M-MLV Reverse Transcriptase

[Cat. No.]

E - 3121 (10,000 units) E - 3122 (50,000 units)

[Lot No.] 0703

[Concentration] 200 units/uL

 Description: Moloney Murine Leukemia Virus(M-MLV) Reverse Transcriptase is an RNA-dependent DNA polymerase. This enzyme is able to use an RNA molecule as a template and synthesize a doublestrand DNA

- Source : M-MLV Reverse Transcriptase is isolated from a E.coli strain containing a recombinant clone.
- Application: First-strand synthesis of cDNA from RNA molecules.
- . Supplied with Enzyme
- M-MLV Reverse Transcriptase (10,000U): 50ul (200U/ul)
- 5X Reaction Buffer (0.5 mL): 50 mM Tris-HCl, 250 mM KCl, 10 mM MgCl₂, pH8.1
- 100mM DTT (0.2 mL)
- Storage condition: 20 mM Tris-Cl (pH7.6), 150 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1 %
 IGEPAL CA-630, 50 % Glycerol, store at -20 °C
- Unit Definition: One unit is defined as the amount of enzyme required to incorporates 1 nmole of dTTP into acid-precipitable material in 10 min at 37 °C using poly(A)•oligo(dT) as template primer.
- Quality Assurance: DNase and RNase activity is not detected after incubation of 1 ug of DNA and RNA with 200 units of M-MLV Reverse Transcriptase for 3 hours at 37 °C – 42 °C.
- Application protocol
- first-strand cDNA synthesis [20 uL reaction volume]

Step 1

Step 2

Total RNA or RNA

→ 1 ug total RNA or 5 ng-100 ng RNA

Oligo dT (or random primer)

→ 10 - 100 pmoles

DEPC water(RNase - and DNase - free)

- → variable volume
- → Denature RNA and primer for 10 min at 65 °C
- → Immediately cool on ice

5X M-MLV RTase reaction buffer	4 uL
100mM DTT	2 uL
dNTP	variable volume
RNase inhibitor	20 units
M-MLV RTase	200 units
Total (Step1 + Step 2)	20 uL

- → cDNA synthesis for 1 hr at 37-42 °C
- Note: Store the buffer in small aliquots at -20 °C to minimize degradation of the DTT

Note

For research use only. Not for use in diagnostic or therapeutic procedures.