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M-MLV Reverse Transcriptase (Rnase H minus)

Cat # ME006
Pack Size: 10000ku
Storage: -20°C
Note: *Make a working aliquots to minimize the number of freeze-thaw cycles . Mix well prior to use. DO NOT VORTEX*

Introduction

MMLV Reverse Transcriptase (Rnase H minus) is an enzyme produced by Gene to Protein Pvt Ltd that is commonly used in molecular biology research for cDNA synthesis from RNA templates. This enzyme is a genetically engineered variant of the Moloney Murine Leukemia Virus (MMLV) Reverse Transcriptase, which lacks RNase H activity. RNase H is an endonuclease that hydrolyzes the RNA strand of RNA-DNA hybrids, leading to the degradation of the RNA template during cDNA synthesis. The absence of RNase H activity in MMLV Reverse Transcriptase (Rnase H minus) allows for the synthesis of full-length cDNA without RNA degradation.

Storage buffer

MMLV Reverse Transcriptase (Rnase H minus) is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl2, and 0.1 mM EDTA. The enzyme should be stored at -20°C for long-term storage. The enzyme is stable for up to 12 months when stored under these conditions.

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.

Application

- First-strand cDNA synthesis.

Procedure for First-strand cDNA synthesis

- Use a 20-µL reaction volume for 1 ng–5 µg of total RNA or 1–500 ng of mRNA. Add the following components to a nuclease free microcentrifuge tube

Component	Volume
Oligo (dT) (500 µg/mL), or 50–250 ng random primers, or 2 pmole gene-specific primer (Not provided)	1 µL
Total RNA, or mRNA	100 ng to 5 µg total RNA, or 1 ng to 500 ng of mRNA
10 mM dNTP Mix (10 mM each dATP, dGTP, dCTP and dTTP at pH 7) (Not Provided)	1 µL
Sterile, distilled water	To 12 µL

- Heat mixture to 65°C for 5 minutes, then quick chill on ice. Collect the contents of the tube by brief centrifugation, then add the following components::

Rnase Inhibitor is particularly essential when using less than 50 ng of starting RNA.

- Mix contents of the tube gently, then incubate at 37°C for 2 minutes.
- Add 1 µL (200 units) of M-MLV RT, then mix by pipetting gently up and down. If using random primers, incubate tube for 10 minutes at 25°C .

Note: If less than 1 ng of RNA is used, reduce the amount of M-MLV RT in the reaction to 0.25 µL (50 units), then add the sterile, distilled water to a final volume of 20 µL.

- Incubate at 37°C for 50 minutes. Heat the reaction at 70°C for 15 minutes to inactivate the enzyme.