invitrogen

PRODUCT INFORMATION SHEET

M-MLV Reverse Transcriptase (200 U/µL)

Catalog Numbers 28025013 and 28025021

Doc. Part No. 28025.PPS **Pub. No.** MAN0001462 **Rev.** B.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. M-MLV RT is isolated from *E. coli* expressing a portion of the *pol* gene of M-MLV on a plasmid. This enzyme is used to synthesize first-strand cDNA up to 7 kb.

Contents and storage

Component	Cat. No. 28025013 (40,000 units)	Cat. No. 28025021 (200,000 units)	Storage conditions
M-MLV Reverse Transcriptase (200 U/µL)[1]	200 µL	1 mL	Store at –20°C (non-frost-free).
5X First-Strand Buffer[2]	1 mL	4 × 1 mL	
0.1M DTT[3]	500 µL	4 × 500 µL	

[1] Storage buffer composition: 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

[2] 250 mM Tris-HCl (pH 8.3 at room temperature), 375 mM KCl, 15 mM $MgCl_2$

[3] Refreeze immediately after use.

Required materials not supplied

RNaseOUT™ Recombinant Ribonuclease Inhibitor (40 units/µL; Cat. No. 10777019).

Before you begin

Thaw 5X First-Strand Buffer and 0.1 M DTT at room temperature just before use

Note: Refreeze immediately after use.

For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

Synthesize first-strand cDNA using M-MLV RT

Use a 20-µL reaction volume 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:

Component	Volume
Oligo $(dT)_{12\text{-}18}$ (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, then quick chill on ice. Collect the contents of the tube by brief centrifugation, then add the following components:

Component	Volume
5X First-Strand Buffer	4 µL
100 mM DTT	2 µL
RNaseOUT™ Recombinant Ribonuclease Inhibitor (40 units/µL)[1]	1 µL

[1] RNaseOUT™ is particularly essential when using less than 50 ng of starting RNA.

3. Mix contents of the tube gently, then incubate at 37°C for 2 minutes.
4. Add 1 µL (200 units) of M-MLV RT, then mix by pipetting gently up and down. If using random primers, incubate tube for 10 minutes at 25°C .

   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), then add the sterile, distilled water to a final volume of 20 µL.
5. Incubate at 37°C for 50 minutes.
6. Heat the reaction at 70°C for 15 minutes to inactivate the enzyme.

(*Optional*) Remove template RNA

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.

1. Add 1 µL (2 units) of *E. coli* RNase H.
2. Incubate for 20 minutes at 37°C.

Prepare and run PCR reaction

1. Add the following components to a PCR reaction tube:

Component	Volume
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	39.1 µL
Total reaction volume	**50 µL**

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

[2] For the PCR reaction, use only 10%, or 2 µL, of the first-strand reaction from “Synthesize first-strand cDNA using M-MLV RT” on page 2. Adding larger amounts of the first-strand reaction may not increase amplification, and may result in decreased amounts of PCR product.

2. Mix gently, then centrifuge the sample.
3. Perform PCR reaction according to the supplier instructions.

Unit definition

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

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Life Technologies Corporation | 5781 Van Allen Way | Carlsbad, California 92008 USA

For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

The information in this guide is subject to change without notice.

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Important Licensing Information: These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

©2022 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified.

ThermoFisher SCIENTIFIC