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Highly sensitive reverse transcriptase

Product composition

Cat. No.	SEJ0214-1	SEJ0214-2
Highly sensitive reverse transcriptase	50 μl	200 μl
5 ×RT Buffer	0.5 ml	1 ml
RNase-free Water	1.5 ml	1.5 ml
Manual	One	One

Product storage and expiration date

Store at -20℃ for more than two years.

Product introduction

Highly sensitive reverse transcriptase is the third generation M-MLV reverse transcriptase obtained by gene modification and recombination technology. Compared with the wild-type M-MLV reverse rate enzyme, this enzyme removed RNase H activity, significantly improved the reverse transcription speed and thermal stability (maximum tolerance temperature was 60℃), and enhanced the tolerance to the complex secondary structure of RNA. Highly sensitive reverse transcriptase has strong anti-interference and high reverse transcriptase efficiency for low-copy RNA template, and is the preferred reverse transcriptase for RT-PCR nucleic acid detection kit.

Product application

1. Construction of cDNA library.
2. RT-PCR reaction and Real Time RT-PCR reaction.
3. Primer extension.
4. RNA sequencing.
5. One-step RT-PCR

Activity unit

Product concentration is 200 U/μl. Definition of activity unit: Using Poly (rA) as template and Oligo (dT) as primer, the amount of enzyme required to catalyze the incorporation of 1 nmoldTTP within 10 minutes at 37℃ was defined as 1 activity unit (U).

purity

The purity of the product was more than 90% by Coomath blue staining SDS-PAGE. The product was free of endonucrenase, exonucrenase and RNase contamination.

User-supplied reagents and items

1．oligo(dT)12-18 (25 μM) or random primers (25 μM) or gene-specific primers (1 μM)
2．dNTPs（10 mM each）
3．Maybe need RNase Inhibitor
4．RNase-free 1.5 ml centrifuge tubes
5．Pipettes and pipette tips (to avoid RNAse contamination, it is necessary to use RNase-free pipette tips with filters)
7．Constant temperature water bath
8．Operating in an RNAse-free laboratory environment: As saliva and skin both contain RNAse, please wear latex gloves and masks throughout the entire RNA extraction process.

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Operation procedure

1. The following reagents are added to RNASE-free sterilized microcentrifuge tubes:

1) 2 μl oligo(dT)$_{12-18}$（25 μM）or 2 μl Random primer（25 μM）or 2 μl Gene specific primers（1 μM）;

2) 0.5-5 μg Total RNA or 50-500 ng mRNA;

* When using less than 0.5 μg of Total RNA (such as reverse transcription of viral RNA), the amount of high-sensitivity reverse transcriptase should be reduced to 0.05-0.5μl, otherwise it may lead to non-specific amplification products in subsequent PCR amplification.
* When using less than 0.5 μg of Total RNA, it is recommended to add 1 μl of RNase Inhibitor (Simgen Cat. No. 8008125).

* If the RNA template needs to be heated at 70°C for 5 minutes to disrupt secondary structures, the addition of RNase Inhibitor should not be omitted.

3) 1 μl dNTPs（10 mM each）;

4) Refill RNaser-free Water to15 μl。

* If the RNA template has a high GC content or complex secondary structures, it is recommended to add the following steps: heat the RNA at 70°C for 5 minutes to disrupt its secondary structures, then quickly place it on ice to prevent the reformation of secondary structures, and finally centrifuge briefly to the bottom of the tube.

2. Add reagents according to the table below:

Step 1 Mixture	**15 μl**
5 ×RT Buffer	**4 μl**
Highly sensitive reverse transcriptase	**1 μl ***
Total	**20 μl**

*When using less than 0.5 μg of Total RNA, the amount of high-sensitivity reverse transcriptase should be reduced to 0.05-0.5 μl.

3. Gently mix the reagents, and when using random primers, incubate at 25°C for 10 minutes.

4.Incubate at 50°C for 30 minutes.

5.Heat at 95°C for 5 minutes, then cool on ice or store at -20°C for future use.

6.Dilute to 50μl with RNase-free Water, and take 2-5μl for PCR amplification.