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LiScript™ One Step Green RT-qPCR Kit

Cat. #: M0032 Size: 250 rxns

Introduction

LiScript™ One Step Green RT-qPCR Kit is specially designed for qPCR detection using RNA (e.g., RNA virus) as templates. Reverse transcriptionand qPCR can be finished in one tube in the presence of Gene Specific Primers (GSP). There is no additional opening/pipetting operations are required, which greatly increasing assay throughput and reducing the risk of contamination. Intergrating the superior performance of LiScript™ Reverse Transcriptase and LiTaq plus DNA Polymerase with the optimized buffer, the lowest RNA template for detection of this kit is 0.1 pg. The specificity-enhancing factor existing in the buffer can effectively reduce the formation of primer dimers and improve the specificity of product. The kit is provided as the formation of master mix, which is convenient to use.

Package Information

Components	M0032
RNase-free ddH_2O	2× 1.25 ml
2× One Step Green Mix	2× 1.25 ml
One Step Green Enzyme Mix	250 µl
50× ROX Reference Dye 1*	100 µl
50× ROX Reference Dye 2*	100 µl

* 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

All materials should be stored at -20°C.

Notes

1. The One Step Green Enzyme Mix contains high concentration of glycerol. Please centrifuge briefly and mix gently before use.
2. When using 2× One Step Green Mix, please mix thoroughly and pipett accurately, avoid strong light exposure, and protect from light.
3. To avoid contamination, please use RNase-free tips and EP tubes.

Protocol

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

2× One Step Green Mix	10 µl
One Step SYBR Green Enzyme Mix	1 µl
50× ROX Reference Dye 1	0.4 µl
Primer 1 (10 µM)	0.4 µl
Primer 2 (10 µM)	0.4 µl
Template RNA	Total RNA: 1 pg - 1 µg
RNase-free ddH_2O	to 20 µl

Note: The volume of each component in the reaction can be adjusted according to the following principles:

Generally, a good result can be obtained when the final concentration of primer in the reaction system is 0.2 µM. If the result is not as expected, the primer concentration can be adjusted between 0.1 - 1.0 µM.

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., dilute to 2 - 5 µl/sample).

The length of the amplification product should be within the range of 100 - 500 bp, especially 100 - 200 bp.

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

Stage 1	Reverse Transcription	Rep: 1	50°C*	3 min**
Stage 2	Initial Denaturation	Rep: 1	95°C	30 sec
Stage 3	Cycling Reaction	Reps: 40	95°C	10 sec
			60°C	30 sec***
Stage 4	Melting Curve	Rep: 1****	95°C	15 sec
			60°C	60 sec
			95°C	15 sec

* 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.

** Reverse transcription can be extended to 15 min, which will increase the yield of cDNA.

*** Please adjust the extension time according to the minimum time limit of data acquisition required by the Real-time PCR instrument: 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.

**** Different instruments have different melting curve acquisition procedures. Just use the default melting curve acquisition program.

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

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