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Datasheet

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ACE Script II 1st Strand cDNA RT Kit

Cat# EP2010 50 rxn
EP2011 100 rxn

Storage at -20 ℃ for one year

INTRODUCTION

The ACE Script 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 at 50℃ is > 240 min. Even at 55℃, the ACE Script II can stay stable for a long time, which significantly benefits the transcription of RNA templates with complex secondary structures. In addition, the ACE Script II improves 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 ACE Script II 1st Strand cDNA RT Kit contains all the components necessary for the 1st strand cDNA synthesis. The products are suitable for PCR and qPCR. The 2X RT buffer 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	CP2010 50rxn	CP2011 100rxn
EA	RNase-free ddH_2O	1 ml	1 ml
EB	2X RT buffer Mix	500 ul	1 ml
EC	ACE Script II Enzyme Mix	100 ul	200 ul
ED	Oligo-$(dT)_{23}$ VN (50uM)	50 ul	100 ul
EE	Random Hexamers (50ng/ul)	50 ul	100 ul

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 (50uM)	1 ul
or Random Hexamers (50ng/ul)	
or Gene Specific Primer (2uM)	
Total RNA	10 pg -5 ug
or Poly A+ RNA	10 pg - 500 ng
RNase-free ddH_2O	To 8 ul

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

A.2: 1st Strand cDNA synthesis

Mix components in a RNase-free PCR tube	
Mixture of A.1	8 ul
2X RT buffer Mix	10 ul
ACE Script II Enzyme Mix	2 ul

Condition		
25℃		5 min
50℃	*	45 min
85℃		5 min

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

B. If the cDNA product will be used for qPCR

Mix components in a RNase-free PCR tube	
2X RT buffer Mix	10 ul
Oligo-$(dT)_{23}$ VN (50uM)	1 ul
Random Hexamers (50ng/ul)	1 ul
Total RNA	1 pg – 1 ug
or Poly A+ RNA	10 pg- 100 ng
ACE Script II Enzyme Mix	2 ul
RNase-free ddH_2O	To 20 ul

Condition		
25℃		5 min
50℃	*	15 min
85℃		5 min

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

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.