M-MLV GIV Reverse Transcriptase

REF: EG24101S

Storage Condition

-20℃

Components

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

Description

M-MLV GIV Reverse Transcriptase is a fourth-generation M-MLV reverse transcriptase obtained through genetic modification and recombination technologies. Compared with the wild-type M-MLV reverse transcriptase, it lacks RNase H activity. Key enhancements include superior thermal stability, with an optimal reaction temperature of 55℃ and sustained 50% activity at 70℃ . Furthermore, it demonstrates improved tolerance to complex secondary structures , accelerated reaction speed (completing reactions in as little as 10 minutes), and high template affinity. These combined properties ensure robust performance with low-copy templates and enable efficient synthesis of full-length cDNA fragments up to 20 kb.

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

Protein Purity

The enzyme is ≥95% pure as determined by SDS-PAGE analysis using Coomassie Blue staining.

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.

Residual Host DNA

ChP(2020) Volume IV, Detection of Exogenous DNA Residues, Method 3 (General Chapter 3407), the residual Escherichia coli host cell DNA content of this product is below 10 copies/20 U.

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~2 μg/20 μl
5× M-MLV First Strand Buffer	4 μl
0.1 M DTT	1 μl
M-MLV GIV 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 55℃ for 10 min; If using random primers, first incubate at 25℃ for 5 min, followed by incubation at 55℃ for 10 minutes.

Note: If the desired cDNA is less than 3 kb, the incubation time can be shortened to 15 minutes. For RNA with high GC content or complex secondary structure, the reverse transcription reaction temperature can be raised to 60℃ ~65℃ .

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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