

Catalog
TBP0153-5000

Unit
5000 U

Description

MMLV Reverse Transcriptase is an enzyme encoded by the Moloney Murine Leukemia Virus. As an RNA-dependent DNA polymerase, it synthesizes the first strand of complementary DNA (cDNA) from a single-stranded RNA template after a primer has been attached. MMLV RT is widely used in molecular biology to generate cDNA for applications such as RT-PCR and first-strand cDNA synthesis.

Features

- **Low RNase H Activity:** Exhibits weak RNase H activity, resulting in high cDNA yield and enabling the synthesis of more full-length cDNA.
- **Thermally Stable:** Optimal reaction temperature is 50°C, with a maximum of 55°C. Effectively overcomes RNA secondary structures, ensuring smooth and efficient reverse transcription.
- **Broad Temperature Range:** Functions efficiently between 37°C and 55°C, maintaining over 80% of peak activity between 42°C and 55°C.
- **Enhanced Amplification Efficiency:** Engineered mutations improve the enzyme's binding affinity to RNA, significantly increasing amplification speed. Produces high-quality cDNA, ideal for cDNA library construction.

Product Details

Storage Buffer: 20 mM Tris-HCl (pH7.5), 200 mM NaCl, 0.25 mM EDTA, 0.01% NP-40(v/v), 2.5 mM DTT, 50% glycerol (v/v).

5×Reaction Buffer: [5×RTBuffer] 250mM Tris-HCl (pH 8.3), 15mM MgCl₂, 375 mM KCl, 50mM DTT.

Storage: -20°C

Components

Component	
MMLV(RNase H-)(200U/uL)	
5×RT Buffer (with DTT)	

Protocol

The first-strand cDNA synthesis

1. Add the following reagents to a RNase free PCR tube at room temperature add the MMLV RT last.

Oligo dT12-18 (1µg/µl)	1µl
or random primer (50-250ng)	1µl
Total RNA (10ng-5µg)	xµl
or mRNA (1-500ng)	
dNTP (10mM each)	1µl
DEPC ddH ₂ O	(14-x)µl

2. Gently mix and incubate 10 Min at 70°C then chill on ice for 2-10min.
3. Centrifuge for a few seconds then Put the tube into ice and add the next composition.

5×RT Buffer	4µl
RNasin (40U/µl)	1µl

4. Gently mix and incubate at 50°C for 2 min (Oligo dT12-18 or sequence especially primer) or at 25°C for 10 min for the random primer.
5. Centrifuge for a few seconds. Add 1µl MMLVRT (200U/µl) Incubate at 50°C for 50min.
6. Inactivate at 70°C for 10min then get the cDNA

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