

GUeasy MMLV Reverse-Transcriptase III (H-)

i. Product Description

M-MLV Reverse Transcriptase (MMLV) is an RNA-mediated DNA polymerase derived from Moloney Murine Leukemia Virus. This enzyme uses RNA (synthesized from cDNA) or ssDNA as a template to make a complementary strand of DNA. Compared with wild-type M-MLV reverse transcriptase, GUeasy MMLV Reverse-Transcriptase III (H-) can effectively synthesize high-quality cDNA by removing RNase H activity through genetic modification and increasing the reverse transcription temperature to 50-60 °C, avoiding the inhibition of cDNA synthesis by the complex secondary structure of RNA.

ii. Advantages

Stability: extremely high stability by high-standard production process.

Tolerance: for common impurities have a strong tolerance.

Compatibility: compatible with animal, plant, virus and other templates.

iii. Operation steps

1) RNA denaturation (Denaturation of RNA helps to open up secondary structures, which can greatly increase the production of first-strand cDNA. Do not omit the denaturation step for fragments larger than 3Kb)

Components	Volume (ul) up to10	
RNase Free ddH₂O		
Total RNA	10pg-5ug	
Oligo (dT) ₁₈ (50 μM)		
or Random Primers (100 μM)	1	
or Gene Specific Primers (2 μM)		

It was then heated at 65° C for 5 min and quickly placed on ice to cool for 2 min. After the reaction solution was collected by centrifugation briefly, the reverse transcription reaction solution in the table below was added and gently blown and mixed.

2) The first strand cDNA synthesis reaction liquid was prepared

Components	Volume (ul)	
The previous reaction liquid	10	
5×GUeasy MMLV Reverse-Transcriptase III buffer	4	
dNTP Mix (10 mM)	1	
GUeasy MMLV Reverse-Transcriptase III (200U/uI)	1	
RNase inhibitor (40 U/µL)	1	
RNase Free ddH ₂ O	up to 20ul	

3) The first strand cDNA synthesis reaction program

Temperature	Time	
25 ℃	5min	
42℃	15-30min	
85℃	5min	



iV. Notes

- 1) When using Random Primers, incubate for 5 min at 25 ℃. This step may be omitted if Oligo (dT)18 or Gene Specific Primers are used.
- 2) Reverse transcription temperature: $42^{\circ}\mathbb{C}$ is recommended. For templates with high GC content or complex secondary structure, the reverse transcription temperature can be increased to $50\text{-}55^{\circ}\mathbb{C}$.
- 3) Heating at 85°C for 5 min was used to inactivate reverse transcriptase.
 - % Reverse transcription products can be immediately used for subsequent PCR or qPCR reaction, or can be stored at -20°C for a short time. If long-term storage is needed, it is recommended to store them at -80°C after packaging to avoid repeated freeze-thaw.

V. Storage condition

Shipped in ice-pack, stored at -20°C, valid for two years.

Vi. Product Specification

Product	Catalog #	Size	Activity
GUeasy MMLV Reverse-Transcriptase III (H-)	RT3003-01	100ul	20000U
GUeasy MMLV Reverse-Transcriptase III (H-)	RT3003-02	200ul	40000U
GUeasy MMLV Reverse-Transcriptase III (H-)	RT3003-05	500ul	100000U

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