

## M-MLV Reverse Transcriptase

(Catalogue number M023, M024)

rev. 04/2025

### 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/ $\mu$ l.

#### Components and packaging

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

#### 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)<sub>12-18</sub> is used as a template.

#### Storage

- At temperature -20°C  $\pm$  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 MgCl<sub>2</sub>, stabilizer.

### Protocol

#### Recommended protocol for 1st-strand cDNA synthesis

- The following components are added to nuclease-free microcentrifuge tube:
  - 4  $\mu$ l PCR H<sub>2</sub>O.
  - 1  $\mu$ l of total RNA template at a concentration 1 mg/ml. When different concentration is used, amount of RNA and H<sub>2</sub>O must be adjusted (final volume 5  $\mu$ l).
  - 0.5  $\mu$ l oligo dT<sub>12-18</sub> primer, or other primer (50  $\mu$ M).
  - 0.5  $\mu$ l 10 mM dNTP Mix (each nucleotide at a concentration of 10 mM).
- Briefly centrifuge the tube, incubate at 65°C for 5 min, quickly chill on ice and add:
  - 2  $\mu$ l 5x concentrated M-MLV RT buffer.
  - 1  $\mu$ l 100 mM DTT.
  - 1  $\mu$ l (25 U) M-MLV RT (after dilution of M-MLV in 1 x M-MLV RT react buffer 1 : 8).
- Incubate 1 h at 37°C.
- 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 MgCl<sub>2</sub>, 10 mM DTT, 0.5 mM dGTP, 0.5 mM dATP, 0.5 mM dTTP, 0.5 mM dCTP, 2.5  $\mu$ M oligo dT<sub>12-18</sub> (or other primer), 100  $\mu$ g 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

