Thermo SCIENTIFIC

PRODUCT INFORMATION

M-MuLV Reverse Transcriptase

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#EP0352

Lot: _ **Expiry Date: _**

Components	#EP0352
M-MuLV Reverse Transcriptase, 20 U/µL	5000 U
5X Reaction Buffer	5 × 1 mL

Store at -20 °C

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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 $MgCl_2$, 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

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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 *or* poly(A) RNA *or* specific RNA	100 ng-5 µg 10-500 ng 0.01 pg-0.5 µg
Primer	Oligo(dT)$_{18}$ (#SO131) *or* Random hexamer (#SO141) *or* gene-specific primer	0.5 µg (100 pmol) 0.2 µg (100 pmol) 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.