M-MuLV Reverse Transcriptase

NEW ENGLAND BioLabs Inc.
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M0253S 027140416041

M0253S

RR Yes

10,000 units **200,000 U/ml** **Lot: 0271404**

RECOMBINANT **Store at –20°C** **Exp: 4/16**

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 $MgCl_2$
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 $MgCl_2$, 10 mM dithiothreitol, 0.5 mM [^{3}H]-dTTP, 0.4 mM poly(rA)•oligo(dT)12–18.

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 ^{3}H DNA (10^5 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.

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