

## Certificate of Analysis

### M-MLV Reverse Transcriptase, RNase H Minus:

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

**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<sub>2</sub> 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.

Part# 9PIM530

Revised 1/24



AF9PIM530 0124M530



**Promega**

#### Promega Corporation

2800 Woods Hollow Road	
Madison, WI 53711-5399	USA
Telephone	608-274-4330
Toll Free	800-356-9526
Fax	608-277-2516
Internet	www.promega.com

## 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 [<sup>3</sup>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<sub>2</sub>, 1mM DTT and 20µM [<sup>3</sup>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 [<sup>32</sup>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.

#### PRODUCT USE LIMITATIONS, WARRANTY, DISCLAIMER

Promega manufactures products for a number of intended uses. Please refer to the product label for the intended use statements for specific products. Promega products contain chemicals which may be harmful if misused. Due care should be exercised with all Promega products to prevent direct human contact.

Each Promega product is shipped with documentation stating specifications and other technical information. Promega products are warranted to meet or exceed the stated specifications. Promega's sole obligation and the customer's sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Promega makes no other warranty of any kind whatsoever, and SPECIFICALLY DISCLAIMS AND EXCLUDES ALL OTHER WARRANTIES OF ANY KIND OR NATURE WHATSOEVER, DIRECTLY OR INDIRECTLY, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, AS TO THE SUITABILITY, PRODUCTIVITY, DURABILITY, FITNESS FOR A PARTICULAR PURPOSE OR USE, MERCHANTABILITY, CONDITION, OR ANY OTHER MATTER WITH RESPECT TO PROMEGA PRODUCTS. In no event shall Promega be liable for claims for any other damages, whether direct, incidental, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of Promega products to perform in accordance with the stated specifications.

© 1999–2024 Promega Corporation. All Rights Reserved.

RNasin is a registered trademark of Promega Corporation.

Coomassie is a registered trademark of Imperial Chemical Industries. Nonidet is a registered trademark of Shell International Petroleum Company, Ltd.

Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.

All specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.

Part# 9PIM530

Printed in USA. Revised 1/24.

Signed by:

R. Wheeler, Quality Assurance

## 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	<u>200 units</u>
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 <sub>2</sub>
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.