

Product Contents

M-MLV Reverse Transcriptase, RNase H Minus:

Part No.	Size (units)
M530B	2,500
M530A	10,000

Enzyme Storage Buffer: M-MLV Reverse Transcriptase is supplied in 20mM Tris-HCl (pH 7.5), 200mM NaCl, 0.1mM EDTA, 1mM DTT, 0.01% Nonidet® P-40 and 50% glycerol.

M-MLV Reverse Transcriptase 5X Reaction Buffer (M531A): When the M-MLV Reverse Transcriptase 5X Reaction Buffer supplied with this enzyme is diluted 1:5, it has a composition of 50mM Tris-HCl (pH 8.3 at 25°C), 75mM KCl, 3mM MgCl₂ and 10mM DTT.

Source: Purified from an *E. coli* strain expressing a recombinant clone (1).

Storage Conditions: See the product information label for storage recommendations. Avoid exposure to frequent temperature changes. See the expiration date on the product information label.

Unit Definition: One unit is defined as the amount of enzyme required to catalyze the transfer of 1nmol of deoxynucleotide into acid-precipitable material in 10 minutes at 37°C. The reaction conditions are: 50mM Tris-HCl (pH 8.3), 7mM MgCl₂, 40mM KCl, 10mM DTT, 0.1mg/ml BSA, 0.5mM [³H]dTTP, 0.025mM oligo(dT)₅₀, 0.25mM poly(A)₄₀₀ and 0.01% NP-40. See the unit concentration on the product information label.

Usage Note: M-MLV Reverse Transcriptase is less processive than AMV Reverse Transcriptase, and therefore, more units of the M-MLV enzyme are required to generate the same amount of cDNA as in the AMV reaction.

Part# 9PIM530

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Quality Control Assays

Contaminant Activity

DNase and RNase Assay: To test for nuclease activity, 50ng of radiolabeled DNA or radiolabeled RNA is incubated with 200 units of M-MLV Reverse Transcriptase, RNase H Minus, in M-MLV Reverse Transcriptase 1X Reaction Buffer for one hour at 37°C, and the release of radiolabeled nucleotides is monitored by scintillation counting of TCA-soluble material. Minimum passing specification is <1% release for DNase and <3% release for RNase.

Endonuclease Assay: To test for endonuclease activity, 1µg of Type I supercoiled plasmid DNA is incubated with 200 units of M-MLV Reverse Transcriptase, RNase H Minus, in M-MLV Reverse Transcriptase 1X Reaction Buffer for one hour at 37°C. Following incubation, the supercoiled DNA is visualized on an ethidium bromide-stained agarose gel to verify the absence of visible nicking or cutting.

Physical Purity: The purity is >90% as judged by SDS-polyacrylamide gels with Coomassie® blue staining.

RNase H Activity: To test for RNase H activity, 20µM of radiolabeled poly(rA):poly(dT) is incubated with 200 units of M-MLV Reverse Transcriptase, RNase H Minus, in M-MLV Reverse Transcriptase 1X Reaction Buffer for one hour at 37°C. The release of radiolabeled nucleotides is monitored by scintillation counting of TCA-soluble material. Minimum passing specification is <1%.

Functional Assay

First-Strand cDNA Synthesis: First-strand cDNAs, of 1.2kb and 7.5kb control RNAs, are synthesized by incubating 200 units of M-MLV Reverse Transcriptase, RNase H Minus, 1µg of each template, an oligo(dT)₁₅ primer and radiolabeled dCTP in M-MLV Reverse Transcriptase 1X Reaction Buffer for one hour at 37°C. The minimum specification is the production of 120ng of first-strand cDNA. As visualized by gel electrophoresis and autoradiography, >90% of the cDNA must migrate at 1.2kb for the 1.2kb RNA, and >25% of the cDNA must migrate at 7.5kb for the 7.5kb RNA.

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Part# 9PIM530

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I. Description

Moloney Murine Leukemia Virus Reverse Transcriptase, RNase H Minus (M-MLV RT), is an RNA-dependent DNA polymerase that can be used in cDNA synthesis with long messenger RNA templates (>5kb). This is a form of M-MLV Reverse Transcriptase that has been genetically altered to remove the associated RNase H activity (2). Although many researchers are successful in using M-MLV RT (H+) for analytical and some preparative cDNA applications, reverse transcriptases lacking RNase H activity provide another option for the preparation of long cDNAs and libraries containing a high percentage of full-length cDNA.

An application of M-MLV RT is first-strand synthesis of cDNA from RNA molecules.

Note: M-MLV Reverse Transcriptase is less processive than AMV Reverse Transcriptase, and therefore, more units of the M-MLV enzyme are required to generate the same amount of cDNA as in the AMV reaction.

II. Standard Application

A. First-Strand Synthesis of cDNA

Materials to Be Supplied by the User

- dATP, 10mM (Cat.# U1201, 100mM)
 - dCTP, 10mM (Cat.# U1221, 100mM)
 - dGTP, 10mM (Cat.# U1211, 100mM)
 - dTTP, 10mM (Cat.# U1231, 100mM)
 - Nuclease-Free Water (Cat.# P1193)
1. A typical procedure uses 1µg of RNA. In a sterile RNase-free microcentrifuge tube, add 1µg of the primer or primer-adaptor per microgram of the mRNA sample in a total volume of ≤15µl in water.
 2. Heat the tube to 70°C for 5 minutes to melt secondary structure within the template.
 3. Cool the tube immediately on ice to prevent secondary structure from reforming, then spin briefly to collect the solution at the bottom of the tube.
 4. Add the following components to the annealed primer/template in the order shown.

M-MLV 5X Reaction Buffer	5µl
dATP, 10mM	1.25µl
dCTP, 10mM	1.25µl
dGTP, 10mM	1.25µl
dTTP, 10mM	1.25µl
M-MLV RT	<u>200 units</u>
Nuclease-Free Water to final volume of	25µl

5. Mix gently by flicking the tube, and incubate for 60 minutes at 37°C for random primers or 42°C for other primers or primer-adaptors.
6. Perform second-strand synthesis using a protocol of your choice. Standard protocols for second-strand synthesis may be found in reference 3.

Notes:

1. The M-MLV RT Reaction Buffer is compatible with enzymes used in a number of downstream applications. Phenol extractions and ethanol precipitations typically are not necessary before performing second-strand synthesis and amplification.
2. If there is concern about possible RNase contamination in the reaction, Recombinant RNasin® Ribonuclease Inhibitor (Cat.# N2511) may be added to the reaction (1u/µl) to preserve RNA integrity.

III. Composition of Buffer

M-MLV RT 5X Reaction Buffer (provided)

250mM	Tris-HCl (pH 8.3 at 25°C)
375mM	KCl
15mM	MgCl ₂
50mM	DTT

IV. References

1. Roth, M.J., Tanese, N. and Goff, S.P. (1985) Purification and characterization of murine retroviral reverse transcriptase expressed in *Escherichia coli*. *J. Biol. Chem.* **260**, 9326–35.
2. Tanese, N. and Goff, S.P. (1988) Domain structure of the Moloney murine leukemia virus reverse transcriptase: mutational analysis and separate expression of the DNA polymerase and RNase H activities. *Proc. Natl. Acad. Sci. USA* **85**, 1777–81.
3. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 8.64.