-Datasheet-

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:
20 mM Tris-HCl, pH 7.4, 100 mM NaCl, 0,1mM EDTA, 50 % Gycerol, 0,1% IGEPAL, 1 mM DTT

Reaction Buffer complete 10X: (Note: Composition and concentration has changed from 5x to 10x)
500 mM Tris-HCl (pH 8.3 at 25°C); 30 mM $MgCl_2$; 750 mM KCl; 100 mM DTT.

Dilution Buffer 1X: 10 mM KH_2PO_4 (pH 7.5); 0,1 mM EDTA; 200 mM NaCl; 7 mM 2-mercaptoethanol; 50% glycerol

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 c. Strand-specific primer or d. oligo dT / random primer for each µg of RNA	1-5 µg 50-500 ng 10 pM 250-500 ng
sterile Water	up to 8 µl
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.
10X reaction buffer	2 µl
dNTP mix (10 mM of each = 40 mM)	1 µl
optional: RNAsin	20-40 units
MMLV Reverase (200 u/µl)	200 units
sterile water	up to 20 µl
combine Mix I and Mix II and gently vortex	

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

Note: MMLV used under standard 37°C reaction conditions is best for synthesis of 7 kb or less.
Increasing reaction temp to 42°C may allow synthesis up to 10 kb. MMLV activity drops drastically above 42°C.

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