ACE Script II One Step qRT-PCR Kit (Probe)

Cat# EP2026

Storage: All components should be stored at -20℃.

INTRODUCTION

The ACE Script II One Step qRT-PCR Kit (Probe) is specially designed for qPCRs that directly use RNA as templates. The reverse transcription and PCR can be finished in one tube, significantly reducing pipetting procedures and the risk of contamination. The ACE Script II Reverse Transcriptase and hot-start Champagne Taq DNA Polymerase contained this kit enables high-sensitive total RNA detection (as little as 1 pg). The ACE Script II One Step qRT-PCR Kit (Probe) is a master mix system. The 2× One Step Q Probe Mix contains an optimized buffer and dNTP/dUTP mix and is suitable for high-sensitive detection systems based on fluorescence labelled probes(i.e. TaqMan).

CONTENTS

Component	EP2026 250 rxn (20 µl/rxn)
RNase free ddH2O	1.25 ml × 2
2× One Step Q Probe Mix [a]	1.25 ml × 2
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. contains dNTP Mix and Mg^{2+} .

b. contains HiScript II Reverse Transcriptase, RNase inhibitor, and Champagne Taq DNA Polymerase.

c. Used to rectify the error of fluorescence signals between different wells. Use 50× ROX Reference Dye 1 for ABI 7900HT/ 7300 Real-Time PCR and System and StepOne Plus™ ; Use 50× ROX Reference Dye 2 for ABI 7500, 7500 Fast Real-Time PCR System and Stratagene Mx3000P. Don't use ROX for neither Roche nor Bio-Rad Real-Time PCR instruments.

STORAGE

All components should be stored at -20℃

PROTOCOL (Using ABI StepOne Plus ™)

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

RNase free ddH2O	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
Gene Specific Primer Forward (10 µM) a	0.4 µl
Gene Specific Primer Reverse (10 µM)	0.4 µl
TaqMan Probe (10 µM) b	0.2 µl
Template RNA c	Total RNA: 1 pg-1 µg

Note: For each component, the volume of can be adjusted according to the following principle:

a. The final concentration of primer is usually 0.2 µM, and if necessary, it can be adjusted between 0.1 µM and 1.0 µM.

b. The final concentration of TaqMan probe can be adjusted between 50 nM and 250 nM.

c. The accuracy of template volumes impacts significant impacts on the qPCR results, due to the high sensitivity this kit. Therefore, to improve experimental repeatability, it is recommended to dilute the template and pipet more volumn to the reaction system.

d. The size of the amplicon should be within the range of 80 bp-200 bp.

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

Standard Program

Stage 1	Reverse Transcription	Reps: 1	50℃ [a]	15 min
Stage 2	Pre-denaturation	Reps: 1	95℃	30 sec
Stage 3	PCR Cycles	Reps: 45	95℃	10 sec
			60℃	30 sec [b]

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

Stage 1	Reverse Transcription	Reps: 1	50℃	5 min
Stage 2	Pre-denaturation	Reps: 1	95℃	30 sec
Stage 3	PCR Cycles	Reps: 45	95℃	5 sec
			60℃	20 sec [c]

Note:

a. For templates with complex secondary structure or high GC-content, the temperature can be increased to 55℃, which will improve the sensitivity and performance.

b. 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; for ABI 7500, ≥ 34 sec.

c. Please check the fast program is compatible for the qPCR instrument.

Tips

1. The 2× One Step Q Probe Enzyme Mix contains glycerol. Before pipetting, please collect the liquid by a brief

centrifugation.

2. To avoid RNase contamination, please keep the experiment area clean, wear clean gloves and masks, and use RNase-free tubes and tips.

PRODUCT USE LIMITATION

These products are intended for research use only.