program

Stage 1	Reverse Transcription	Rep: 1	50°C[a]	5 min
Stage 2	Initial Denaturation	Rep: 1	95°C	30 sec
Stage 3	Cycles	Reps: 40	95°C	5 sec
			60°C	15 sec[b]

a. For templates with complex secondary structure or high GC content, the temperature of reverse transcription can be increased to 55°C, which will improve the amplification efficiency and sensitivity.

b. The reaction time, heat and cooling speed of each stage in fast program should be adjusted according to the Real Time PCR instrument actually used and your own needs.

3. Confirm the curve of Real Time PCR after the reaction is completed, and plot a standard curve.