Ver.24021

M-MLV Reverse Transcriptase

Cat.No.ODR23

Description: M-MLV Reverse Transcriptase, encoded by Moloney Murine Leukemia Virus is an RNA-dependent DNA polymerase that synthesizes the complementary first strand cDNA from a single-stranded RNA template to which a primer has been hybridized. M-MLV Reverse Transcriptase which is the mutated form of M-MLV Reverse Transcriptase rendering the enzyme thermostable with no RNase H activity. It can retain 100% activity at 50℃; also retains more than 80% activity even at 55℃.

Product contents:

Contents	Volume
M-MLV Reverse Transcriptase (200U/μL)	5000U
5X M-MLV Buffer (50mM DTT included)	200μL

Storage

- Store at –20°C

Features

RNase H activity	Negative
Concentration	200U/μL
Expiration date (at -20ºC)	2 years

Application

The first-strand cDNA synthesis; RT-PCR.

Note:

Thaw 5X RT Buffer at room temperature just before use and refreeze immediately after use.

Protocol

First-strand cDNA synthesis (reaction volume 20μL)

1. Prepare the following mixture in a PCR tube.

RNA	10ng – 1μg total RNA 10ng – 0.5μg mRNA	- μg
Primer	1μL Oligo $(dT)_{18}$ /1μL Random hexamer or 0.5μL Oligo $(dT)_{18}$ + 0.5μL Random hexamer or 1μL Gene-specific Primer (15 – 20μM)	1μL
10mM dNTP		1μL
RNase-free water to		15.5μL

Note: Prepare aliquots of Oligo$(dT)_{18}$ and Random Hexamer to avoid multiple freeze thaw cycles.

2. Heat the mixture at 65ºC for 5 minutes and cool down immediately on ice for 1 minute. Spin down the tube by centrifugation briefly.
   Note: Use heating block or PCR machine. DO NOT use water bath.
3. In the same tube, add the following reaction mixture:
   (Total reaction volume- 20µL)

Reagents	Volume
5X M-MLV Buffer (Included 50mM DTT)	4µL
M-MLV Reverse Transcriptase	0.5µL

4. Mix gently by pipetting.
   Note: For random hexamer, incubate the combined reaction mixture at 25ºC for 5 minutes and then proceed with the next step. For oligo(dT)$_{18}$ or gene-specific primers, directly proceed with the next step.
5. Incubate at 50ºC for 60 minutes.
6. Inactivate the reaction mixture by heating at 95ºC for 5 minutes.

   Note: Use heating block or PCR machine. DO NOT use water bath.
7. The product can be used for PCR immediately or stored at -20ºC.
   Note: As a recommendation; cDNA product should compose 10% of total reaction volume of PCR.