

Certificate of Analysis

M-MLV Reverse Transcriptase, RNase H Minus, Point Mutant:

Part No.	Size (units)
M368A	2,500
M368B	10,000
M368C	50,000

Description: Moloney Murine Leukemia Virus Reverse Transcriptase, RNase H Minus, Point Mutant (M-MLV RT (H-)) is an RNA-dependent DNA polymerase that can be used in cDNA synthesis with long RNA templates (>5kb). The lack of RNase H activity is germane to this application in that this activity can start to degrade template when incubation times are long, as they may need to be when making long cDNAs. 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 (H-) is first-strand synthesis of cDNA from RNA molecules.

Enzyme Storage Buffer: M-MLV Reverse Transcriptase, RNase H Minus, Point Mutant 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 MgCl₂ and 10mM DTT.

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

Storage Temperature: See the Product Information Label for storage recommendations and expiration information.

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, 0.01% NP-40, 0.5mM [3H]dTTP, 0.025mM oligo(dT)₅₀ and 0.25mM poly(A)₄₀₀. See the unit concentration on the Product Information Label.

Usage Note: M-MLV Reverse Transcriptase, RNase H Minus, Point Mutant is less processive than AMV Reverse Transcriptase, so 8 units of the M-MLV enzyme are generally required to generate the same amount of cDNA as 1 unit of AMV Reverse Transcriptase.

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Promega

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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, Point Mutant 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, Point Mutant 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 determined by SDS-polyacrylamide gels with Coomassie® blue staining.

Functional Assay

First-Strand cDNA Synthesis: First-strand cDNAs, of 1.2kb and 6.5kb control RNAs, are synthesized by incubating 200 units of M-MLV Reverse Transcriptase, RNase H Minus, Point Mutant; 1µg of each template; an oligo(dT)₁₅ primer and radio-labeled 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 6.5kb for the 6.5kb RNA.

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Part# 9PIM368

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Signed by:

J. Stevens, Quality Assurance

I. Standard 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)
- Primer

1. A typical procedure uses 1µg of mRNA. 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 for 5 minutes to prevent secondary structure from reforming, then centrifuge 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 RT 5X Reaction Buffer	5µl
dATP, 10mM	1.25µl
dCTP, 10mM	1.25µl
dGTP, 10mM	1.25µl
dTTP, 10mM	1.25µl
M-MLV RT (H-)	1µl (200 units)
Nuclease-Free Water to final volume of	25µl
5. Mix gently. Incubate a reaction using Oligo(dT)₁₅ at 40°C or a reaction using Random Hexamers at room temperature for the initial 10 minutes, then 40–55°C for the final 50 minutes (see Note 1). For a reaction using other primers, incubate at 40–55°C for 60 minutes.
6. Perform second-strand synthesis using a protocol of your choice. Standard protocols for second-strand synthesis may be found in reference 2.

Notes:

1. Incubation at elevated temperatures (i.e., 55°C) can decrease RNA secondary structure and enhance cDNA synthesis of some templates.
2. 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.
3. 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.

II. Two-Step RT-PCR

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)
- PCR Core System I or II (Cat.# M7660, M7665)
- Oligo(dT)₁₅ (Cat.# C1101), Random Hexamers (Cat.# C1181) or gene-specific primer

A. First-Strand cDNA Synthesis

1. A typical procedure uses between 1ng–1µg total RNA or 10pg–1µg mRNA. In a sterile RNase-free microcentrifuge tube, add 0.5µg Oligo(dT)₁₅, 50–250ng random hexamers, or 2pmol of a gene-specific primer to the RNA in a total volume of ≤14µl.
2. Heat the tube to 70°C for 5 minutes, then cool quickly on ice for 5 minutes.

3. Add the following components to the annealed primer/template in the order shown:

M-MLV RT 5X Reaction Buffer	5µl
dATP, 10mM*	1.25µl
dCTP, 10mM*	1.25µl
dGTP, 10mM*	1.25µl
dTTP, 10mM*	1.25µl
M-MLV RT (H-)**	1µl (50–100 units)
Nuclease-Free Water to final volume of	25µl

*Alternatively, use 1.25µl of PCR Nucleotide Mix in place of the separate dNTPs.

Use 50 units of enzyme unless using RNA >500ng. Enzyme can be diluted in 1X Reaction Buffer or Enzyme Storage Buffer and used immediately. **Do not store diluted enzyme for later use.

4. Mix gently. Incubate a reaction using Oligo(dT)₁₅ at 40°C or a reaction using Random Hexamers at room temperature for the initial 10 minutes, then 40–55°C for the final 50 minutes (see Note 1). For a reaction using gene-specific primers, incubate at 40–55°C for 60 minutes.
5. Inactivate the reaction by heating for 15 minutes at 70°C. The cDNA can now be used as a template for amplification by PCR.

Notes:

1. Incubation at elevated temperatures (i.e., 55°C) can decrease RNA secondary structure and enhance cDNA synthesis of some templates.
2. Amplification of some PCR targets >1kb may require removal of RNA complementary to the cDNA. To remove RNA, add 1µl (2 units) RNase H (Cat.# M4281) and incubate for 20 minutes at 37°C.
3. The reverse transcription of some GC-rich RNA templates may be enhanced by the addition of 1M betaine (final) or 5% DMSO (final) to the reverse transcription step.

B. Second-Strand cDNA Synthesis and PCR Amplification

1. Use 1–10µl of the first-strand cDNA reaction in the subsequent PCR amplification. Combine the following in a PCR tube:

10X PCR Reaction Buffer (without MgCl ₂)	5µl
25mM MgCl ₂ *	3µl
PCR Nucleotide Mix, 10mM each	1µl
amplification primers	50pmol each
Taq DNA Polymerase (5u/µl)	0.5µl
first-strand cDNA reaction	1–10µl
Nuclease-Free Water to final volume of	50µl

*Optimal concentration of MgCl₂ needs to be determined empirically for each template/primer pair.

2. Mix gently and proceed to thermal cycling according to your specific experiment.

III. Composition of Buffers

M-MLV RT 5X Reaction Buffer		10X PCR Reaction Buffer	
250mM Tris-HCl (pH 8.3 at 25°C)		100mM Tris-HCl (pH 9.0 at 25°C)	
375mM KCl		500mM KCl	
15mM MgCl ₂		1.0% Triton® X-100	
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) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 8.64.