Product Contents

M-MLV Reverse Transcriptase:

Part No.	Size (units)
M170A	10,000
M170B	50,000

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 @ 25°C), 75mM KCl, 3mM $MgCl_2$ and 10mM DTT.

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

Storage Conditions: Store at –20°C. 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 $MgCl_2$, 40mM KCl, 10mM DTT, 0.1mg/ml BSA, 0.5mM [^{3}H]dTTP, 0.025mM oligo(dT), 0.25mM poly(A) 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. Thus, starting with 1µg of mRNA in a first-strand cDNA synthesis, 200 units of the M-MLV enzyme are recommended as opposed to 25 units of the AMV enzyme.

Quality Control Assays

Activity Assay

First-Strand cDNA Synthesis: 200 units of enzyme are used to produce cDNA from 1µg of a 1.2kb control RNA, using [^{32}P] dCTP as a tracer. The minimum specification is 120ng of first strand cDNA made from 1µg of RNA. The cDNA product must be >90% full length.

In addition, a mix of different RNA species is used in a reverse transcription reaction (run at 42°C with 200 units of enzyme, 20 units of Recombinant RNasin® Ribonuclease Inhibitor and 1µg RNA per reaction). Prominent bands of 8 different cDNA products ranging in length from 0.5 to 8kb must be observed by gel electrophoresis and autoradiography.

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 in 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 both DNase and RNase.

Endonuclease Assay: To test for endonuclease activity, 1µg of Type I supercoiled plasmid DNA is incubated with 500 units of M-MLV Reverse Transcriptase in 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 (analysis on 0.4µg of DNA).

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

Part# 9PIM170

Revised 6/05

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Part# 9PIM170
Printed in USA. Revised 6/05

Usage Information

I. Description

Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT) is an RNA-dependent DNA polymerase that can be used in cDNA synthesis with long messenger RNA templates (>5kb). M-MLV RT is the preferred reverse transcriptase for long mRNA templates because the RNase H activity of M-MLV RT is weaker than the commonly used Avian Myeloblastosis Virus (AMV) reverse transcriptase.

Application of M-MLV RT includes:

- 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. First-Strand Synthesis of cDNA

Materials to Be Supplied by the User

(Buffer composition is provided in Section III.)

- Recombinant RNasin® Ribonuclease Inhibitor (Cat.# N2511)
- 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. The following procedure uses 2µg of RNA. In a sterile RNase-free microcentrifuge tube, add 0.5µg of the primer or primer-adaptor per microgram of the mRNA sample in a total volume of ≤15µl in water. Heat the tube to 70°C for 5 minutes to melt secondary structure within the template. 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.
2. Add the following components to the annealed primer/template in the order shown.

   Note: Do not alter the ratio of primer to mRNA.

M-MLV 5X Reaction Buffer	5µl
dATP, 10mM	1.25µl
dCTP, 10mM	1.25µl
dGTP, 10mM	1.25µl
dTTP, 10mM	1.25µl
Recombinant RNasin® Ribonuclease Inhibitor	25 units
M-MLV RT	200 units
Nuclease-Free Water to final volume	25µl

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. The extension temperature may be optimized between 37 and 42°C.
4. Perform second-strand synthesis using a protocol of your choice. Standard protocols for second-strand synthesis may be found in reference 2.

   Note: 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.

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. 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.

Part# 9PIM170
Printed in USA. Revised 6/05