M-MLV Reverse Transcriptase

Catalog Number 28025-013 and 28025-021

Doc. Part No. 28025.PPS **Pub. No.** MAN0001462 **Rev.** A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product 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. The enzyme is used to synthesize first-strand cDNA up to 7 kb.

Contents and storage

Component	Quantity	Storage conditions
M-MLV RT (200 U/µL)	40,000 units (Cat. no. 28025-013) 200,000 units (Cat. no. 28025-021)	• Store at –20°C (non-frost-free). • Refreeze 5X First-Strand Buffer and 0.1 M DTT immediately after use.
5X First-Strand Buffer[1]	1 mL	
DTT	0.1 M	

[1] 250 mM Tris-HCl (pH 8.3 at room temperature), 375 mM KCl, 15 mM $MgCl_2$

Required materials not provided

RNaseOUT™ Recombinant Ribonuclease Inhibitor (40 units/µL; Cat. no. 10777-019).

Before you begin

- Prepare 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
- Thaw 5X First-Strand Buffer and 0.1 M DTT at room temperature just before use Note: Refreeze immediately after use.

Synthesize first-strand cDNA 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:

Oligo $(dT)_{12-18}$ (500 µg/mL), or 50–250 ng random primers, or 2 pmole gene-specific primer	1 µL
Total RNA, or mRNA	1 ng to 5 µg total RNA, or 1 ng to 500 ng of mRNA
10 mM dNTP Mix (10 mM each dATP, dGTP, dCTP and dTTP at neutral pH)	1 µL
Sterile, distilled water	To 12 µL

2. Heat mixture to 65°C for 5 minutes and quick chill on ice. Collect the contents of the tube by brief centrifugation and add:

5X First-Strand Buffer	4 µL
0.1 M DTT	2 µL
RNaseOUT™ Recombinant Ribonuclease Inhibitor (40 units/µL)	1 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 minutes.
4. Add 1 µL (200 units) of M-MLV RT, and mix by pipetting gently up and down. If using random primers, incubate tube at 25°C for 10 minutes.

   Note: 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.
5. Incubate 50 minutes at 37°C.
6. Inactivate the reaction by heating at 70°C for 15 minutes.

   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 minutes.

Prepare PCR reaction

Use only 10% of the first-strand reaction (2 µL of the reaction from "Synthesize first-strand cDNA using M-MLV RT" on page 2) 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:

10X PCR Buffer [200 mM Tris-HCl (pH 8.4), 500 mM KCl]	5 µL
50 mM $MgCl_2$[1]	1.5 µL
Amplification primer 1 (10 µM)	1 µL
Amplification primer 2 (10 µM)	1 µL
Taq DNA polymerase (5 U/µL)	0.4 µL
cDNA[2]	2 µL
Autoclaved, distilled water	38.1 µL

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

[2] From "Synthesize first-strand cDNA using M-MLV RT" on page 2.

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 minutes to denature.
4. Perform 15 to 40 cycles of PCR. Annealing and extension conditions are primer and template dependent and must be determined empirically.

Unit definition

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

Limited product warranty

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