Certificate of Analysis

M-MLV Reverse Transcriptase, RNase H Minus:

Part# 9PIM530
Revised 1/24

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

AF9PIM530 0124M530

Description: Moloney Murine Leukemia Virus Reverse Transcriptase, RNase H Minus [M-MLV RT (H–)], 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 (1). 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.

Applications of M-MLV RT (H–), are first-strand cDNA synthesis from RNA molecules and RT-PCR.

Enzyme Storage Buffer: M-MLV RT (H–) 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), 75mM KCl, 3mM $MgCl_2$ and 10mM DTT.

Source: *E. coli* cells expressing a recombinant clone.

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

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

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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 RT (H–) in M-MLV Reverse Transcriptase 1X Reaction Buffer for 1 hour at 37°C, and the release of radiolabeled nucleotides is monitored by scintillation counting of TCA-soluble material. The 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 RT (H–) in M-MLV Reverse Transcriptase 1X Reaction Buffer for 1 hour at 37°C. Following incubation, the 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: One unit of RNase H is defined as the amount of enzyme required to produce 1nmol of acid-soluble ribonucleotides from [^{3}H]poly(rA):poly(dT) in 20 minutes at 37°C in a reaction containing 20mM HEPES-KOH (pH 7.8), 50mM KCl, 10mM $MgCl_2$, 1mM DTT and 20µM [^{3}H]poly(rA):poly(dT). When 1,000 units of M-MLV RT (H–) are tested under these conditions, the result is below the limit of detection.

Functional Assay

First-Strand cDNA Synthesis: Two hundred units of M-MLV RT (H–) are used to produce cDNA from 1µg of 1.2kb and 6.5kb control RNAs in separate reactions, using [^{32}P] dCTP as a tracer. The specification is the conversion of >12% of mRNA to cDNA. Full-length cDNA is observed by gel electrophoresis and autoradiography.

Signed by:

R. Wheeler, Quality Assurance

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Usage Information

1. Standard First-Strand cDNA Synthesis

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 ≤14µl 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	5.00µl
dATP, 10mM	1.25µl
dCTP, 10mM	1.25µl
dGTP, 10mM	1.25µl
dTTP, 10mM	1.25µl
M-MLV RT	200 units
Nuclease-Free Water to final volume of	25.00µl

3. 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.
4. Perform second-strand synthesis using a protocol of your choice. Standard protocols for second-strand synthesis may be found in reference 2.

Notes:

a. 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.

b. If you are concerned about possible RNase contamination in the reaction, add Recombinant RNasin® Ribonuclease Inhibitor (Cat.# N2511) to the reaction (1u/µl) to preserve RNA integrity.

2. 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

3. References

1. 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.
2. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) In: *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 8.64.

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