

AccurSTART II U⁺ One Step RT-qPCR Super Premix (Fast & One Tube)



Q631

Version 25.1

Product Description

AccurSTART II U⁺ One Step RT-qPCR Super Premix (Fast & One Tube) is a single-tube probe-based RT-qPCR dedicated premix that is designed for single or multiplex qPCR detection using RNA (e.g. RNA viruses) as a template. This premix employs enzyme-engineered hot-start technology, coupled with an upgraded high-affinity Taq polymerase antibody, ensuring efficient enzyme inactivation and precise activation. It also features a thermostable Reverse Transcriptase and an improved chemical buffer system, allowing primers and probes to be pre-mixed in advance. The prepared working solution can be stably stored at low temperatures for a long time, and samples can be added directly without the need for extra pipetting or tube-opening. The premix is compatible with fast cycling protocols, significantly shortening reaction time and enabling rapid, fully pre-mixed amplification. Additionally, the inclusion of a dUTP/UDG anti-contamination system which is active at room temperature, effectively eliminates carryover contamination from previous amplifications, thereby ensuring the accuracy of test results.

Components

Components	Q631-01 200 rxns (20 µl/rxn)	Q631-02 1,000 rxns (20 µl/rxn)	Q631-03 5,000 rxns (20 µl/rxn)
■ U ⁺ One Step RT-qPCR Probe 5 × Master Mix ^a	800 µl	4 × 1 ml	20 ml
■ 50 × ROX Reference Dye 1 ^b	80 µl	400 µl	2 × 1 ml
■ 50 × ROX Reference Dye 2 ^b	80 µl	400 µl	2 × 1 ml

a. It contains dNTP/dUTP Mix, Mg²⁺, Reverse Transcriptase, RNase inhibitor, Heat-labile UDG, and Taq DNA Polymerase.

b. It is used to normalize fluorescence signal variation between wells. For ABI 7900HT/7300 Real-Time PCR System and StepOnePlus, use 50 × ROX Reference Dye 1. For ABI 7500, 7500 Fast Real-Time PCR System, and Stratagene Mx3000P, use 50 × ROX Reference Dye 2. ROX is not required when using Real-Time PCR instruments from Roche or Bio-Rad.

Storage

Store at -30 ~ -15°C and protect from light. Ship at ≤0°C.

Applications

It is applicable to the detection of various RNA nucleic acids from animals, plants, and microorganisms (including viruses).

Notes

1. U⁺ One Step RT-qPCR Probe 5 × Master Mix contains a high concentration of glycerol. Before use, briefly centrifuge the tube to collect the glycerol at the bottom, then gently pipette up and down to mix thoroughly before accurate pipetting.
2. For the preparation of the reaction solution, use RNase-free pipette tips, EP tube, and other consumables to minimize the risk of contamination and ensure the integrity of target RNA.

Experiment Process (Using ABI QuantStudio 3 as the Testing Instrument)

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

Components	Volume
RNase-free ddH ₂ O	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 can be adjusted according to the following principles:

- ▲ In general, a final primer concentration of 0.2 µM provides good amplification results. If the amplification is suboptimal, primer concentration can be adjusted within the range of 0.1 - 1.0 µM.
- ▲ The final probe concentration can be adjusted between 50 - 250 nM.
- ▲ qPCR is highly sensitive. Accurate quantification of the template input is essential for reliable quantitative results. It is recommended to dilute the template (e.g., to 2 - 5 µl per sample) before adding it to the reaction mix, which can effectively improve the reproducibility of the experiment.
- ▲ The recommended amplicon length is between 80 - 200 bp.

2. The pre-mixed working solution can be prepared according to the following formulation.

Components	Volume
RNase-free ddH ₂ O	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 working solution can be stored at -20°C for long-term storage. To start the reaction, simply add 5 µl of template.
- ▲ The working solution volume multiplier can be adjusted according to the amount of template added. Increasing the multiplier can further enhance the stability of the premixed solution.

3. Reaction Program

Standard Program

Stage	Program	Reps	Temp	Time	Ramp Rate
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	Cycling Reaction	Reps: 45	95°C	10 sec	1.6°C/sec
			60°C	30 sec	1.6°C/sec

Fast Program

On the "Experiment Properties" interface, select the "Fast" mode under "Run Mode". The amplification program is as follows:

Stage	Program	Reps	Temp	Time	Ramp Rate
Stage 1	Reverse Transcription	Rep: 1	55°C	2 min	3.19°C/sec ^a
Stage 2	Initial Denaturation	Rep: 1	95°C	30 sec	3.19°C/sec
Stage 3	Cycling Reaction	Reps: 45	95°C	1 sec	3.19°C/sec
			60°C	10 sec ^b	2.45°C/sec ^a

a. The ramp rate can be adjusted according to the maximum limitation of the instrument.

b. The extension time can be adjusted based on the instrument's fastest capability. An extension time of at least 5 sec is recommended.

4. After the reaction, verify the amplification curves and proceed with standard curve generation and other analyses.

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