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DATA SHEET

Version: 2
Revision date: 05/06/2023

1. Identification

Product name **MMLV Reverse Transcriptase**
10000 U (200 U/µL)

Cat. No **P0073**

2. Description

MMLV Reverse Transcriptase (MMLV-RT) , encoded by Moloney Murine Leukemia Virus (MMLV) is an RNAdependent 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.

3. Protein information

Purity	Purity is >95% by SDS-PAGE
Storage buffer	20mM Tris-HCl (pH 7.5), 100mM NaCl, 0.1mM EDTA, 1mM DTT, 0,01% Triton X-100 and 50% glycerol
Reaction Buffer (10X)	500 mM Tris-HCl, pH 8.3, 750 mM KCl, 30 mM MgCl2, 100 mM DTT, 2.5 mM spermidine
Biological activity	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.

4. Storage specifications

Store at -20˚C*. Avoid exposure to constant temperature changes.
*Upon thawing, if any precipitate is observed, pulse vortex until the precipitate is completely resuspended.

5. Applications

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

6. Further information

Product Use Limitations This product is developed, designed and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Disclaimer The information provided in this Data Sheet is correct to the best of our knowledge and belief at the date of publication. This information is intended only as a guide and should not be taken as a warranty or quality specification. Canvax Reagents S.L.U. shall not be held liable for any damage resulting from handling or from contact with the above product.

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PROTOCOL

1. **Mix in the tube:**

   - ✓ 10 pg - 5 µg of the total RNA (or 10 pg – 0,5 µg 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.**

   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.**