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Datasheet

Ver.1 Date : 20180222

ACE Script II One Step qRT-PCR Kit

Cat# EP2026 –100 rxn

Storage at -20 ℃ for one year

INTRODUCTION

The ACE Script II One Step qRT-PCR Kit 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 HiScript II Reverse Trancriptase 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 is a master mix system. The 2× One Step Q Probe Mix contains an opimized buffer and dNTP/dUTP mix and is suitable for high-sensitive detection systems based on fluorescence labelled probes (i.e. TaqMan).

CONTENTS

No	Component	**EP2024 – 500 rxn**
AA	RNase free ddH_2O	1.25 ml x 2
AB	2× One Step Q Probe Mix [a]	1.25 ml x 2
AC	One Step Q Probe Enzyme Mix [b]	250 µl
AD	50X ROX Reference Dye 1 [c]	100 µl
AE	50X 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 Step One 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:

2× One Step Q Probe Mix	10 µl
One Step Q Probe Enzyme Mix	1 µl
50X 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
RNase free ddH_2O	To 20 ul

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

a. The final concentration of primer is usually 0.2μM,and if necessary, it can be adjusted between 0.1μM and1.0μM.

b. The final concentration of TaqMan Probe primer 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. Put the sample in a qPCR instrument and run the following program for qPCR:

Standard Program

Stage	Temp.	Time	Cycle
Reverse Transcription	50℃ [a]	15 min	1
Pre-denaturation	95℃	30 s	1
PCR Cycles	95℃	10 s	45
	60℃	30 s [b]	

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

Stage	Temp.	Time	Cycle
Reverse Transcription	50℃	15 min	1
Pre-denaturation	95℃	30 s	1
PCR Cycles	95℃	5 s	45
	60℃	20 s	

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

c. time should be ≥ 31 sec; for ABI 7500, ≥ 34 sec; and for ABI Step One Plus™, ≥ 10 sec.

d. 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.