

M-MLV Reverse Transcriptase

(Catalogue number M023, M024)

rev. 01/2022

Description

Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT) is an enzyme for cDNA synthesis from single-stranded RNA in the presence of oligonucleotide primer.

Technical data

Enzyme concentration

- M-MLV RT is supplied at a concentration 200 U/μl.

Components and packaging

- 1 tube with M-MLV RT; 2 000 U/10 μl.
- 1 tube with 5x concentrated M-MLV RT react buffer, 200 μl.
- 1 tube of 100 mM dithiothreitol (DTT), 100 μl.

Unit definition

- One unit of M-MLV RT is defined as the amount of the enzyme which incorporates 1 nmol of dTTP into acid precipitable material during 10 min at 37°C; poly(A)-oligo (dT)₁₂₋₁₈ is used as a template.

Storage

- At temperature -20°C ± 5°C.

Purity

- The enzyme is free of nucleases and is suitable for performing cDNA synthesis.

Composition

- Storage buffer: 20 mM Tris-HCl (pH 7.5 at 22°C), 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.01% Nonidet P-40, 50% (vol/vol) glycerol.
- 5x concentrated M-MLV RT buffer (1st-strand buffer): 250 mM Tris-HCl (pH 8.3 at 22°C), 375 mM KCl, 15 mM MgCl₂, stabilizer.

Protocol

Recommended protocol for 1st-strand cDNA synthesis

1. The following components are added to nuclease-free microcentrifuge tube:
 - 4 μl PCR H₂O.
 - 1 μl of total RNA template at a concentration 1 mg/ml. When different concentration is used, amount of RNA and H₂O must be adjusted (final volume 5 μl).
 - 0.5 μl oligo dT₁₂₋₁₈ primer, or other primer (50 μM).
 - 0.5 μl 10 mM dNTP Mix (each nucleotide at a concentration of 10 mM).
2. Briefly centrifuge the tube, incubate at 65°C for 5 min, quickly chill on ice and add:
 - 2 μl 5x concentrated M-MLV RT buffer.
 - 1 μl 100 mM DTT.
 - 1 μl (25 U) M-MLV RT (after dilution of M-MLV in 1 x M-MLV RT react buffer 1 : 8).
3. Incubate 1 h at 37°C.
4. Heat the reaction tube at 80°C for 15 min to denature M-MLV-RT.

Final concentrations are: 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl₂, 10 mM DTT, 0.5 mM dGTP, 0.5 mM dATP, 0.5 mM dTTP, 0.5 mM dCTP, 2.5 μM oligo dT₁₂₋₁₈ (or other primer), 100 μg RNA/ml, 2500 U M-MLV-RT/ml.

Cat. No.	Product name and specification	Amount
M023	M-MLV Reverse Transcriptase	2 000 U
M024	M-MLV Reverse Transcriptase	5x 2 000 U

