



Product Name

Cat. No.

Pack Size

MuLV Reverse Transcriptase
100 Reactions

Cat# BB-E0040

20,000 U (100 µl)

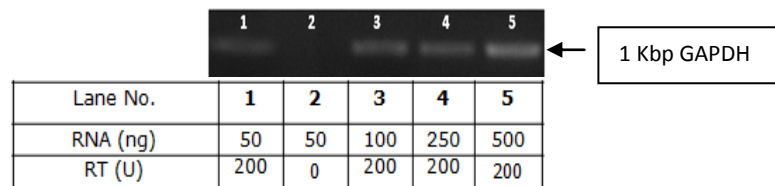
Description: Murine Leukemia Virus Reverse Transcriptase (MuLV RT) is an RNA-dependent DNA polymerase that can be used in cDNA synthesis with long messenger RNA templates (upto 5 Kbp). The enzyme is isolated from *E.coli* expressing a portion of *pol* gene of MuLV on a plasmid. The RNase H activity of MuLV RT is weaker than the commonly used Avian Myeloblastosis Virus (AMV) reverse transcriptase.

Reagents supplied:

- MuLV RT (200 U/µl), 100 µl
- 5x RT Buffer (250 mM Tris-HCl, pH 8.4; 375 mM KCl; 15 mM MgCl₂), 400 µl

Storage Buffer: 20mM Tris-HCl (pH 7.5), 100mM NaCl, 0.1mM EDTA, 1mM DTT, 50% glycerol (v/v).

Storage Instruction: -20°C



Reverse Transcription done with the 200 U of MuLV-RT using above mentioned amount of total RNA in a 20 µl reaction volume. 2ul RT product used for the GAPDH PCR.

First-Strand cDNA Synthesis Using RT

- A 20µl volume can be used for 50 ng-1 µg of Total RNA or 1-500 ng of mRNA
- Add the following components to a nuclease-free microcentrifuge tube

Oligo (dT) ₁₂₋₁₈ (500 µg/ml) or 50-250 ng random primers or 2 pmole gene-specific primer (GSP)	1 µl
1 ng to 5 ng total RNA or 1-500 ng of mRNA	x µl
1 µl dNTP Mix (10mM each)	1 µl
Sterile, distilled water	To 12 µl

- Heat mixture to 65°C for 5 min. & quick chill on ice. Collect the contents of the tube by brief centrifugation and add (suggestion to make a master mix):

5 X First-Strand Buffer	4 µl
0.1 M DTT	2 µl
RNase Inhibitor	1 µl

- Mix contents of the tube gently. Keep it at room temp for 5 min.
- Add 1 µl of RT (200U/µl) and mix by pipetting gently up and down
- Incubate at 42°C for 50 min
- Inactivate the reaction by heating at 70°C for 15 min

