

M-MLV Reverse Transcriptase

SAMPLE

Ref : NB-03-0130 5 000U
NB-03-0131 10 000U

Contents

	NB-03-0130	NB-03-0131
M-MLV (200 U/ µl)	25 µl	50 µl
5X First-Strand Buffer	100 µl	200 µl

Description

The 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.

Source

Purified from an *E. coli* strain expressing a recombinant clone.

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.

Applications

Generation of first strand cDNA for use in:

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

Storage Buffer

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

Store at -20°C.

5xFirst-Strand Buffer

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

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
Primers	oligo (dT) ₁₅ primers (50 μ M) or Random Hexamer primers (50 μ M)	1 μ l 1 μ l
DEPC-treated water		to 13.4 μ l
Total volume		13.4 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)	0.6 μ l
M-MLV	1 μ l

4. Mix gently and centrifuge

5. For oligo(dT)₁₅, 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.

The product of the first strand cDNA synthesis can be used directly in PCR or qPCR. The volume of first strand cDNA synthesis reaction mixture should not comprise more than

1/10 of the total PCR reaction volume. Normally, 2 μ l of the first strand cDNA synthesis reaction mixture is used as template for subsequent PCR in 50 μ l total volume.

Quality Control

This product has passed the following quality control assays: SDS polyacrylamide gel analysis for purity; functional absence of endodeoxyribonuclease, 3' and 5' exodeoxyribonuclease, and ribonuclease activities, yield and length of cDNA product.

PRODUCT USE LIMITATION.

This product is developed, designed and sold exclusively for research purposes and in vitro use only.

The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.