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Datasheet

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ACE Script II 1st Strand cDNA RT Kit (+gDNA wiper)

Cat# EP2012 50 rxn
EP2013 100 rxn

Storage at -20 ℃ for one year

INTRODUCTION

The ACE Script II Reverse Transcriptase (+gDNA Wiper) is designed for the 1st strand cDNA synthesis with genomic DNA removal treatments. The ACEScript II Reverse Transcriptase is a new generation reverse transcriptase optimized from the M-MLV (RNase H-) Reverse Transcriptase. The half-life of ACE Script II RTase at 50℃ is > 240 min. Even at 55℃, the ACE Script II RTase can stay stable for a long time, which significantly benifits the transcription of RNA templates with complex secondary structures. In addition, the ACE Script II RTase has a improved template affinity and cDNA synthesis efficiency. It has a good resistance to most RT PCR inhibitors and is suitable for long-fragment cDNA amplification (as long as 20 kb).

The residual genomic DNA in RNA template can be removed rapidly and completely after a treatment (42℃ for 2 min) with the 4× gDNA Wiper. The 10× RT Mix contains an optimized buffer and dNTPs. The ACE Script II Enzyme Mix contains the ACE Script II Reverse Transcriptase and the RNase inhibitor. The Oligo- $(dT)_{23}$VN has a better affinity to Ploy A^+ RNA than Oligo-$(dT)_{18}$. In addition, random hexamers and gene-specific primers (GSP) are also optional.

CONTENTS

No	Component	EP2012 50rxn	EP2013 100rxn
FA	RNase-free ddH_2O	1 ml	1 ml
FB	4X gDNA Wiper Mix	200 µl	400 µl
FC	2X RT buffer Mix[a]	120 µl	240 µl
FD	ACE Script II Enzyme Mix[b]	100 µl	200 µl
FE	Oligo-$(dT)_{23}$ VN (50 µM)	50 µl	100 µl
FF	Random Hexamers (50ng/µl)	50 µl	100 µl

a. Contains dNTPs
b. Contains RNase inhibitor

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.

Primer selection (Oligo-$(dT)_{23}$ VN, Random hexamers, or GSP)

A. If the cDNA product will be used for **PCR**

■ For eukaryotic RNA templates, generally, use Oligo-$(dT)_{23}$ VN to obtain the highest yield of full-length cDNA.

■ Use gene-specific primer (GSP) to obtain the highest specificity. However, switch to Oligo-$(dT)_{23}$ VN or random

hexamers if GSP fails in the 1st-strand cDNA synthesis.

■ Random hexamers with the lowerst specificity can be used for RNA templates, including mRNA, rRNA, and tRNA. Use random hexamers when Oligo-$(dT)_{23}$ VN or GSP fails in cDNA synthesis due to complex secondary structure, high GC content, or prokaryotic RNA template.

B. If the cDNA product will be used for **qPCR**

■ Use the mixture of Oligo-$(dT)_{23}$ VN or random hexamers.

A. If the cDNA product will be used for PCR

A.1: RNA Denaturation : incubate 65℃ for 5 min and then chill on ice immediately for 2 min.

Mix components in a RNase-free PCR tube	
Oligo-$(dT)_{23}$ VN (50 μM)	1 μl
or Random Hexamers (50ng/ul)	
or Gene Specific Primer (2 μM)	
Total RNA	10 pg -5 μg
or Poly A+ RNA	10 pg - 500 ng
RNase-free ddH_2O	To 12 **μ**l

Note: RNA denaturation benefits the cDNA yield. However, for cDNA <3 kb, please skip the denaturation step.

A.2 Removal of Genomic DNA : Add 4 μl of 4X gDNA Wiper to the mixture of Step 1.1 (12 μl), mix thoroughly, and incubate at 42℃ for 2 min.

A.3 1st Strand cDNA synthesis

Mix components in a RNase-free PCR tube	
Mixture of step A.2	16 μl
10X RE Buffer Mix	2 μl
ACE Script II Enzyme Mix	2 μl

Condition	
25℃ [a]	5 min
50℃ [b]	45 min
85℃	2 min

c. Only necessary when using random hexamers. Please skip this step when using Oligo-(dT)23VN or Gene Specific Primers (GSP).
d. For templates with complex secondary structure or high GC-content, the temperature can be increased to 55℃, which will benefit the yield.

A.4 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.

B. If the cDNA product will be used for qPCR

B.1 Removal of Genomic DNA : Mix the following components in a RNase-free microtube by pipetting, and incubate at 42℃ for 2 min.

Mix components in a RNase-free PCR tube	
4X gDNA Wiper Mix	4 µl
Oligo-$(dT)_{23}$ VN (50µM)	1 µl
Random Hexamers (50ng/µl)	1 µl
Total RNA	10 pg – 1 µg
or Poly A+ RNA	10 pg- 100 ng
RNase-free ddH_2O	To 16 µl

B.2

Mix components in a RNase-free PCR tube	
Mixture of Step B.1	16 µl
10X RT Buffer Mix	2 µl
ACE Script II Enzyme Mix	2 µl

B.3 1st Strand cDNA synthesis

Condition	
25℃	5 min
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.

B.4 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.

PRODUCT USE LIMITATION

These products are intended for research use only.