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

Cat# EP2028

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

INTRODUCTION

The ACE Script II One Step qRT-PCR Kit (SYBR) is designed for SYBR Green I-based 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 Trancriptase and Champagne Taq DNA Polymerase contained in this kit and an optimized buffer system enables high-sensitive total RNA detection (as little as 1 pg). The ACE Script II One Step qRT-PCR Kit (SYBR) is a master mix system. The 2× One Step SYBR Green Mix contains an optimized buffer, dNTPs, specificityenhancer factors, and SYBR Green I. The One Step SYBR Green Enzyme Mix contains HiScript II Reverse Transcriptase, RNase inhibitors, and Champagne Taq DNA polymerase .

CONTENTS

Component	EP2028 250 rxn (20 µl/rxn)
RNase free ddH2O	1.25 ml × 2
2× One Step SYBR Green Mix [a]	1.25 ml × 2
One Step SYBR Green 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, Specificity-Enhancer Factors, and SYBR Green I.

b. contains ACE Script 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 PlusTM ; 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 PlusTM)

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

RNase free ddH2O	to 20 µl
2× One Step SYBR Green Mix	10 µl
One Step SYBR Green Enzyme Mix	1 µl
50× ROX Reference Dye	0.4 µl
Gene Specific Primer Forward (10 µM) [a]	0.4 µl
Gene Specific Primer Reverse (10 µM)	0.4 µl
Template RNA [b]	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 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 b to the reaction system.

c. The size of the amplicon should be within the range of 100 bp-500 bp or 100 bp-200 bp as preferred

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

Stage 1	Reverse Transcription	Reps: 1	50℃ [a]	3 min [b]
Stage 2	Pre-denaturation	Reps: 1	95℃	30 sec
Stage 3	PCR Cycles	Reps: 40	95℃	10 sec
			60℃	30 sec [c]
Stage 4	Melting Curve	Reps: 1	Default	

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 time for reverse transcription can be extended to 15 min, which will benefit the yield of cDNA.

c. 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.

Tips

1. The One Step SYBR Green Enzyme Mix contains glycerol. Therefore, before pipetting, please collect the liquid by a brief centrifugation.
2. Vortex the 2× One Step SYBR Green Mix before pipetting, and protect it from light.
3. 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.