



PRODUCT INFORMATION

M-MuLV Reverse Transcriptase

Pub. No. MAN0012025

Rev. Date 17 June 2016 (Rev. B.00)

#EP0352

Lot: _ Expiry Date: _

Components	#EP0352
M-MuLV Reverse Transcriptase, 20 U/ μ L	5000 U
5X Reaction Buffer	5 \times 1 mL

Store at -20 °C

www.thermofisher.com

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

Description

M-MuLV Reverse Transcriptase (RT) is an RNA- and DNA-dependent DNA polymerase. The enzyme possesses a ribonuclease H activity specific to RNA in RNA-DNA hybrids (1, 2). M-MuLV RT incorporates modified nucleotides.

Source

E.coli cells with a cloned fragment of the *pol* gene encoding Moloney Murine Leukemia Virus reverse transcriptase.

Applications

- First strand cDNA synthesis for RT-PCR and real-time RT-PCR, see protocol on back page.
- Synthesis of cDNA for cloning and expression.
- Generation of labeled cDNA probes for microarrays.
- DNA labeling (3).
- Analysis of RNA by primer extension (3).

Definition of Activity Unit

One unit of the enzyme incorporates 1 nmol of dTMP into a polynucleotide fraction in 10 min at 37 °C.

Storage Buffer

The enzyme is supplied in: 50 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 1 mM EDTA, 5 mM DTT, 0.1% Triton X-100 and 50% glycerol.

5X Reaction Buffer

250 mM Tris-HCl (pH 8.3 at 25 °C), 250 mM KCl, 20 mM MgCl₂, 50 mM DTT.

Inhibition and Inactivation

- Inhibitors: metal chelators, inorganic phosphate, pyrophosphate and polyamines (3).
- Inactivated by heating at 70 °C for 10 min.

Note

M-MuLV RT has significantly lower RNase H activity than Avian Myeloblastosis Virus (AMV) reverse transcriptase.

CERTIFICATE OF ANALYSIS**Endodeoxyribonuclease Assay**

No detectable degradation was observed after incubation of supercoiled plasmid DNA with M-MuLV Reverse Transcriptase.

Ribonuclease Assay

No detectable degradation was observed after incubation of [3H]-RNA with M-MuLV Reverse Transcriptase.

Labeled Oligonucleotide (LO) Assay

No detectable degradation after incubation of single-stranded or double-stranded radiolabeled oligonucleotides with M-MuLV Reverse Transcriptase.

Functional Assay

M-MuLV Reverse Transcriptase was tested for use in the first strand cDNA synthesis.

Quality authorized by: 

Jurgita Zilinskiene

(continued on back page)

Protocol for First Strand cDNA Synthesis

The following protocol is optimized to generate first-strand cDNA for use in two-step RT-PCR.

Mix and briefly centrifuge all components after thawing, keep on ice.

1. Add into sterile, nuclease-free tube on ice in the indicated order:

Template RNA	total RNA	100 ng-5 µg
	or poly(A) RNA	10-500 ng
	or specific RNA	0.01 pg-0.5 µg
Primer	Oligo(dT) ₁₈ (#SO131)	0.5 µg (100 pmol)
	or Random hexamer (#SO141)	0.2 µg (100 pmol)
	or gene-specific primer	15-20 pmol
DEPC-treated water (#R0601)		to 11.5 µL

2. **Optional:** If RNA template is GC rich or is known to contain secondary structures, mix gently, centrifuge briefly and incubate at 65 °C for 5 min, chill on ice, briefly centrifuge and place on ice.

3. Add the following components in the indicated order:

5X reaction buffer	4 µL
Thermo Scientific RiboLock RNase Inhibitor (#EO0381)	0.5 µL (20 U)
dNTP Mix, 10 mM each (#R0191)	2 µL (1 mM final concentration)
M-MuLV Reverse Transcriptase	2 µL (40 U)
Total volume	20 µL

Mix gently and centrifuge briefly.

4. If oligo(dT)₁₈ primer or gene-specific primer is used, incubate 60 min at 37 °C.
If random hexamer primer is used, incubate 10 min at 25 °C followed by 60 min at 37 °C.
For transcription of GC rich RNA reaction temperature can be increased to 45 °C.
5. Terminate the reaction by heating at 70 °C for 10 min.
Do not heat-inactivate enzyme prior to analysis of long cDNA to avoid cleavage.

Note

- The reverse transcription reaction product can be directly used in PCR or stored at -20 °C.
- Use 2 µL of the reaction mix to perform PCR in 50 µL volume.

References

1. Verma, I.M., Reverse transcriptase, The Enzymes (Boyer, P.D., ed), Academic Press Inc., vol. 14, 87-103, 1981.
2. Gerard, G.F. and D'Alessio, J.M., Methods in Molecular Biology, 16, Humana Press, Totowa, N.J., 73-93, 1993.
3. Sambrook, J., Russell, D.W., Molecular Cloning: A Laboratory Manual, the third edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001.

LIMITED USE LABEL LICENSE: Internal Research and Development Use Only.

The purchase of this product conveys to the buyer the limited, non-exclusive, non-transferable right (without the right to resell, repackage, or further sublicense) to use this product for internal research and development purposes. No other license is granted to the buyer whether expressly, by implication, by estoppel or otherwise. In particular, the purchase of the product does not include or carry any right or license to use, develop, or otherwise exploit this product commercially and no rights are conveyed to the buyer to use the product or components of the product for purposes including but not limited to provision of services to a third party, generation of commercial databases or clinical diagnostics. This product is sold pursuant to authorization from Thermo Fisher Scientific and Thermo Fisher Scientific reserves all other rights. For information on purchasing a license for uses other than internal research and development purposes, please contact outlicensing@lifetech.com or Out Licensing, Life Technologies Inc., 5791 Van Allen Way, Carlsbad, California 92008.

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively *for research purposes and in vitro use only*. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. Please refer to www.thermofisher.com for Material Safety Data Sheet of the product.

© 2016 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries.