Product Manual

Product Name	M-MLV Reverse Transcriptase
Source	Recombinant expression in Escherichia coli
Catalog Number	CSB-DEM025
Physical Form	Liquid
Enzyme Activity	200 U/μL
Storage Conditions	-20 ±5°C
Molecular Weight	74 kDa
Storage Buffer	20 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.01% NP-40, 50% glycerol
Activity Definition	1 unit (U) is the amount required to incorporate 1 nmol of dTTP into acid-insoluble material in a 50 μL reaction system with Poly(rA)•Oligo d(T)15 as the template at 37°C for 10 minutes.
Quality Control	No detectable endonuclease, DNase, and RNase activities. PCR testing shows no residual host genomic DNA.
Shelf Life	24 months

Product Descriptionp

M-MLV Reverse Transcriptase is a genetically engineered reverse transcriptase that reduces RNase H activity and improves thermal stability. It can synthesize the first strand cDNA at higher temperatures compared to wild-type M-MLV. This product exhibits normal activity at 50-60°C, with higher specificity, higher yield, and the ability to synthesize cDNA up to 12 kb in length.

Product components

Component No.	Component Name	Specifications		
1	5×First-Strand Buffer	0.4mL	2mL	4mL
2	M-MLV Reverse Transcriptase	20000U	100000U	200000U

Operation Instructions

First Strand cDNA Synthesis (Simple Procedure)：

Add and mix the components listed in the table below and incubate at 55°C for 0.5 hours.

If using random primers, it is recommended to preheat the reaction mixture at 25°C for 5 minutes before the 55°C reaction.

Component	Addition amount
ddH_2O	Up to 20 μL
5× First-Strand Buffer	4 μL
RNA Template	50 pg -1 μg*
50 μM d(T)23VN or 60 μM random primers	2 μL
10 mM dNTPs	1 μL
RNase Inhibitor (40 U/μL)	0.2 μL
M-MLV Reverse Transcriptase (200 U/μL)	1 μL

Note:

1. 1 ng-1 μg total RNA template or 50 pg-100 ng Poly(A)-RNA. Inactivate the enzyme at 95°C for 3 minutes. For downstream
2. PCR applications, the volume of reverse transcription product should not exceed 1/10 of the total PCR reaction volume.

First Strand cDNA Synthesis (Standard Procedure)

If denatured template RNA is required, use the following protocol.Add RNA template and d(T)23VN primer to an RNase-free PCR tube.

Component	Volume
RNA Template	50 pg -1 μg*
50 μM d(T)23VN or 60 μM random primers	2 μL
10 mM dNTPs	1 μL
ddH_2O	Up to 10 μL

Note: 1 ng-1 μg total RNA template or 50 pg-100 ng Poly(A)-RNA.

Incubate the mixture at 65°C for 5 minutes to denature the RNA template/primer, briefly centrifuge, and quickly place on ice for 2 minutes. Then add the following components to the PCR tube:

Component	Volume
ddH_2O	Up to 20 μL
Denatured RNA Template/Primer from the previous step	10μL
5× First-Strand Buffer	4 μL
100 mM DTT	2 μL
RNase Inhibitor (40 U/μL)	0.2 μL

After thorough mixing, incubate at 55°C for 0.5 hours. If using random primers, it is recommended to preheat the reaction mixture at 25°C for 5 minutes before the 55°C reaction. Heat inactivate the enzyme at 95°C for 3 minutes, and store the reverse transcription product at -20°C. For downstream PCR applications, the volume of reverse transcription product should not exceed 1/10 of the total PCR reaction volume.

One-Step RT-qPCR Reaction

For testing, it is recommended to use our optimized One Step RT-qPCR KIT. In general, the recommended concentration of M-MLV Reverse Transcriptase for one reaction is 0.5-2U/μL. The amplification conditions are as follows:

Recommended PCR reaction program

Temperature	Time	Cycles
55-60°C	5-10 min	1
95°C	3 min	1
95°C	15-30 s	40-45
45-68°C	15-60 s	
68°C	1 kb/min	