invitrogen™

M-MLV Reverse Transcriptase

Cat. Nos.	**Size:**
28025-013	**40,000 units**
28025-021	**200,000 units**
Conc: 200 U/µl	**Store at -20°C (non-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). The enzyme is used to synthesize first-strand cDNA up to 7 kb.

Components

M-MLV RT

5X First-Strand Buffer [250 mM Tris-HCl (pH 8.3 at room temperature), 375 mM KCl, 15 mM $MgCl_2$]

0.1 M DTT

Storage Buffer

20 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.01% (v/v) NP-40, 50% (v/v) glycerol

Storage Conditions

Store all components at -20°C in a non-frost-free freezer.

Additional Components

Thaw 5X First-Strand Buffer and 0.1 M DTT at room temperature just prior to use and refreeze immediately.

RNaseOUT™ Recombinant Ribonuclease Inhibitor (40 units/µl) is available separately from Invitrogen (Cat. No.: 10777-019).

Part No. 28025.PPS Doc. Rev: 100702

First-Strand cDNA Synthesis Using M-MLV RT

A 20-µl reaction volume can be used for 1 ng–5 µg of total RNA or 1–500 ng of mRNA.

1. Add the following components to a nuclease-free microcentrifuge tube:
   1 µl oligo $(dT)_{12\text{-}18}$ (500 µg/ml), or 50–250 ng random primers, or 2 pmole gene-specific primer
   1 ng to 5 µg total RNA or 1 ng to 500 ng of mRNA
   1 µl 10 mM dNTP Mix (10 mM each dATP, dGTP, dCTP and dTTP at neutral pH)
   Sterile, distilled water to 12 µl
2. Heat mixture to 65°C for 5 min and quick chill on ice. Collect the contents of the tube by brief centrifugation and add:
   4 µl 5X First-Strand Buffer
   2 µl 0.1 M DTT
   1 µl RNaseOUT™ Recombinant Ribonuclease Inhibitor (40 units/µl) (**Note:** When using less than 50 ng of starting RNA, the addition of RNaseOUT™ is essential.)
3. Mix contents of the tube gently and incubate at 37°C for 2 min.
4. Add 1 µl (200 units) of M-MLV RT,[a] and mix by pipetting gently up and down. If using random primers, incubate tube at 25°C for 10 min.
5. Incubate 50 min at 37°C.
6. Inactivate the reaction by heating at 70°C for 15 min.

The cDNA can now be used as a template for amplification in PCR. However, amplification of some PCR targets (>1 kb) may require the removal of RNA complementary to the cDNA. To remove RNA complementary to the cDNA, add 1 µl (2 units) of *E. coli* RNase H and incubate at 37°C for 20 min.

[a] If less than 1 ng of RNA is used, reduce the amount of M-MLV RT in the reaction to 0.25 µl (50 units), and add the sterile, distilled water to 20-µl final volume.

PCR Reaction

Use only 10% of the first-strand reaction (2 µl of the reaction from the previous page) for PCR. Adding larger amounts of the first-strand reaction may not increase amplification and may result in decreased amounts of PCR product.

1. Add the following to a PCR reaction tube for a final reaction volume of 50 µl:

5 µl	10X PCR Buffer [200 mM Tris-HCl (pH 8.4), 500 mM KCl]
1.5 µl	50 mM $MgCl_2$[b]
1 µl	10 mM dNTP Mix
1 µl	amplification primer 1 (10 µM)
1 µl	amplification primer 2 (10 µM)
0.4 µl	*Taq* DNA polymerase (5 U/µl)
2 µl	cDNA (from first-strand reaction)
38.1 µl	autoclaved, distilled water

2. Mix gently and layer 1–2 drops (~50 µl) of silicone oil over the reaction. *(Note: the addition of silicone oil is unnecessary in thermal cyclers equipped with a heated lid.)*
3. Heat reaction to 94°C for 2 min to denature.
4. Perform 15 to 40 cycles of PCR. Annealing and extension conditions are primer and template dependent and must be determined empirically.

[b] For best results, determine the optimal concentration of $MgCl_2$ empirically for each template-primer pair.

Quality Control

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.

Unit Definition

One unit incorporates 1 nmole of dTTP into acid-precipitable material in 10 min at 37°C using poly(A)•oligo$(dT)_{25}$ as template-primer (4).

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.

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