

User's Manual and Instructions

M-MLV Reverse Transcriptase

Catalog Number: Z5040002 and Z5040002-100K

Concentration:	200 u/μl
Storage Buffer:	20 mM Tris-HCl (pH 7.5), 1 mM DTT, 0.05% Triton X-100, 1 mM Na ₂ EDTA, 0.1 M NaCl and 50% glycerol.
Reaction Buffer (5x):	250 mM Tris-HCl (pH 8.3), 375 mM KCl, 15 mM MgCl ₂ , and 50 mM DTT
Unit Definition:	One unit of the enzyme incorporates 1 nmole dTTP into acid-precipitable material in 10 minutes at 37°C, using poly (A): oligo dT as a template:primer.
Quality Control:	Nonspecific Endonuclease/Nickase Activity: None detected after 14-16 hour incubation of 100 units of enzyme with 300 ng of supercoiled plasmid DNA, analyzed on agarose gel. Exonuclease Activity: None detected after 14-16 hour incubation of 100 units of enzyme with 40 ng of ³² P-labeled Sau3A fragments of pUC19, analyzed by PAGE. Protease Activity: None detected after 14-16 hour incubation of 1200 units of enzyme with 1 μg of protease substrate, analyzed by fluorescence.

Storage and Handling: -20°C

Protocol

First-Strand Synthesis of cDNA

Materials to Be Supplied by the User

- Recombinant RNasin Ribonuclease Inhibitor (Cat.# Z5040001)
- dATP, 10mM (Cat.# K6011101-400 , 100mM)
- dCTP, 10mM (Cat.# K6011103-400 , 100mM)
- dGTP, 10mM (Cat.# K6011102-400 , 100mM)
- dTTP, 10mM (Cat.# K6011104-400 , 100mM)
- Nuclease-Free Water

1. The following procedure uses 2 μg of RNA. In a sterile RNase-free microcentrifuge tube, add 0.5 μg of the primer or primer-adaptor per microgram of the mRNA sample in a total volume of 15 μl 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 5 μ l; dATP, 10mM 1.25 μ l; dCTP, 10mM 1.25 μ l; dGTP, 10mM 1.25 μ l; dTTP, 10mM 1.25 μ l; RNasin Ribonuclease Inhibitor 25 units; M-MLV RT 200 units; Nuclease-Free Water to final volume 25 μ l

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.

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.