

M-MLV Reverse Transcriptase

M-MLV 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, higher yield of cDNA and more full-length cDNA product up to 12 kb.

Size: 10,000 U

Concentration: 200U/ul

Cat N°	Size	Storage/Shelf life
FS-RT-1032	10,000 U	-20°C/one year

Kit Components:

FS-RT-1033	Size
M-MLV Reverse Transcriptase (200U/ul)	50 ul
5X First-Strand Buffer	500 ul
100 mM DTT (10X)	200 ul

Product Source:

The gene encoding a mutant M-MuLV Reverse Transcriptase (RNase H-) is expressed in *E. coli* and purified to near homogeneity.

Unit Definition:

One unit is defined as the amount of enzyme that will incorporate 1 nmol of dTTP into acid-insoluble material in 10 minutes at 37°C using poly(rA)•oligo(dT)18 as template, with a total reaction volume of 50 µl.

Reaction Conditions:

1X First-Strand Reaction Buffer, 10 mM DTT, 200 units M-MLV Reverse Transcriptase, supplemented with 0.5 mM dNTPs (not included) and 5 µM dT23VN (not included). Incubate at 42°C for 50 minutes. If random primers are used, a 10-minute incubation at room temperature is recommended before transferring to 42°C.

1X First-Strand Reaction Buffer:

50 mM Tris-HCl, 75 mM KCl, 3 mM MgCl₂, pH 8.3 @ 25°C

Storage Temperature: -20°C

Storage Conditions:

20 mM Tris-HCl, 100 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 0.01% IGEPAL® CA-630, pH 7.5 @25°C

Heat Inactivation: 65°C for 20 min

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Instructions

➤ First Strand cDNA Synthesis (Quick Protocol)

Thaw components on ice and mix by inverting several times.

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
Nuclease-free H ₂ O	Up to 20 µl
5X First-Strand Buffer	4 µl
d(T) ₂₃ VN (50 µM) or Random Primer Mix (60 µM)	2 µl
100 mM DTT	2 µl
RNase Inhibitor (40 U/µl)	0.2 µl
Template RNA	up to 1 µg*
10 mM dNTP	1 µl
M-MLV RT (200 U/µl)	1 µl

* 1 ng-1 µg total RNA or 50 pg-100 ng poly(A)-RNA

** M-MLV Reverse Transcriptase can be used at 42–48°C.

2. Inactivate the enzyme at 65°C for 20 minutes. For downstream PCR application, the volume of cDNA product should not exceed 1/10 of the PCR reaction volume.

➤ First Strand cDNA Synthesis (Standard Protocol)

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

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

Component	Volume
Total RNA	up to 1 µg*
d(T) ₂₃ VN (50 µM) or Random Primer Mix (60 µM)	2 µl
10 mM dNTP	1 µl
Nuclease-free H ₂ O	Up To 10 µl

* 1 ng-1 µg total RNA or 50 pg-100 ng poly(A)-RNA

** M-MLV Reverse Transcriptase can be used at 42–48°C.

2. Denature sample RNA/primer for 5 minutes at 65°C. Spin briefly and put on ice immediately.

3. Add the following components to the tube

Component	Volume
Nuclease-free H ₂ O	Two Steps Total Up to 20 µl
5X First-Strand Buffer	4 µl
100 mM DTT	2 µl
RNase Inhibitor (40 U/µl)	0.2 µl
M-MLV RT (200 U/µl)	1 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 65°C for 20 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.

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- First strand cDNA Synthesis (No-RT Negative Control Reaction)

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

Component	Volume
Template RNA	up to 1 µg*
d(T)23VN (50 µM) or Random Primer Mix (60 µM)	2 µl
5X First-Strand Buffer 4 µl	4 µl
100 mM DTT	2 µl
10 mM dNTP Mix	1 µl
RNase Inhibitor (40 U/µl)	0.2 µl
Nuclease-free H ₂ O	Up to 20 µl

* 1 ng-1 µg total RNA or 50 pg-100 ng poly(A)-RNA

QC Process:

- Purity is above 95% detected by SDS-PAGE.
- No exonuclease, nuclease, RNase contamination.
- Host genomic DNA is no residual detected by PCR.

Additional Products:

RNase Inhibitor (40,000 U/mL) is available separately.

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