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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-1032	10,000 U (50 preps)	-20°C/one year
FS-RT-1033	40,000 U (200 preps)	-20°C/one year

Kit Components

Component	FS-RT-1032	FS-RT-1033
M-MLV- Reverse Transcriptase	10,000U (50ul)	40,000U (200ul)
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 μM) or random primer (10 μM) or 10 μM of 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.

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Operation steps

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

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

*0.5-5 μg Total RNA or 50-500 ng mRNA.When using less than 0.5ug 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℃ 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.

2. Mix gently and evenly. When Random Primers 9 is used as a primer, it takes 10 minutes to keep at 25 ℃. If Oligo (dT)18 or Gene Specific Primers are used, this step can be omitted.

4. Keep at 42℃ for 60 minutes.

5. Heating at 95°c for 5 minutes, it is cooled on ice or stored at - 20℃

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