

High-Accuracy Reverse Transcriptase

REF: EG21104S

Storage Condition

-20°C

Components

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

Description

High-Accuracy Reverse Transcriptase is a RNase H-deficient Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase cloned and expressed through genetic modification and recombinant technology. It coupled with a reversibly-bound aptamer that inhibits Reverse Transcriptase activity below 40 °C , thereby enhancing product specificity. Through genetic engineering, the enzyme's optimal reaction temperature has been increased to 50°C , providing stronger extension capability and making it suitable for longer cDNA synthesis.

Definition of Activity Unit

The enzyme quantity needed to incorporate 1 nmol of [³H] dTTP in 10 minutes at 37°C 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°C , there was no significant change of the DNA substrate by agarose gel electrophoresis.

Exonuclease Activity

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

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
dNTP Mix (10 mM Each)	1 μl
Nuclease-Free Water	To 13 μl

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

2. Incubate the above mixture at 65°C for 5 minutes, then quickly place it on ice for 1 minute to cool.

3. Add to the reaction mixture:

Reagent	Amount
5× M-MLV First Strand Buffer	4 μl
0.1 M DTT	1 μl
High-Accuracy Reverse Transcriptase	1 μl
(Optional) RNase Inhibitor (40 U/μl)	1 μl

4. Mix gently and spin down.

5. If using Oligo(dT)₂₀ or gene-specific primers, incubate at 50°C for 30 minutes. If using random primers, first incubate at 25 °C for 5 minutes, followed by incubation at 50°C for 30 minutes.

6. Terminate the reaction by incubating at 85°C for 5 minutes.

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

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