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/µL)

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.

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 $MgCl_2$, 50 mM DTT)
- ✓ 1 µl of dNTP mix (10 mM of each dNTP)
- ✓ MMLV Reverse Transcriptase (200 u/µl) – 200 units
- ✓ H_2O – up to 20 µl

4. Incubate the mixture at 37 – 55 °C [1] for 30 – 120 min[2].

[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