FISHER MOLECULAR BIOLOGY

FS-RT-4020 cDNA First Strand Synthesis Kit

Introduction

cDNA First Strand Synthesis Kit is ideal for routine molecular biology applications, basic gene expression analysis, using high quality total RNA and mRNA extractions
It is optimized for long and ***full length cDNA Synthesis (up to 10kb)***

The enzyme mix combines Reverse Transcriptase and RNase Inhibitor, and the reaction mix contains an optimized buffer. The 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 [$d(T)_{23}VN$] forces the primer to anneal to the beginning of the poly A 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.

Kit components	FS-RT-4020 100 Tests	FS-RT-4021 200 Tests
cDNA Enzyme Mix (10X)	200 µL	2X 200 µL
cDNA Reaction Mix (2X)	1 ml	2X 1 ml
Oligo d(T)23VN * (50 µM) **	200 µL	2x200 µL
Random Primer Mix (60 µM) **	200 µL	2x200 µL
dNTPs (10 mM each)	100 µL	2x 100 µL
Nuclease-free H2O	1,25 ml	2x 1,25 ml

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

First Strand cDNA Synthesis Reaction

1. 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 However, this step can be omitted in many cases (unpublished results).

2. 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 min.incubation can be used for up to 5 kb cDNA synthesis.

Choice of Primers for Reverse Transcription

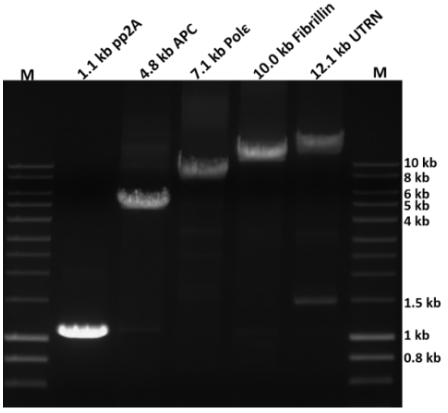
1. Oligo-dT primer is preferred for most applications because it ensures that all cDNA copies terminate at the 3´ end of the mRNA and produces the longest contiguous cDNA. An anchored oligo-dT primer [d(T)23VN] forces the primer to anneal to the start of the polyA tail, thereby preventing priming at internal sites in the polyA tail (1).

2. 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.

3. 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.

4. Primer concentration (recommended):

Primer	Oligo $d(T)_{23}VN$	Random Primer Mix	Specific Primer
Final Conc.	5 µM	6 µM	0.1-1 µM



Using 1µg of HeLa cell total RNA as template, reverse transcription was performed with cDNA First-Strand Synthesis Kit, and 1µL of cDNA was used as template to obtain clear and specific electrophoresis bands for genes of different lengths.

Quality control

The performance of cDNA First Strand 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.

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Protocols

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

Easy Protocol

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

Components	1x Reaction
Template RNA	up to 1 μg
d(T)$_{23}$ VN (50 μM)*	2 μL
10 mM dNTPs	1 μL
cDNA Reaction Mix (2X)	10 μl
cDNA Enzyme Mix (10X)	2 μL
Nuclease-free H2O	to 20 μL

* *Note: According to different requirements, primers can be added according to the recommended primer concentrations: Oligo d(T)23 VN (5 μM), random primers (6 μM), and specific primers (0.1-1 μM).

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

Standard protocol helps to denature the secondary structure of complex RNA templates, improve the efficiency of reverse transcription, and increase the yield of cDNA products

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

Components	1 x Reaction
Total RNA 1–5 μL	(up to 1 μg)
d(T)$_{23}$ VN (50 μM)*	2 μL
10 mM dNTPs	1 μl
Nuclease-free H2O	to 8 μL

*Note: According to different requirements, primers can be added according to the recommended primer concentrations: Oligo d(T)23 VN (5 μM), random primers (6 μM), and specific primers (0.1-1 μM).

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.

Components	1 x Reaction
cDNA Reaction Mix (2x)	10 μl
cDNA 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.

A. No-RT Negative Control Reaction

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

Components	1x Reaction
Template RNA	up to 1 μg
d(T)23 VN (50 μM)	2 μL
cDNA Reaction Mix (2X)	10 μL
10 mM dNTPs	1 μL
Nuclease-free H2O	20 μL

General Information for Successful cDNA Synthesis

1. Intact RNA of high purity is essential for sensitive RT-PCR detection.

2. Total RNA or mRNA can be used in the reverse transcription reaction. Total RNA is generally sufficient for most RT-PCR analyses.

Trouble-shooting Guide

Low Yield of cDNA

1. 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

3. Phenol/chloroform extraction and ethanol extraction can remove contaminant proteins such as proteases

4. Some target RNA may contain strong pauses for RT; Use random priming instead of d(T)$_{23}$VN.

5. Use sufficient amount of RNA.