

M-MLV (H-) Reverse Transcriptase

Catalog # R021



Version 5.1

Vazyme biotech co., ltd.

Introduction

The wildtype Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase has the following activities: RNA-dependent DNA polymerase, DNA-dependent DNA polymerase, and RNase H. The M-MLV (H-) Reverse Transcriptase is a single-site mutant of M-MLV which contains no RNase H activity. Compared with M-MLV mutants obtained via deletion of the RNase H domain, this product, which retains a complete protein structure and polymerase activities, can be used for the synthesis of longer cDNA or the preparation of cDNA library.

Contents of Kits

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

Storage

All components should be stored at -20°C.

Unit Definition

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

Protocol

1. Mix the following components in a RNase-free centrifuge tube:

RNase free ddH ₂ O	to 20 µl
5x 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
Template RNA	Total RNA: 100 pg-5 µg Poly (A)* RNA: 10 pg-500 ng

2. Programs for the 1st-strand cDNA synthesis:

For oligo (dT)₁₈

42°C	45 min*
70°C	15 min

For Random Hexamers

25°C	10 min
42°C	45 min*
70°C	15 min

For Gene Specific Primers

42°C	45 min*
70°C	15 min

* Can be optimized between 30 min and 60 min. Longer time is helpful to obtain longer cDNA (> 5 kb).

3. Incubate at 70°C for 15 min to inactivate the reverse transcriptase. The cDNA can be used for PCR or be stored at -20°C immediately. For PCR, it is recommended that the volume of cDNA ≤ 1/10 of total PCR reaction system volume.



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