

5X ACEScript II 1st Strand cDNA RT SuperMix

Cat# EP2014 – 100 rxn

Storage at -20 °C

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 **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-based 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)
CA	5X ACEScript II RT All-Mix	400 µl
CB	RNase-free ddH2O	1 ml x 2
CC	5X No RT Control Mix	40 µl

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 integrity 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. 1st-strand cDNA synthesis

Mix the following components in a RNase-free PCR tube

5X ACEScript II RT All-Mix	4 μ l
Template RNA ^c	Total RNA : 1pg-500ng
RNase-free ddH2O	To 20 μ l

* To avoid residual genomic DNA in RNA Template, set a negative control for each experiment.

2. Reverse transcription (Standard or Fast program).

a. Standard program

Temp.	Time
25°C	10 min
50°C*	30 min
85°C	5 min

b. Fast program

Temp.	Time
50°C*	15 min
85°C	2 min

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

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

TIPS

- Both 5X ACEScript II RT All-Mix and 5 \times No RT Control Mix contain glycerol. Therefore, before pipetting, please collect the liquid by a brief centrifugation.
- It is recommended that in a 20 μ l reverse transcription reaction system, the amount of total RNA is \leq 500 ng. However, for target genes with low expression levels, the amount of total RNA can be \leq 1 μ g.
- Use RNase-free water to dissolve total RNA. **DO NOT** use TE, for the EDTA in TE inhibits the reverse transcription reaction.
- If the Ct value difference between No RT Control and Experimental Group is $<$ 5, which indicates that the template RNA has been contaminate by genomic DNA, it is suggested to use **5X ACE Script II 1st Strand cDNA RT SuperMix (+gDNA wiper) (ACE Biolabs, EP2015)** to eliminate genomic DNA in RNA templates.
- For reverse transcription, the Fast Program is suitable for most RT- qPCR. Generally there is no difference between the results of using Fast Program and that of using Standard Program. However, please switch to Standard Program if the amplification efficiency is poor or the Ct value is too high.

PRODUCT USE LIMITATION

These products are intended for research use only.