M-MLV Reverse Transcriptase

Catalog No: P4041 (5000 U)

Concentration: 200U/µl

Contents:

M-MLV	25µl
5xfirst-strand buffer	100µl

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.

Source

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

Storage Buffer

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

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.

5xfirst-Strand Buffer

250mM Tris-HCl (pH 8.3 at 25°C), 375mM KCl,15mM $MgCl_2$

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

I. First-Strand cDNA Synthesis using M-MLV RT

A 20-µl reaction volume can be used for 1ng–5µg 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 500ng 1-5 µg
Prime	oligo $(dT)_{15}$ primer(50µM) or random hexamer primer(50µM)	1 µl 1 µl
DEPC-treated water		to 13.4 µl
Total volume		13.4 µl

2. Mix gently, centrifugebriefly 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 µl
dNTPs(10 mM each)	1 µl
RNasin (40U/µl)	0.6 µl
M-MLV	1 µl

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.

II. PCR Reaction

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 µl of the first strand cDNA synthesis reaction mixture is used as template for subsequent PCR in 50 µl 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.