

HiScript II U⁺ One Step qRT-PCR Probe Kit

Q223

Version 24.1



Product Description

HiScript II U⁺ One Step qRT-PCR Probe Kit is specially designed for qPCR detection using RNA (e.g., RNA virus) as templates. Using gene specific primers (GSP), the reverse transcription and qPCR can be finished in one tube. No additional opening/pipetting operations are required, greatly increasing assay throughput and reducing the risk of contamination. Combining the superior performance of HiScript II Reverse Transcriptase and hot-start Champagne Taq DNA Polymerase, equipping with an optimized buffer, the detection sensitivity of HiScript II U⁺ One Step qRT-PCR Probe Kit can reach 0.1 pg of total RNA or <10 copies of RNA templates. This product is provided as master mix. The 2 × One Step U⁺ Mix contains an optimized buffer and dNTP mix, and is suitable for high-specificity detection systems based on fluorescence labelled probes (e.g., TaqMan). 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		Q223-01 250 rxns (20 µl/rxn)
<input type="checkbox"/>	RNase-free ddH ₂ O	2 × 1.25 ml
<input checked="" type="checkbox"/>	2 × One Step U ⁺ Mix ^a	2 × 1.25 ml
<input checked="" type="checkbox"/>	One Step U ⁺ Enzyme Mix ^b	250 µl
	50 × ROX Reference Dye 1 ^c	100 µl
	50 × ROX Reference Dye 2 ^c	100 µl

a. It contains dNTP/dUTP Mix and Mg²⁺.

b. It contains HiScript II Reverse Transcriptase, RNase inhibitor, Heat-labile UDG, and Champagne 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 and microorganisms (viruses, etc.).

Notes

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

Experiment Process (Using ABI StepOne Plus as a test machine)

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

Components	Volume
RNase-free ddH ₂ O	to 20 µl □
2 × One Step U ⁺ Mix	10 µl ■
One Step U ⁺ Enzyme Mix	1 µl ■
50 × ROX Reference Dye 1	0.4 µl
Gene Specific Primer Forward (10 µM)	0.4 µl
Gene Specific 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 and add it to the reaction system (e.g., dilute to 2 - 5 µl/sample).
- ▲ The size of the amplified products should be within the range of 80 - 200 bp.

2. Run the One Step qRT-PCR program as follows:

Standard Program (to achieve the highest amplification sensitivity)

Stage 1	Reverse Transcription	Rep: 1	55°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 ^b

Fast Program (suitable for most One Step qRT-PCR amplifications)

Stage 1	Reverse Transcription	Rep: 1	55°C ^a	5 min
Stage 2	Initial Denaturation	Rep: 1	95°C	30 sec
Stage 3	Cycles	Reps: 45	95°C	5 sec
			60°C	20 sec ^c

- 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. Please adjust the extension time according to the minimum time limit of data acquisition required by the Real-time PCR instrument used: For ABI 7700 and 7900HT, the extension time should be ≥30 sec; for ABI 7000 and 7300, the extension time should be ≥31 sec; for ABI 7500, the extension time should be ≥34 sec.
- c. Please check if the fast program is compatible with the qPCR instrument.

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

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