SIGMA-ALDRICH®

sigma-aldrich.com

3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

M-MLV Reverse Transcriptase,
recombinant,
expressed in *E. coli*

Product Code **M 1302**

Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

M-MLV (Moloney Murine Leukemia Virus) Reverse Transcriptase is a DNA polymerase that uses DNA, single-stranded RNA, or an RNA:DNA hybrid (using 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] M-MLV is used for the preparation of cDNA libraries or for first strand cDNA synthesis for use in RT-PCR† reactions.

Unit Definition: One unit incorporates 1 nmole of TTP into acid precipitable material in 10 minutes at 37 °C.[2]

Components

- M-MLV Reverse Transcriptase,
  Product Code M1427
  200 units/µl in 20mM Tris-HCl (pH 7.5), 200mM NaCl, 0.1mM EDTA, 1mM DTT, 0.01% Nonidet® P-40 and 50% glycerol.
  Provided as 40,000 or 200,000 units
- 10X M-MLV Reverse Transcriptase Buffer,
  Product Code B8559
  500 mM Tris-HCl, pH 8.3, with 500 mM KCl, 30 mM MgCl2, and 50 mM DTT
  Provided as 0.5 mL vials, 1 vial/40,000 units and 4 vials/200,000 units

Reagents Required but Not Provided

(Product Codes have been given where appropriate.)

- Deoxynucleotide Mix, Product Code D7295,
  10 mM dATP, 10 mM dCTP, 10 mM dGTP, 10 mM dTTP
- Water, Product Code W1754
- Choice of specific primer (user defined), anchored oligo $d(T)_{23}$ primers (Product Code O4387) or random nonamers (Product Code R7647).
- RNA template
- Ribonuclease Inhibitor, Product Code R2520

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Procedure

The following procedure uses 1-5 µg of total RNA or approximately 50 ng of mRNA.

1. In a clean, nuclease-free 0.5 ml or 0.2 ml thin-walled microcentrifuge tube, add the following:

   1 µl 10 mM dNTP mix
   1 µl Specific primer, random nonamers or oligo$(dT)_{23}$ (final concentration 1-5 µM)
   1 µl RNA template
   g.s. Nuclease-free water
   10 µl Total volume

2. Mix gently and briefly centrifuge to collect all components to the bottom of the tube.

3. Incubate at 70 °C for 10 minutes.

4. Remove the tubes and place on ice.

5. Add the remaining components to the microcentrifuge tube:

   2 µl 10X M-MLV Reverse Transcriptase Buffer
   1 µl M-MLV Reverse Transcriptase
   0.5 µl RNase Inhibitor (40 units/µl)
   6.5 µl Nuclease-free water
   20 µl Final Volume

6. Incubate at 37 °C for 50 minutes.
   Optional step: Incubate reaction at room temperature for 10 minutes prior to the 37 °C incubation for 50 minutes. This will ensure elongation of random primers or anchored oligo$(dT)_{23}$ primers before the higher reverse transcriptase temperature.

7. The cDNA strand has now been produced. Heat the reaction tube between 80 °C and 94 °C for 10 minutes to denature the M-MLV reverse transcriptase.

References

1. Gerard, G.F., *et al*., Influence on stability in *Escherichia coli* of the carboxy-terminal structure of cloned Moloney murine leukemia virus reverse transcriptase. *DNA,* **5**:271-9 (1986).

2. Houts, G.E., *et al*.. Reverse transcriptase from avian myeloblastosis virus. *J. Virol*. **29:**517-22 (1979).

3. Howland, P., *et al.* Positive- and negative-acting promoter sequences regulate cell type-specific expression of the rat synapsin I gene. Mol. Brain Res. **11**:345-53.

4. Gerard, G.F. and D'Alessio, J.M. *Methods in Molecular Biology* Vol. 16: Enzymes of Molecular Biology, (Buwell, M. Ed.) Humana Press, Totowa, N.J., p. 73 (1993)

†The PCR process is covered by patents owned by Hoffman-LaRoche, Inc.

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