

ABscript II cDNA First Strand Synthesis Kit

Catalog: RK20400

Size: 50 RXN / 100 RXN

1. Introduction

ABScript II cDNA First Strand Synthesis Kit features two optimized mixes, ABScript II Enzyme Mix and ABScript II Reaction Mix. The enzyme mix combines ABScript II Reverse Transcriptase and RNase Inhibitor, and the reaction mix contains an optimized buffer. ABScript II Reverse Transcriptase is a recombinant M-MuLV reverse transcriptase with reduced RNase H activity and increased thermostability. It can be used to synthesize first strand cDNA at higher temperatures than the wild type M-MuLV. The enzyme is active up to 48 °C, which provides higher specificity and higher yield of cDNA.

The kit also provides two optimized primers for reverse transcription and nuclease-free water. An anchored Oligo-dT primer [d(T)₂₃VN] forces the primer to anneal to the beginning of the polyA tail. The optimized Random Primer Mix provides random and consistent priming sites covering the entire RNA templates including both mRNAs and non-polyadenylated RNAs. The first strand cDNA product generated is up to 10 kb.

2. List of Components

Components	Cat.No.	50 RXN	100 RXN
ABScript II Enzyme Mix (10X)	RM21451	100 µl	200 µl
ABScript II Reaction Mix (2X)	RM21450	600 µl	1.25 ml
Oligo d(T) ₂₃ VN * (50 µM)**	RM20115	100 µl	200 µl
Random Primer Mix (60 µM)**	RM20116	100 µl	200 µl
dNTPs (10 mM each)	RM20120	50 µl	100 µl
Nuclease-free H ₂ O	RM20214	1.25 ml	1.25 ml

*V = A, G or C; N = A, G, C or T **Contains 1 mM dNTP.

Store all reagents at -20 °C.

3. Quality Controls

The performance of ABScript II First Strand cDNA Synthesis Kit is

tested in an RT reaction using Jurkat total RNA with primer d(T)₂₃VN. The length of cDNA achieved is verified by detection of a 9.2 kb amplicon of fibrillin gene.

4. First Strand cDNA Synthesis

Reaction

- Denaturation of RNA and primer at 65–70 °C for 5 minutes can remove secondary structures that may impede long cDNA synthesis. However, this step can be omitted in many cases (unpublished results).
- We recommend incubation at 42 °C for one hour for maximum yield and length. However, many targets can be detected after a much shorter incubation time. For example, 10 minutes incubation can be used for up to 5 kb cDNA synthesis.

5. Choice of Primers for Reverse

Transcription

- Oligo-dT priming is preferred for most applications because it ensures that all cDNA copies terminate at the 3' end of the mRNA and produces the longest contiguous cDNA. An anchored oligo-dT primer [d(T)₂₃VN] forces the primer to anneal to the start of the polyA tail, thereby preventing priming at internal sites in the polyA tail (1).
- The Random Primer Mix is an optimized mix of hexamer and dNTP. It provides random priming sites covering the entire RNA templates including both mRNAs and non-polyadenylated RNAs (such as ribosomal RNAs). The Random Primer Mix yields shorter cDNAs on average and can be used for the detection of multiple short RT-PCR products. Random Primer Mix offers good performance in a wide range of RNA templates.
- When a gene-specific primer is used in a cDNA synthesis reaction, the cDNA product can be used only for amplification of that transcript. This priming method gives good results

when the amount of RNA is limiting (below 10 ng) and only one particular cDNA is desired.

- Recommended primer concentration:

PRIMER	OLIGO d(T) ₂₃ VN	RANDOM PRIMER MIX	SPECIFIC PRIMER
Final Conc.	5 μM	6 μM	0.1–1 μM

6. First Strand cDNA Synthesis

Protocols

Thaw kit components on ice and mix by inverting several times.

Easy Protocol

- Mix the following components and incubate at 42 °C for 1 hour. If Random Primer Mix is used, an incubation step at 25 °C for 5 minutes is recommended before the 42 °C incubation.

1x RXN	
Nuclease-free H ₂ O	to a total volume of 20 μl
Template RNA	up to 1 μg
d(T) ₂₃ VN	2 μl
10 mM dNTPs	1 μl
ABScript II Reaction Mix (2X)	10 μl
ABScript II Enzyme Mix (10X)	2 μl
Total	20 μl

- Inactivate the enzyme at 80 °C for 5 minutes. For downstream PCR application, the volume of cDNA product should not exceed 1/10 of the PCR reaction volume.

Standard Protocol

If denaturation of template RNA is desired, use the following protocol.

- Mix RNA sample and primer d(T)₂₃VN in a sterile RNase-free microfuge tube.

1x RXN	
Total RNA	1–6 μl (up to 1 μg)
d(T) ₂₃ VN (50 μM)	2 μl
10 mM dNTPs	1 μl
Nuclease-free H ₂ O	to a total volume of 8 μl
Total	8 μl

- Denature sample RNA/d(T)₂₃VN for 5 minutes at 65 °C. Spin briefly and put promptly on ice.
- Add the following components.

1x RXN	
ABScript II Reaction Mix (2X)	10 μl
ABScript II Enzyme Mix (10X)	2 μl

- Incubate the 20 μl cDNA synthesis reaction at 42 °C for one hour. If Random Primer Mix is used, an incubation step at 25 °C for 5 minutes is recommended before the 42 °C incubation.
- Inactivate the enzyme at 80 °C for 5 minutes. The cDNA product should be stored at –20 °C. In general, the volume of cDNA product should not exceed 1/10 of the PCR reaction volume.

No-RT Negative Control Reaction

Mix the following components and incubate at 42 °C for 1 hour.

1x RXN	
Template RNA	up to 1 μg
d(T) ₂₃ VN (50 μM)	2 μl
ABScript II Reaction Mix (2X)	10 μl
10 mM dNTPs	1 μl
Nuclease-free H ₂ O	to a total volume of 20 μl
Total	20 μl

7. General Information for Successful cDNA Synthesis

- Intact RNA of high purity is essential for sensitive RT-PCR detection.
- Total RNA or mRNA can be used in the reverse transcription reaction. Total RNA is generally sufficient for most RT-PCR analyses.

8. Trouble-shooting Guide

Low Yield of cDNA

- Check the integrity of the RNA by denaturing agarose gel electrophoresis (2).
- RNA should have a minimum A260/A280 ratio of 1.7 or higher. Ethanol precipitation followed by a 70% ethanol wash can remove most contaminants such as EDTA and guanidinium. Precipitation with lithium chloride can remove polysaccharides (2).
- Phenol/chloroform extraction and ethanol extraction can remove contaminant proteins such as proteases (2).

- Some target RNA may contain strong pauses for RT; Use random priming instead of d(T)₂₃VN.
- Use sufficient amount of RNA.

9. References

- 1) Liao, J. and Gong, Z. (1997) *Biotechniques* 23, 368–370.
- 2) Sambrook, J. and Russel, D.W. (2001). *Molecular Cloning: A Laboratory Manual*, (3rd ed.), (pp. 8.46–8.53 and 11.37–11.42). Cold Spring Harbor: Cold Spring Harbor Laboratory Press.