Médico ™ M-MLV Reverse Transcriptase (recombinant)

Cat No.: MD-MLV-25 & MD-MLV-50
Size : 25 preps & 50 preps

Contents :

Contents	MD-MLV-25(25 preps)	MD-MLV-50(50 preps)
M-MLV	100 µl (5,000U)	200 µl (10,000U)
5x First-Strand Buffer	100 µl	200 µl

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.

Recommended storage condition:

● Store at -20°C.

Description :

Médico ™ M-MLV Reverse Transcriptase (RT) is a genetically modified M-MLV RT. It differs from the M-MLV RT by its structure and catalytic properties. The enzyme possesses an RNA-dependent and DNA-dependent polymerase activity and a ribonuclease H activity specific to RNA in RNA-DNA hybrids. Médico ™ M-MLV Reverse Transcriptase has significantly lower RNase H activity than Avian Myeloblastosis Virus (AMV) reverse transcriptase.

Source :

E.coli cells with a cloned fragment of the pol gene encoding Moloney Murine Leukemia Virus reverse transcriptase.

Application :

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.

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

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.

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) mRNAor specific RNA	1 to 500 ng or 1-5 µg
Prime	oligo (dT)15 primer or Random hexamer primer	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℃ for less than a week. For longer storage, -70℃ is recommended.

II. PCR Amplification of First Strand cDNA

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.

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