

**CERTIFICATE OF ANALYSIS**

# M-MuLV Reverse Transcriptase

**#EP0352**      5000 u

**Lot:**                      **Expiry Date:**

Concentration:      20 u/μl  
Supplied with:      5 x 1 ml of 5X Reaction Buffer

**Store at -20°C**

In total 6 vials.

## Description

M-MuLV Reverse Transcriptase (RT) is an RNA- and DNA-dependent DNA polymerase. It can use either RNA or DNA to prime DNA synthesis from an RNA or DNA template. The enzyme possesses a ribonuclease H activity specific to RNA in RNA-DNA hybrids (1, 2).

## Source

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

## Features

- High yields of first strand cDNA up to 9 kb.
- Optimum of activity at 37°C.
- Incorporates modified nucleotides (e.g. Cy3-, Cy5-, rhodamine-, aminoallyl-, fluorescein-labeled nucleotides).

## Applications

- First strand cDNA synthesis, see protocol on back page.
- RT-PCR and real-time RT-PCR.
- Synthesis of cDNA for cloning and expression.
- Generation of labeled cDNA probes for micro-arrays.
- 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 (adsorbed on DE-81) in 10 min at 37°C.

Enzyme activity is assayed in the following mixture: 50 mM Tris-HCl (pH 8.3), 6 mM MgCl<sub>2</sub>, 10 mM DTT, 40 mM KCl, 0.5 mM dTTP, 0.4 MBq/ml [<sup>3</sup>H]-dTTP, 0.4 mM polyA·oligo (dT)<sub>12-18</sub>.

## 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 MgCl<sub>2</sub>, 50 mM DTT.

## Inhibition and Inactivation

- Inhibitors: metal chelators, inorganic phosphate, pyrophosphate and polyamines (2).
- Inactivated by heating at 70°C for 10 min.

## Note

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

## QUALITY CONTROL ASSAY DATA

### Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 200 units of the enzyme with 1 µg of pBR322 DNA in 50 µl of reaction buffer for 4 hours at 37°C.

### Exodeoxyribonuclease Assay

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 200 units of the enzyme with 1 µg of sonicated *E. coli* [<sup>3</sup>H]-DNA in 50 µl of reaction buffer for 4 hours at 37°C.

### Labeled Oligonucleotide (LO) Assay

No detectable degradation of a single-stranded and double-stranded labeled oligonucleotide was observed after incubation with 200 units of the enzyme for 4 hours at 37°C.

### Ribonuclease Assay

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 200 units of the enzyme with 1 µg of [<sup>3</sup>H]-RNA in 50 µl of reaction buffer for 4 hours at 37°C.

### Functional Assay

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

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 $\mu$ g
	<i>or</i> poly(A) RNA	10-500 ng
	<i>or</i> specific RNA	0.01 $\mu$ g-0.5 $\mu$ g
Primer	Oligo(dT) <sub>18</sub> (#S0131)	0.5 $\mu$ g (100 pmol)
	<i>or</i> Random hexamer (#S0141)	0.2 $\mu$ g (100 pmol)
	<i>or</i> gene-specific primer	15-20 pmol
DEPC-treated water (#R0601)		to 12.5 $\mu$ l

- 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 $\mu$ l
RiboLock™ RNase Inhibitor (#E00381)	0.5 $\mu$ l (20 u)
dNTP Mix, 10 mM each (#R0191)	2 $\mu$ l (1 mM final concentration)
M-MuLV Reverse Transcriptase	2 $\mu$ l (40 u)
<b>Total volume</b>	<b>20 <math>\mu</math>l</b>

Mix gently and centrifuge briefly.

- 4 If oligo(dT)<sub>18</sub> 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  $\mu$ l of the reaction mix to perform PCR in 50  $\mu$ l 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.

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### 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.fermentas.com](http://www.fermentas.com) for Material Safety Data Sheet of the product.

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## Related Products

- Oligo(dT)<sub>18</sub> Primer #S0131
- Random Hexamer Primer #S0141
- dNTP Mix, 10 mM each #R0191
- dNTP Mix, 25 mM each #R1121
- Aminoallyl-dUTP #R0091
- Biotin-11-dUTP #R0081
- Fluorescein-12-dUTP\* #R0101
- RiboLock™ RNase Inhibitor #E00381
- DreamTaq™ DNA Polymerase #EP0701
- Taq DNA Polymerase (recombinant) #EP0401
- Pfu DNA polymerase (native)\* #EP0571
- Pfu DNA polymerase (recombinant)\* #EP0501
- PCR Master Mix (2X) #K0171
- Maxima™ Hot Start Taq DNA Polymerase #EP0601
- TrueStart™ Taq DNA Polymerase #EP0611
- PyroStart™ Fast PCR Master Mix (2X) #K0211
- High Fidelity PCR Enzyme Mix\* #K0191
- Long PCR Enzyme Mix\* #K0181
- DNase I, RNase-free #EN0521
- RNase H, *E.coli* #EN0201
- DNA Polymerase I, *E.coli* #EP0041
- DEPC-treated Water #R0601
- Water, nuclease-free #R0581

\* not available in the USA