AccurSTART U+ One Step RT-qPCR Super PreMix (ONE TUBE)

Q621

Version 24.1

Product Description

AccurSTART U+ One Step RT-qPCR Super PreMix (ONE TUBE) is a one-tube probe-based RT-qPCR master mix suitable for single or multiplex quantitative PCR detection using RNA as a template (such as RNA virus). The enzyme in this master mix uses a hot-start technology based on enzyme modification, with a new generation of high-affinity Taq enzyme antibodies, to achieve efficient blocking and precise release. Combined with one-step dedicated Reverse Transcriptase and optimized buffer, it supports pre-mixing of primers and probes in advance and the mixture can be stored for a long time at low temperature. The sample to be tested can be added directly when using, no additional tube opening/pipetting operation. This product is also compatible with fast programs, which greatly shortens the detection 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	Q621-01 200 rxns (20 μl/rxn)	Q621-02 1,000 rxns (20 μl/rxn)	Q621-03 10,000 rxns (20 μl/rxn)
RNase-free ddH_2O	1 ml	10 ml	100 ml
U+ One Step RT-qPCR Probe 5 × Master Mix[a]	800 μl	4 × 1 ml	40 ml
50 × ROX Reference Dye 1[b]	80 μl	400 μl	4 × 1 ml
50 × ROX Reference Dye 2[b]	80 μl	400 μl	4 × 1 ml

a. It contains dNTP/dUTP Mix, Mg^{2+}, Reverse Transcriptase, RNase inhibitor, Heat-labile UDG, and Taq DNA Polymerase.

b. 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 and microorganisms (viruses, etc.).

Notes

1. U+ One Step RT-qPCR Probe 5 × Master 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. Mix the following components in an RNase-free centrifuge tube:

Components	Volume
RNase-free ddH_2O	To 20 μl
U$^+$ One Step RT-qPCR Probe 5 × Master Mix	4 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:

▲Generally, the final concentration of primer in the reaction system is 0.2 μM to obtain better amplification effect. When the reaction performance is poor, the primer concentration 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.

▲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. diluted to 2 - 5 μl/sample) and add it to the reaction system.

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

The full premix reaction solution can be prepared according to the following system:

Components	Volume
RNase-free ddH_2O	To 15 μl
U$^+$ One Step RT-qPCR Probe 5 × Master Mix	4 μl
Primer Forward (10 μM)	0.4 μl
Primer Reverse (10 μM)	0.4 μl
Taqman Probe (10 μM)	0.2 μl

▲ The prepared solution can be stored at -20°C for a long time, and the reaction can be started by directly adding 5 μl of template when using.

▲ The solution concentration can be adjusted according to the amount of template added, and increasing the solution concentration can further improve the stability of the full premix.

2. Reaction Program

Standard Program (for the optimal amplification sensitivity)

Stage 1	Reverse Transcription	Rep: 1	55°C	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 (suitable for most One Step qRT-PCR applications)

Stage 1	Reverse Transcription	Rep: 1	55°C	5 min
Stage 2	Initial Denaturation	Rep: 1	95°C	30 sec
Stage 3	Cycles	Reps: 45	95°C	5 sec
			60°C	15 sec[a]

a. Please conduct preliminary experiments for the first attempt to confirm whether the fast program is compatible with the qPCR instrument.

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