



M-MLV Reverse Transcriptase

Cat. No. 28025 -013

Lot No. _____ 40,000 units; 200 U/ μ l

Exp. Date: _____. Store at -20 °C (not frost-free).

Description :

Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT) uses single-stranded RNA or DNA in the presence of a primer to synthesize a complementary DNA strand. This enzyme is isolated (1) from *E. coli* expressing a portion of the *pol* gene of M-MLV on a plasmid (2, 3).

Components :

28025 -013 M-MLV RT	Lot No. _____
Y00146 5X First Strand Buffer	Lot No. _____
Y00147 0.1 M DTT	Lot No. _____

Unit Definition :

One unit of M-MLV RT incorporates 1 nmol dTTP into acid-precipitable material in 10 minutes at 37 °C, using poly(A)•oligo (dT)₂₅ as template•primer (4).

Storage Buffer :

20 mM Tris-HCl (pH 7.5)
1 mM DTT
0.01% (v/v) Nonidet-P40
0.1 mM Na₂EDTA
0.1 M NaCl
50% (v/v) glycerol

5X First Strand Buffer :

250 mM Tris-HCl (pH 8.3)
375 mM KCl
15 mM MgCl₂
This buffer does NOT contain actinomycin D, nucleotides, or DTT.
Refer to Functional Assay Conditions on reverse side further details.

Quality Control Assays : See Back Page

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This product is distributed for laboratory research use only. CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

For technical questions about this product, call the Life Technologies TECH-LINE™ (800) 828-6686.

Quality Control Assays :

This product has passed the following quality control assays:
SDS-polyacrylamide gel analysis for purity; functional absence of
endodeoxyribonuclease, 3' and 5' exodeoxyribonuclease, and ribonuclease activities;
yield and length of cDNA product.

Store the 5X First Strand Buffer and 0.1M DTT at -20 °C. Thaw the solutions at
room temperature just prior to use and refreeze immediately.

The enclosed buffers were assayed with the enzyme and met quality control
specifications.

Functional Assay Conditions :

50 mM Tris-HCl (pH 8.3)
75 mM KCl
10 mM DTT
3 mM MgCl₂
0.5 mM each dGTP, dATP, dTTP, dCTP
(1-10 μCi of [α-³²P]dCTP added as a tracer)
10 μg/ml oligo (dT)₁₂₋₁₈
20 μg/ml mRNA
200 units M-MLV RT
Reaction Volume: 20 μl
Incubation: 60 minutes at 37 °C

Use 200 units of M-MLV RT per μg of RNA (5) in a standard assay. However,
reaction volume and amounts of enzyme and mRNA should be tailored to the second-
strand method.

References:

1. D'Alessio, J. M., and Gerard, G. F. (unpublished).
2. Kotewicz, M., D'Alessio, J., Driftmeier, K., Blodgett, K., and Gerard, G. (1985) *Gene* 35 , 249.
3. Gerard, G. F., D'Alessio, J. M., Kotewicz, M. L., and Noon, M. C. (1986) *DNA* 5:4 , 271.
4. Houts, G. E., Miyagi, M., Ellis, C., Beard, A., and Beard, J. W. (1979) *J. Virol.* 29 , 517.
5. Gerard, G. (1985) *Focus* 7:1 , 1.