

M-MLV (H-) Reverse Transcriptase



R021

Version 23.1

Product Description

M-MLV (H-) Reverse Transcriptase is an M-MLV mutant with loss of RNase H activity obtained by site-directed mutation. Compared with wild-type M-MLV, the M-MLV (H-) Reverse Transcriptase exhibits higher thermostability and greater processivity, making it a better choice in long cDNA synthesis and high-temperature RT reaction. M-MLV (H-) Reverse Transcriptase possesses terminal nucleotidyl transferase (TdT) activity and can be used in 5' RACE or other experiments.

Components

Components	R021-01 10,000 U
■ 5 × RT Buffer	500 µl
■ M-MLV (H-) Reverse Transcriptase (200 U/µl)	50 µl

Storage

Store at -30 ~ -15°C and transport at ≤0°C.

Applications

It is applicable for reverse transcription of animal, plant and microbial RNA.

Source

A recombinant *E. coli* strain carrying M-MLV (H-) reverse transcriptase gene.

Unit Definition

One unit (U) is defined as the amount of enzyme that incorporates 1 nmol of dTTP into acid-insoluble material in 10 min at 37°C with Poly (rA)·Oligo (dT) as the template/primer.

Notes

For research use only. Not for use in diagnostic procedures.

Prevent RNase contamination

Please keep the experimental area clean; wear clean gloves and masks during operation; consumables such as centrifuge tubes and pipette tips used in the experiment must be RNase-free.



Experiment Process

Prepare the following mixtures in RNase-free centrifuge tubes:

Components	Volume
RNase-free ddH ₂ O	to 20 µl
5 × RT Buffer	4 µl ■
dNTP Mix (10 mM each)	1 µl
Oligo (dT) ₁₈ (50 µM)	
or Random hexamers (50 ng/µl)	1 µl
or Gene Specific Primers (2 µM)	
RNase inhibitor (40 U/µl)	1 µl
M-MLV (H-) Reverse Transcriptase (200 U/µl)	1 µl ■
Total RNA	100 pg - 5 µg
or Poly (A) ⁺ RNA	10 pg - 500 ng

Run the following program for 1st strand cDNA synthesis:

For Oligo (dT)₁₈

Temperature	Time
42°C	45 min*
85°C	5 min

For Random hexamers

Temperature	Time
25°C	10 min
42°C	45 min*
85°C	5 min

For Gene Specific Primers

Temperature	Time
42 ~ 50°C	45 min*
85°C	5 min

* It can be adjusted between 30 ~ 60 min, and extended reverse transcription time may help to obtain longer cDNA (>5 kb).

Incubate at 85°C for 5 min to inactivate the reverse transcriptase. The cDNA can be used for PCR immediately or be stored at -20°C. For PCR, it is recommended that the volume of cDNA should be $\leq 1/10$ of total volume.

