

M-MLV Reverse Transcriptase

Reverse Transcriptase is a reverse transcriptase (M-MLV-Reverse Transcriptase) obtained by genetic engineering technology to recombine Moloney murine leukemia virus. It has good heat resistance, can withstand reaction temperatures up to 55 °C, Efficient synthesis of full-length first-strand cDNA up to 13kb, suitable for reverse transcription of complex secondary structure RNA templates, provides broader gene representation and superior qRT-PCR sensitivity.

Cat N°	Size	Storage/Shelf life
FS-RT-1032A	10,000 U	-20°C/one year
FS-RT-1033A	50,000 U	-20°C/one year

Kit Components

Component	FS-RT-1032A	FS-RT-1033A
M-MLV- Reverse Transcriptase	10,000U	40,000U
5×RT Buffer	0.5 mL	1mL
RNase-Free Water	1.5 mL	1.5 mL

Kit application

1. First strand cDNA synthesis as a template for RT-PCR and real-time RT-qPCR
2. Construction of a full-length cDNA library
3. Antisense RNA synthesis

Advantages

1. No RNase H activity
2. Excellent specificity
3. Highly efficient synthesis of full-length first-strand cDNA up to 13 kb
4. Can withstand up to 55 ° C reaction temperature
5. Suitable for complex secondary structure RNA template reverse transcription

Active unit

The M-MLV Reverse Transcriptase concentration is 200U/μl.

A unit of activity (U) is defined as: Poly (A) as the template and Oligo (dT) as the primer, Reaction at 37°C for 10 minutes can mix 1 nmole of dTTP into the amount of enzyme required for acid-insoluble substances.

Purity:

The purity was greater than 90% by Coomassie blue staining SDS-PAGE. The product was free of endonuclease, exonuclease and RNase contamination.

Required Reagents but not supplied

- a. oligo(dT)12-18 (10 mM) or random primer (10 mM) or 2 p mole gene-specific primers
- b. dNTPs (10 mM each)
- c. RNase Inhibitor may be required
(when the amount of starting RNA is less than 0.5 μg, it is recommended to add RNase Inhibitor)
- d. RNase-free 1.5ml centrifuge tube
- e. Pipettes and tips (to avoid RNase contamination, RNase-free pipette tips with filter cartridges must be used)
6. Disposable gloves, masks and other protective equipment
7. Constant temperature water bath
8. In the laboratory without RNase:
Because of the RNase in the saliva and skin, wear latex gloves and a mask during the whole process of RNA extraction.

For Research Use Only

Operation steps

Add the following reagents to a sterilized microcentrifuge tube without RNase(on ice)

Reagent	Usage amount
oligo(dT) ₁₈ (10 mM) or Random Primers 9 (10 mM) or Gene Specific Primers (10 mM)	1.0 µL
	or 1.0 µL
	or 1.0 µL
dNTPs(10mM each)	1.0 µL
RNA*	0.5-5 µg
RNase-Free Water	to 15.0µL

*0.5-5 µg Total RNA or 50-500 ng mRNA. When using less than 0.5µg Total RNA (such as reverse transcription of viral RNA), the amount of M-MLV Reverse Transcriptase should be reduced to 0.05-0.5 µl, which may result in subsequent PCR amplification to produce non-specific amplification products.

1. The mixture is heated at 70°C for 5 minutes and then cooled rapidly on ice. After a short period of centrifugation, the following components are added:

Reagent	Usage amount
Step 1 Reaction Solution	
5*RT Buffer	4.0 µL
M-MLV Reverse Transcriptase	1.0 µL
RNase Inhibitor (Optional) *	1.0 µL

*It is recommended to add 1µl RNase Inhibitor when the dosage is less than 0.5µg Total RNA.

- Mix gently and evenly. When Random Primers 9 is used as a primer, it takes 10 minutes to keep at 25 °C. If Oligo (dT)₁₈ or Gene Specific Primers are used, this step can be omitted.
- Keep at 42°C for 60 minutes.
- Heating at 95°C for 5 minutes, it is cooled on ice or stored at - 20°C

* Reverse transcription products can be immediately used for subsequent PCR or qPCR reactions, or can be stored at - 20°C for a short time. If long-term storage is required, it is recommended that the products be stored at -80 °C after packaging to avoid repeated freezing and thawing.

For Research Use Only

FISHER MOLECULAR BIOLOGY

36 Terry Drive
Trevose, PA 19048 - USA