5X ACEScript II 1st Strand cDNA RT SuperMix (+gDNA wiper)

Cat# EP2015 – 100 rxn

Storage at -20 ℃

INTRODUCTION

The **ACEScript II 1st Strand cDNA RT SuperMix** is specially designed for 2-step RT-qPCR. The 5× SuperMix contains all necessary components needed for reverse transcription, including Buffer, dNTPs, ACEScript II Reverse Transcriptase, RNase inhibitor, and Random primers/Oligo-dT primer mix.

The ACEScript II Reverse Transcriptase, optimized from M-MLV (RNase H-) Reverse Transcriptase, is a new generation reverse transcriptase with highly improved heat stability and cDNA synthesis efficiency. The residual genomic DNA in RNA template can be removed rapidly and completely with the 4X gDNA wiper. The **ACEScript II 1st Strand cDNA RT SuperMix** has been specially optimized for qPCR. For example, the ratio of Random primers/Oligo-dT primer is optimized to enable cDNA synthesis at any region of the template RNA and to ensure the repeatability of qPCR results. The cDNA products are compatible for SYBR- or probe-basded qPCR, such as 2X ACE SYBR® qPCR Master Mix (ACE Biolabs, EP2016) and 2X ACE SYBR® color qPCR Master Mix (ACE Biolabs, EP2019).

CONTENTS

No	Component	**EP2014 – 100 rxn (20µl/rxn)**
DA	5X ACEScript II RT All-Mix[1]	400 µl
DB	4X gDNA wiper	400 µl
DC	5X No RT Control Mix[2]	40 µl
DD	RNase-free ddH_2O	1 ml x 2

1. contains Buffer, dNTP, APOScript Reverse Transcriptase, RNase inhibitor, and Random primers/Oligo dT primer mix.

2. contains no APOScript Reverse Transcriptase, used for control.

ADDITIONAL MATERIALS REQUIRED

1. RNase-free microtube (1.5 ml) or PCR tube (0.2 ml).
2. Thermocycler (PCR instrument) or water bath.
3. Ice bath

PROTOCOL

Note: 1. Use high quality total RNA with high intergrity for reverse transcription.
2. To avoid RNase contamination, please keep the experiment area clean, wear clean gloves and masks, and use RNase-free tubes and tips.

1. **Removal of Genomic DNA :** Mix the following components thoroughly in an RNase-free PCR tube and incubate at 42℃ for 2 min.

4X gDNA wiper	2μl
Template RNA	Total RNA 1 pg-500 ng
RNase-free ddH2O	To 8 **μ**l

2. Mix the following components in a RNase-free PCR tube

5X ACEScript II RT All-Mix	4 μl
mixture of step 1	8 μl
RNase-free ddH2O	8 μl
Total Volume	To 20 **μ**l

No RT Control (Optional): No RT Control is a negative control which contains no Reverse Transcriptase and is used to indicate whether there is residual genomic DNA in RNA template.

3. **Reverse transcription**

Temp.	Time
50℃*	15 min
85℃	2 min

*For templates with complex secondary structure or high GC-content, the temperature can be increased to 55℃, which will benefit the yield.

The products can be used for PCR immediately or be stored at -20℃ for 6 months. However, it is recommended to stored at -80℃ and make aliquots to avoid repeated freezing and thawing.

TIPS

1. Both 5X ACEScript II RT All-Mix and 5× No RT Control Mix contain glycerol. Therefore, before pipetting, please collect the liquid by a brief centrifugation.
2. It is recommended that in a 20 μl reverse transcription reaction system, the amount of total RNA is ≤ 500 ng. However, for target genes with low expression levels, the amount of total RNA can be ≤ 1μg.
3. Use RNase-free water to dissolve total RNA. **DO NOT** use TE, for the EDTA in TE inhibits the reverse transcription reaction.

PRODUCT USE LIMITATION

These products are intended for research use only.