M-MLV Reverse Transcriptase

Cat. No.
R 7501 (10,000 units)
R 7502 (50,000 units)
R 7503 (100,000 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 from *E. coli* expressing a portion of the *pol* gene of M-MLV on a plasmid(1,2). The enzyme is used to synthesize first-strand cDNA up to 7 kb.

Kit Size

Component	10,000 U	50,000 U	100,000 U
M-MLV Reverse Transcriptase	50 µl	5 x 50 µl	5 x 100 µl
5 x Reaction Buffer	1ml	5 x 1ml	10 x 1ml

Storage Buffer

20 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.01% (v/v) Nonidet P-40, 50% (v/v) glycerol, in DEPC treated water

5 X Reaction Buffer

250 mM Tris-HCl (pH 8.3 at 25°C), 375 mM KCl, 15 mM $MgCl_2$, 0.1 M DTT

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 (3).

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)12-18 (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 Reaction Buffer
   - 2 µl 0.1 M DTT
   - 1 µl Ribonuclease Inhibitor (40 units/µl)

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.

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 [100 mM Tris-HCl (pH 8.3), 500 mM KCl]
   - 1.5 µl 50 mM MgCl2
   - 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. For best results, determine the optimal concentration of MgCl2 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.

References

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