

Introduction

LiScript™ One Step Probe RT-qPCR Kit is specially designed for qPCR that directly use RNA (e.g. virus RNA) as template. Using gene specific primers (GSP), the reverse transcription and qPCR can be finished in one tube, significantly reducing pipetting procedures and the risk of contamination. This kit can prevent the contamination of PCR product. Combining the superior performance of LiScript™ Reverse Transcriptase and hot-start LiTaq DNA Polymerase, with an optimized buffering system, the detection sensitivity of LiScript™ One Step qRT-PCR Probe Kit can reach 0.1 pg of total RNA or less than 10 copies of RNA templates and has higher thermal stability. LiScript™ One Step qRT-PCR Probe Kit is provided in Master Mix. The 5 × One Step Mix contains an optimized buffer and dNTP Mix, and is suitable for high-specificity detection systems based on fluorescence labelled probes.

Package Information

Components	M0033
RNase-free ddH ₂ O	2× 1 ml
5× One Step Mix	600 µl
One Step Enzyme Mix	150 µl
50× ROX Reference Dye 1*	60 µl
50× ROX Reference Dye 2*	60 µl

* Used to rectify the error of fluorescence signals between different 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

All materials should be stored at -20°C.

Notes

- One Step 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.

Protocol

1. Prepare the reaction solution in a RNase-free PCR tube as follows:

5× One Step Mix	6 µl
One Step Enzyme Mix	1.5 µl
50× ROX Reference Dye 1	0.6 µl
Forward Primer (10 µM)	0.6 µl
Reverse Primer (10 µM)	0.6 µl
TaqMan Probe (10 µM)	0.3 µl
Template RNA	Total RNA: 1 pg - 1 µg
RNase-free ddH ₂ O	to 30 µl

Note: For each component, the recommended volume can be adjusted as follows:

Generally, the final concentration of primer should be 0.2 µM. If necessary, it 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.

The accuracy of template volumes have significant impacts on the qPCR results, due to the high sensitivity of qPCR. Therefore, to improve the experimental repeatability, it is recommended to dilute the template and pipet more volume (e.g. diluted to 2 - 5 µl/sample) to the reaction system.

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

2. Place the sample in a qPCR instrument and run the following program for One Step qRT-PCR:

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 60°C	10 sec 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 60°C	5 sec 20 sec***



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LiScript™ One Step Probe RT-qPCR Kit

Cat. #: M0033 Size: 100 rxns

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

** The extension time varies between different qPCR instruments 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; and for ABI 7500, ≥ 34 sec;

*** Please check if the fast program is compatible with the qPCR instrument.

3. Data analysis of the Real Time PCR amplification curve and the standard curve, etc.