MMLV-RT enzyme

Storage Conditions: Store at -20ºC. It is stable a year from the date of purchase if stored and handled properly.

Quality Control:

1. Nonspecific Endonuclease/Nickase Activity: None detected after 14-16 hour incubation of 100 units of enzyme with 300 ng of supercoiled plasmid DNA, analysed on agarose gel.
2. Exonuclease Activity: None detected after 14-16 hour incubation of 100 units of enzyme with 40 ng of 32P-labeled Sau3A fragments of pUC19, analysed by PAGE.
3. Protease Activity: None detected after 14-16 hour incubation of 1200 units of enzyme with 1 µg of protease substrate, analysed by fluorescence.

Source: *E.coli*

Size and concentration: 16,000 units or 100,000 units with 1 ml or 2.5 ml 5x reaction buffer, respectively at 200 units/µl concentration.

Storage buffer: 20 mMTris-HCl (pH 7.5), 1 mMDTT, 0.05% (v/v)Triton X-100, 0.1 mMEDTA, 0.1 MNaCl and 50% (v/v) glycerol.

Reaction buffer (5x): 250 mMTris-HCl (pH 8.3), 375 mMKCl, 15 mMMgCl2, and 50 mMDTT

Unit definition: One unit of the enzyme incorporates I nmole of dTTPinto acid-precipitable material in 10 minutes at 37°C using poly (A): oligo (dT)25 as template-primer.

PROTOCOL

First-Strand cDNA Synthesis
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

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template. Cool the tube immediately on ice to prevent secondary structure from reforming and 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 µl; dATP, 10mM 1.25 µl; dCTP, 10mM 1.25 µl; dGTP, 10mM 1.25 µl; dTTP, 10mM 1.25 µl; RNasin.
Ribonuclease Inhibitor 25 units; M-MLV RT 200 units; Nuclease-Free Water to final volume 25 µl.

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.

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.
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.