

Data sheet

MMLV Reverse Transcriptase

Cat. No:P0073 (10.000 units)

Cat. No: P0074 (5X10.000 units)

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.

Application

- RT PCR
- Synthesis of cDNA
- mRNA 5'-end Mapping by Primer Extension Analysis
- End-labeling of DNA
- Dideoxynucleotide Sequencing

Kit Contents

Components

MMLV Reverse Transcriptase (200U/ μ L)

Reaction Buffer 5X

Storage

Store at -20°C.

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.

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 in the tube:

- ✓ 1 - 5 µg of the total RNA (or 50 – 500 ng of poly(A)-RNA)
- ✓ 10 pmole of strand-specific primer (or 250 – 500 ng of oligo-dT for each µg of RNA)
- ✓ add water up to 8 µl

2. Incubate the mixture 10 min at 70 °C, then 10 – 15 min at room temperature (for the specific primer) or place on ice in case of oligo-dT or random primer.

3. Add into the mixture:

- ✓ 4 µl of 5x Reaction Buffer (250 mM Tris-HCl pH 8.3; 500 mM KCl, 15 mM MgCl₂, 50 mM DTT)
- ✓ 1 µl of dNTP mix (10 mM of each dNTP)
- ✓ MMLV Reverse Transcriptase (200 u/µl) – 200 units
- ✓ H₂O – up to 20 µl

4. Incubate the mixture at 37 – 55 °C¹ for 30 – 120 min².

¹ depends on the RNA: Higher temperatures (up to 55 °C) for higher structured RNA; Try to adjust the pH to 8.8.

² 30 min for cDNA with 500 bp; 115 min for 1.5 kb

5. Heat the mixture 10 min at 65 – 70 °C to inactivate the MMLV Reverse Transcriptase.

6. Use the mixture for PCR or other applications.

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and in vitro use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. Please refer to www.canvaxbiotech.com for Material Safety Data Sheet of the product.

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