HiScript II One Step qRT-PCR Probe Kit

Q222

Version 22.1

Product Description

HiScript II One Step qRT-PCR Probe Kit is specially designed for qPCR detection using RNA (e.g., RNA virus) as templates. Reverse transcription and 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 increase assay throughput and reduce the risk of contamination. Intergrating the superior performance of HiScript II Reverse Transcriptase and hot-start Champagne Taq DNA Polymerase with the optimized buffer, the lowest RNA template for detection of this kit is 0.1 pg or 10 copies. This kit is provided as the formation of master mix. The 2 × One Step Mix contains an optimized buffer and dNTP mix, which is suitable for high-specificity detection systems that based on fluorescence labeled probes (e.g., TaqMan).

Components

	Components	Q222-01 250 rxns (20 μl/rxn)
☐	RNase-free ddH_2O	2 × 1.25 ml
■	2 × One Step Q Probe Mix[a]	2 × 1.25 ml
■	One Step Q Probe 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 mix and Mg^{2+}.

b. It contains HiScript II Reverse Transcriptase, RNase inhibitor 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. Do not use ROX for Roche and Bio-Rad Real-Time PCR instruments.

Storage

Store at -30 ~ -15℃ and transport at ≤0℃.

Applications

It is applicable for detection of various RNA nucleic acids of animals, plants and microorganisms (viruses, etc.).

Notes

For research use only. Not for use in diagnostic procedures.

1. One Step Q Probe Enzyme Mix contains high concentration of glycerol. Please centrifuge briefly and mix gently before use.

2. To avoid contamination, please use RNase-free tips and EP tubes.

Experiment Process (Using ABI StepOne Plus)

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

RNase-free ddH_2O	to 20 μl	☐
2 × One Step Q Probe Mix	10 μl	■
One Step Q Probe 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	
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, 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.

▲ 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 add the template to the reaction system after dilution (e.g., dilute to 2 - 5 μl/sample).

▲ The size of the amplification product should be within the range of 80 - 200 bp.

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

Standard Program (for the optimal amplification sensitivity)

Stage 1	Reverse Transcription	Rep:1	50℃[a]	15 min
Stage 2	Initial Denaturation	Rep:1	95℃	30 sec
Stage 3	Cycling Reaction	Reps: 45	95℃	10 sec
			60℃	30 sec[b]

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

Stage 1	Reverse Transcription	Rep:1	50℃[a]	5 min
Stage 2	Initial Denaturation	Rep:1	95℃	30 sec
Stage 3	Cycling Reaction	Reps: 45	95℃	5 sec
			60℃	20 sec[c]

a. For templates with complex secondary structure or high GC content, the temperature of reverse transcription can be increased to 55℃, 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: 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. To achieve the best results, it is strongly recommended to do a pilot experiment to determine whether the fast program is suitable for the qPCR instrument or not.

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