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MMLV Reverse Transcriptase

Cat. No: XV0073P (10,000 U)

Cat. No: XV0074P (5 x 10,000 U)

Introduction

MMLV Reverse Transcriptase (MMLV-RT), encoded by Moloney Murine Leukemia Virus (MMLV) is an RNA-dependent DNA polymerase that synthesizes the cDNA first strand from a single-stranded RNA template to which a primer has been hybridized. MMLV-RT will also extend primers hybridized to single-stranded DNA. Second strand cDNA synthesis can be achieved from some mRNA templates without an additional DNA polymerase.

Source: Purified from E. coli strain harboring a plasmid that directs the synthesis of modified form of MMLV-RT.

MW: 69 KDa

Unit definition: One unit of the enzyme incorporates 1 nmol dTTP into acid-precipitable material in 10 minutes at 37°C, using poly(A) oligo dT as a template primer.

Enzyme concentration: 200 U/µl

Enzyme Storage Buffer: MMLV Reverse Transcriptase is supplied in 20mM Tris-HCl (pH 7.5), 100mM NaCl, 0.1mM EDTA, 1mM DTT, 0.01% Triton X-100 and 50% glycerol.

Application

- RT PCR
- Synthesis of DNA
- mRNA 5'-end mapping by primer extension analysis
- End-labeling of DNA
- Dideoxynucleotide sequencing

Kit Contents

- MMLV Reverse Transcriptase
- 10X Reaction Buffer*

Storage

Upon receipt of the kit, immediately store the components at -20 °C.

*After thawing, if any precipitate is observed, pulse vortex until the precipitate is completely resuspended.

Quality Control

MMLV RT is free of detectable RNase, and DNase (exo- and endonuclease) activities.

Purity: >90% as judged by SDS-polyacrylamide gels with blue staining.

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Protocol

1. Mix the following in the tube:
    - 10 pg - 5 µg of the total RNA (or 10 pg – 500 ng of mRNA/poly(A)RNA)
    - 1 µl of Oligo(dT)12-18 (50-60 µM); → 2.5-3 µM final conc.

      Or random hexamer (50-250 ng/µL); → 2.5-12.5 ng/µL final conc.

      Or gene-specific primer (2 µM) → 0.1 µM final conc.
    - 1 µl of dNTP Mix (10 mM each) → 0.5 mM final conc.
    - add Nuclease-free water up to 16 µl
2. Incubate the mixture 5 min at 65 °C. Chill on ice for at least 1 min, briefly centrifuge again and place on ice.
3. Add into the mixture:
    - 2 µl of 10x Reaction Buffer (500 mM Tris-HCl, pH 8.3, 750 mM KCl, 30 mM MgCl2, 100 mM DTT)
    - 1 µl of Ribonuclease Inhibitor (not provided)
    - 1 µl MMLV Reverse Transcriptase (200 u/µl) – 200 units

      Final Volume→ 20 µl
4. Incubate the mixture at 37 °C for 50 min.

   Note: When using random-hexamer primers, incubate first at 25°C for 10 min and then at 37 °C for 50 min.
5. Heat the mixture 15 min at 70 °C to inactivate the MMLV Reverse Transcriptase.
6. Store cDNA product at -20 °C or proceed to downstream applications.