

M-MLV (H-)Reverse Transcriptase

Cat No: YT4502

Size: 10000 U

Concentration: 200U/ μ l

for research use only

< store at -20°C >

Contents: M-MLV (10,000U) 50 μ l
5X first-strand Buffer 200 μ l

Description :

Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT) uses single-stranded RNA or DNA in the presence of a primer to synthesize a complementary DNA strand. This enzyme is isolated from E. coli expressing a portion of the pol gene of M-MLV on a plasmid. The enzyme is used to synthesize first-strand cDNA up to 5 kb.

Unit Definition :

One unit incorporates 1 nmole of dTTP into acid-precipitable material in 10 min at 37°C using poly(A)•oligo(dT) 25 as template-primer.

Storage Buffer

20mM Tris-HCl (pH 7.5), 200mM NaCl, 0.1mM EDTA, 1mM DTT, 0.01% NP-40, 50% glycerol

5xfirst-Strand Buffer

250mM Tris-HCl (pH 8.3 at 25°C), 375mM KCl, 15mM MgCl₂ 50mM DTT

Applications:

Generation of first strand cDNA for use in:

- PCR, see Protocol for First-strand cDNA Synthesis;
- Second strand cDNA synthesis.
- DNA labeling.
- Real-time PCR;
- Analysis of RNA by primer extension.

Protocol:

First-Strand cDNA Synthesis Using M-MLV RT

A 20- μ l reaction volume can be used for 1ng–5 μ g of total RNA or 1–500ng of mRNA.

1. Add the following reagents into a sterile, nuclease-free tube on ice in the indicated order:

Template RNA	Poly (A) mRNA Or specific RNA	1 to 500 ng 1-5 μ g
Prime	oligo (dT)18 primer(50 μ M) or Random hexamer primer(50 μ M)	1 μ l 1 μ l
DEPC-treated water		To 13.5 μ l
Total Volume		13.5 μ l

2. Mix gently, centrifuge briefly and incubate at 70°C for 5 min. Chill on ice, spin down and place the vial back on ice.

3. Prepare the following cDNA Synthesis Mix, add the following components in the indicated order:

5x first-strand buffer	4 μ l
dNTPs(10 mM each)	1 μ l
RNasin (40U/ μ l)	0.5 μ l
M-MLV	1 μ l

4. Mix gently and centrifuge

5. For oligo(dT)18, incubate for 60 min at 42°C. For random hexamer primed synthesis, incubate for 60 min at 37°C .

6. Terminate the reaction by heating at 70°C for 5 min. The reverse transcription reaction product can be used immediately in second strand cDNA synthesis reactions or stored at -20°C for less than a week. For longer storage, -70°C is recommended.