ProtoScript® II First Strand cDNA Synthesis Kit

NEB #E6560S/L **30/150 reactions** **Version 4.0_6/23**

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Kit Components

All kit components can be stored for one year at -20°C except where noted.

ProtoScript II Enzyme Mix (10X)

ProtoScript II Reaction Mix (2X)

Oligo d(T)23 VN* (50 µM)**

Random Primer Mix (60 µM)**

Nuclease-free H_2O

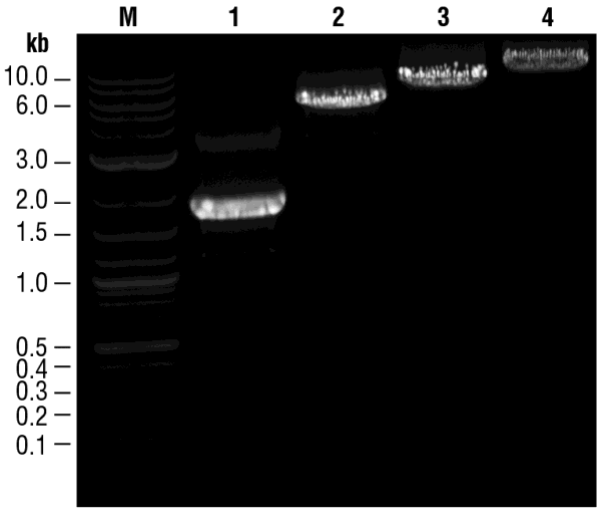
* V = A,G or C; N = A, G, C or T
** Contains 1 mM dNTP

Introduction

ProtoScript II First Strand cDNA Synthesis Kit features two optimized mixes, ProtoScript II Enzyme Mix and ProtoScript II Reaction Mix. The enzyme mix combines ProtoScript II Reverse Transcriptase and Murine RNase Inhibitor, while the reaction mix contains dNTPs and an optimized buffer. ProtoScript 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, providing 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 [$d(T)_{23}VN$] 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.

Figure 1: cDNA Synthesis of Jurkat RNA with the ProtoScript II First Strand cDNA Synthesis Kit.



First strand cDNA synthesis was carried out in the presence of 1X ProtoScript II Reaction Mix and 1X ProtoScript II Enzyme Mix at 42°C using 250 ng of Jurkat total RNA. Four amplicons from different genes were amplified using 1X LongAmp® Taq 2X Master Mix (NEB #M0287). Lane 1, a 1.9 kb amplicon from SDHA gene; Lane 2, a 5.5 kb from the guanine nucleotide exchange factor; Lane 3, a 7.3 kb amplicon from Xrn-1 gene; and Lane 4, a 9.2 kb amplicon from fibrillin gene. Marker M is 1 kb Plus DNA Ladder (NEB #N3200).

Quality Controls

The performance of Protoscript II First Strand cDNA Synthesis Kit is tested in an RT reaction using Jurkat total RNA with primer $d(T)_{23}VN$. The length of cDNA achieved is verified by detection of a 9.2 kb amplicon of fibrillin gene.

First Strand 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.

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 [$d(T)_{23}VN$] 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 $d(T)_{23}VN$ primers. 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 concentration	5 µM	6 µM	0.1–1 µM

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

Easy Protocol

1. 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.

COMPONENT	VOLUME
Template RNA	up to 1 µg
$d(T)_{23}VN$	2 µl
ProtoScript II Reaction Mix (2X)	10 µl
ProtoScript II Enzyme Mix (10X)	2 µl
Nuclease-free H_2O	**to a total volume of 20 µl**

2. 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.

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

COMPONENT	VOLUME
Total RNA	1–6 µl (up to 1 µg)
$d(T)_{23}VN$	2 µl
Nuclease-free H_2O	**to a total volume of 8 µl**

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

ProtoScript II Reaction Mix (2X)	10 µl
ProtoScript II Enzyme Mix (10X)	2 µl

4. 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.
5. 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.

COMPONENT	VOLUME
Template RNA	up to 1 µg
$d(T)_{23}VN$	2 µl
ProtoScript II Reaction Mix (2X)	10 µl
Nuclease-free H_2O	**to a total volume of 20 µl**

General Information for Successful cDNA Synthesis

Template RNA

- 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. However, if desired mRNA can be easily obtained using a polyA Spin mRNA Isolation Kit (NEB #S1560) or Magnetic mRNA Isolation Kit (NEB #S1550).
- The amount of RNA required for detection depends on the abundance of the transcript-of-interest. In general 1 ng to 1 µg total RNA or 0.050 ng to 100 ng mRNA are recommended.

Troubleshooting Guide

PROBLEM	SOLUTION(S)
Low yield of cDNA	Check the integrity of the RNA by denaturing agarose gel electrophoresis (2) RNA should have a minimum A_{260}/A_{280} 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 $d(T)_{23}VN$
	Use sufficient amount of RNA

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.

Appendix

Supplied Components:

1X ProtoScript II Enzyme Mix:

2.5 units/µl ProtoScript II Reverse Transcriptase
1.0 units/µl Murine RNase Inhibitor

1X ProtoScript II Reaction Mix:

50 mM Tris-Acetate (pH 8.3)
75 mM KAcetate
3.1 mM $Mg(OAc)_2$
2 mM DTT
0.5 mM dNTPs each

Ordering Information

NEB #	PRODUCT	SIZE
E6560S/L	ProtoScript II First Strand cDNA Synthesis Kit	30/150 reactions

COMPANION PRODUCTS

NEB #	PRODUCT	SIZE
S1560S	polyA Spin mRNA Isolation Kit	100/500 units
S1550S	Magnetic mRNA Isolation Kit	1,600/8,000/8,000 units
S1419S	Oligo d(T)$_{23}$ Magnetic Beads	25 mg
E6400S	ProtoScript *Taq* RT-PCR Kit	30 reactions
M0314S/L	Murine RNase Inhibitor	3,000/15,000 units
S1330S	Random Primer Mix	1.0 A_{260} units
M0287S/L	LongAmp *Taq* 2X Master Mix	100/500 reactions
M0270S/L	*Taq* 2X Master Mix	100/500 reactions

Revision History

REVISION	DESCRIPTION	DATE
1.0		2/12
1.1		6/13
1.2		5/14
1.3		11/14
1.4		11/15
1.5		12/17
2.0		6/18
3.0	Updated to new manual format.	1/20
4.0	Updated concentration of ProtoScript Enzyme Mix. Updated table formatting and legal footer.	6/23

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