HiScript II U+ One Step qRT-PCR Probe Kit

Catalog # Q223-01

Version 5.1

Vazyme biotech co., ltd.

1. Introduction

HiScript II U+ One Step qRT-PCR Probe Kit is designed for quantitative PCR with RNA (such as RNA virus) as templates. Both cDNA synthesis and PCR are performed in a single tube using gene-specific primers (GSP), without extra opening pipes and pipetting, thus it increases the quantification throughput and minimizes the chance of contamination. The kit introduces dUTP/UDG anti-contamination system. Heat-labile UDG can degrade contamination with U rapidly at room temperature and will be inactivated at 55℃ during reverse transcription, without affecting efficiency and sensitivity of RT-PCR. Integrating the advantages of HiScript II Reverse Transcriptase, Champagne Taq DNA Polymerase and optimized buffer system, One Step qRT-PCR Probe Kit enables highly sensitive detection as low as 0.1 pg of total RNA or RNA with less than 10 copies. The kit is provided as convenient Master Mix. 2×One-Step U+ Mix contains optimized buffer, dNTP/dUTP Mix, and detection system for fluorescent probes (such as TaqMan®). One-Step U+ Enzyme Mix contains proportion-optimized HiScript II Reverse Transcriptase, RNase inhibitor, Heat-labile UDG and Champagne Taq DNA Polymerase.

2. Package Information

Components	Q223-01 100 rxn (50 μl/rxn)
RNase free ddH_2O	1.25 ml × 2
2 × One Step U$^+$ Mix [a]	1.25 ml × 2
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. Contains dNTP/dUTP Mix and Mg^{2+}

b. Contains HiScript II Reverse Transcriptase, RNase inhibitor, Heat-labile UDG and Champagne Taq DNA Polymerase

c. Used to calibrate the error of fluorescence signals between different wells. Use 50×ROX Reference Dye 1 for ABI 7900HT/7300 Real-Time PCR System and StepOne Plus™. Use 50×ROX Reference Dye 2 for ABI 7500, 7500 Fast Real-Time PCR System and Stratagene Mx3000P. The ROX is not needed for Roche or Bio-Rad Real-Time PCR instruments.

3. Storage

Stored at -20℃.

4. Application examples (The tested instrument is ABI StepOnePlus™)

1. Assemble reactions in an RNase free tube as follows:

RNase free ddH_2O	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) [a]	0.4 μl
Gene Specific Primer Reverse (10 μM) [a]	0.4 μl
TaqMan Probe (10 μM) [b]	0.2 μl
Template RNA [c]	Total RNA: 0.1 pg-1 μg

Notes: The volume of the reagents can be adjusted according to the following principles.

a. The final concentration of primers is recommended to be 0.2 μM. If it doesn't work well, adjust the concentration between 0.1 μM-1.0 μM.

b. Final concentration of probe can be adjusted between 50 nM-250 nM.

c. The qPCR test is very sensitive, and the accuracy of the addition of template volume dramatically affects the final quantification results. It is recommended to dilute the template (e.g. dilute to 2-5 μl/sample) before addition to improve the reproducibility.

d. The length of amplicon should be 80 bp-200 bp.

2. Perform One Step qRT-PCR using the following cycling conditions:

Stage 1	Reverse transcription	Reps: 1	55 ℃	15 min
Stage 2	Pre-denaturation	Reps: 1	95 ℃	5 min
Stage 3	Cycling reaction	Reps: 45	95 ℃	10 sec
			60 ℃	30 sec *

*Extension time should be adjusted according to the limited collecting time of qPCR instruments: at least 30 sec for ABI 7700 and 7900HT; at least 31 sec for ABI 7000 and 7300; at least 34 sec for ABI 7500.

3. Please check Real Time PCR amplification curves and make standard curve. Don't detect by electrophoresis to avoid product contamination.

6. Notes

1. One Step U+ Enzyme Mix contains high-concentration of glycerol. Please collect the liquid by a brief centrifugation before pipetting. Mix thoroughly by pipetting up and down and then absorb the accurate volume.
2. To avoid RNase contamination, please use RNase-free tubes and tips.