

# M-MuLV Reverse Transcriptase



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M0253S 027130615061

## M0253S



**10,000 units 200,000 U/ml Lot: 0271306**

**RECOMBINANT Store at -20°C Exp: 6/15**

**Description:** Moloney Murine Leukemia Virus (M-MuLV, MMLV) Reverse Transcriptase is an RNA-directed DNA polymerase. This enzyme can synthesize a complementary DNA strand initiating from a primer using either RNA (cDNA synthesis) or single-stranded DNA as a template (1-4). M-MuLV Reverse Transcriptase lacks 3' → 5' exonuclease activity.

**Source:** The gene encoding M-MuLV Reverse Transcriptase is expressed in *E. coli* in a vector that results in 16 additional amino acids at the N-terminus and 13 amino acids at the C-terminus.

This construct results in a fully functional Reverse Transcriptase protein with a functional RNase H domain.

Supplied in: 150 mM NaCl, 50 mM Tris-HCl (pH 7.6), 0.1 mM EDTA, 1 mM dithiothreitol, 0.1% NP-40 and 50% glycerol.

**Reagents Supplied with Enzyme:**  
10X Reverse Transcriptase Reaction Buffer

**Reaction Conditions:** 1X Reverse Transcriptase Reaction Buffer, supplemented with dNTPs (not included). Incubate at 37°C or 42°C.

### 1X Reverse Transcriptase Reaction Buffer:

75 mM KCl  
50 mM Tris-HCl  
3 mM MgCl<sub>2</sub>  
10 mM dithiothreitol  
pH 8.3 @ 25°C

**Unit Definition:** One unit is defined as the amount of enzyme required to incorporate 1 nmol of dTTP into an acid-insoluble form in 10 minutes at 37°C using poly(rA)-oligo(dT) as template primer.

**Unit Assay Conditions:** 75 mM KCl, 50 mM Tris-HCl (pH 8.3) 3 mM MgCl<sub>2</sub>, 10 mM dithiothreitol, 0.5 mM [<sup>3</sup>H]-dTTP, 0.4 mM poly(rA)-oligo(dT)<sub>12-18</sub>.

**Quality Assurance:** M-MuLV Reverse Transcriptase is tested for its ability to synthesize full length cDNAs from crude or purified RNA templates. Purified free of detectable levels of RNase, endonuclease and exonuclease activities.

### Quality Control Assays

**16-Hour Incubation:** Incubation of 100 units of enzyme with 1 µg of φX174 RF I DNA in 50 µl assay buffer at 37°C for 16 hours resulted in no detectable degradation of DNA as determined by gel electrophoresis.

**DNA Exonuclease Activity:** Incubation of 100 units of enzyme for 4 hours at 37°C in 50 µl assay buffer with 1 µg of mixed single and double-stranded sonicated <sup>3</sup>H DNA (10<sup>5</sup> cpm/µg), released < 0.15% of the radioactivity.

**RNase Activity:** Incubation of a 10 µl reaction containing 100 units of M-MuLV Reverse Transcriptase with 40 ng of RNA transcripts for 2 hours at 37°C resulted in no detectable degradation of the RNA as determined by gel electrophoresis.

**Heat Inactivation:** 65°C for 20 minutes.

### References:

1. Verma, I.M. (1975) *J. Virol.* 15, 843-854.
2. Gerard, G.F. and Grandgenett, D.P. (1975) *J. Virol.* 15, 785-797.
3. Roth, M.J., Tanese, N. and Goff, S.P. (1985) *J. Biol. Chem.* 260, 9326-9335.
4. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, (2nd ed.), (pp. 5.52-5.55, 8.11-8.17). Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

CERTIFICATE OF ANALYSIS

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