M-MLV GIII Reverse Transcriptase

REF: EG15124S

Storage Condition

-20℃

Components

Component	Amount
M-MLV GIII Reverse Transcriptase(200 U/μl)	50 μl
5× M-MLV First Strand Buffer	500 μl
0.1 M DTT	100 μl

Description

M-MLV GIII Reverse Transcriptase is a third-generation M-MLV reverse transcriptase obtained through genetic modification and recombinant technology. This enzyme has removed RNase H activity relative to wild-type M-MLV reverse transcriptase and significantly improved thermal stability (optimal reaction temperature is 50℃), thereby greatly enhancing the specificity and length of first-strand cDNA synthesis (up to 12 kb), and improving tolerance to complex RNA secondary structures.

Definition of Activity Unit

The enzyme quantity needed to incorporate 1 nmol of [^{3}H] dTTP in 10 minutes at 37℃ using Poly(A)-Oligo(dT) as template/primer is defined as 1 unit of activity.

Quality Control Assays

Residual Host DNA

The product was tested by TaqMan qPCR with primers specific for the *E.coli* 16S rDNA , and the results show that the *E.coli* genome residues less than 1 copy/10 copies.

Endonuclease Activity

The product was tested in a reaction containing a supercoiled plasmid DNA substrate. After incubation for 4 hours at 37℃, there was no significant change of the DNA substrate by agarose gel electrophorresis.

Exonuclease Activity

The product was tested in a reaction containing DNA substrate. After incubation for 16 hours at 37℃ , there was no significant change of the DNA substrate by agarose gel electrophorresis.

Protocol

First-strand cDNA synthesis:

1. Prepare the following reaction system on ice:

Reagent		Amount
Primer		X μl
Oligo(dT)$_{20}$	The final concentration is 2.5 μM	
Or Random Primer	The final concentration is 2.5 ng/μl	
Or Gene-specific Primers	The final concentration is 0.25 μM	
Template RNA[a]		50 ng~1 μg/20 μl
5× M-MLV First Strand Buffer		4 μl
0.1 M DTT		1 μl
M-MLV GIII Reverse Transcriptase (200 U/μl)		1 μl
dNTP Mix (10 mM Each)		1 μl
(Optional) RNase Inhibitor (40 U/μl)		1 μl
Nuclease-Free Water		To 20 μl

a. It is recommend to use high-quality RNA extracted using a kit that removes genomic DNA contamination as a template.

2. Mix gently and spin down.

3. If using Oligo(dT)$_{20}$ or gene-specific primers, incubate at 50℃ for 30 min; If using random primers, first incubate at 25℃ for 5 min, followed by incubation at 50℃ for 30 minutes.

Note: If the desired cDNA is less than 3 kb, the incubation time can be shortened to 15 minutes.

4. Terminate the reaction by incubating at 85℃ for 5 minutes.

5. Place the cDNA solution on ice for use in subsequent experiments.

Note: The cDNA solution can be stored at -20℃ for up to six months, Long-term storage is recommended at -80℃ .

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