MMLV Reverse Transcriptase

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

Description:
MMLV Reverse Transcriptase, encoded by Moloney Murine Leukemia Virus (MMLV RT) 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.

Concentration: 200 u/µl

Storage Buffer:
200 mM potassium phosphate (pH 7.2), 0.2% Triton X-100, 2 mM DTT and 50% glycerol

Reaction Buffer 5X:
250 mM Tris-HC1(pH8.3), 375 mM KCl, 15 mM $MgCl_2$ and 50 mM DTT

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:

Endonuclease Activity: 1 µg of Type 1 supercoiled plasmid DNA is incubated with 500 units of enzyme in 1X reaction buffer for one hour at 37°C. The supercoiled DNA is visualized on an ethidium bromide-stained agarose gel to verify absence of nicking or cutting.

Nuclease Activity: 50 ng of radio labelled DNA or RNA is incubated with 200 units of enzyme in 1X reaction buffer for one hour at 37°C, resulting in <1% release for both DNase and RNase.

Purity: >90% as judged by SDS-polyacrylamide gels with blue staining. MMLV RT is free of detectable RNase, and DNase (exo- and endonuclease) activities.

Usage:
Standard Protocol:We recommend to prepare 2 Mixes

Mix I

Component	Amount/conc.
a. Total RNA or b. PolyA RNA	1-5 µg 50-500 ng
c. Strand-specific primer or d. oligo dT / random primer for each µg of RNA	10 pM 250-500 ng
sterile Water	up to 8 µl

.. a good decision ..

Incubation	Temperature
10 min	70 °C
10 - 15 min (for c. specific primers) or 5 min (for d. oligo dT / random primer)	room temperature place on ice

Mix II

Component	Amount/conc.
5X reaction buffer	4 µl
dNTP mix (10 mM of each = 40 mM)	1 µl
optional: RNAsin	20-40 units
MMLV Reverase (200 u/µl)	200 units
combine Mix I and Mix II	8 µl (Mix I)
sterile water (and gently vortex)	up to 20 µl

Step	Temperature
30 - 115 min [1.)]	37 - 55°C [2.)]
10 min (Inactivation of enzyme)	65-70°C

[1.)] 30 min for cDNA with 500 bp; 115 min for 1,5 kb
[2.)] depends on the RNA: Higher temperatures (up to 55 °C) for higher structured RNA; Try to adjust the pH to 8.8

Transportation: on blue ice

Storage: at -20°C for 24 months

Ordering information:

Cat.-no	Description	Amount
105-100	MMLV Reverse Transcriptase	10.000 units
105-250	MMLV Reverse Transcriptase	50.000 units

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