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Hypersensitive Reverse Transcriptase Instructions

Composition

Cat. No.	8106050	8106200
Hypersensitive Reverse Transcriptase	50 μl	200 μl
5×RT Buffer	0.5 ml	1 ml
RNase-free Water	1.5 ml	1.5 ml
Instructions	1	1

Storage

It can be stored for up to two years at -20℃.

Technical Support

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Introduction

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

Applications

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

Unit Definition

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

Purity

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

Equipment and Reagents to Be Supplied by User

1. oligo(dT)$_{12\text{-}18}$ (25 μM) or random primers (25 μM) or gene specific primers (1 μM).
2. dNTPs (10 mM each, Simgen Cat.No.7701100).
3. RNase Inhibitor (Simgen Cat.No.8008125) may be required.
4. RNase-free microcentrifuge tubes .
5. Pipettes and tips (To avoid RNase contamination, RNase-free tips with filter must be selected).
6. Protective equipment such as disposable latex gloves and paper towels.
7. Water bath.
8. A laboratory without RNase: Because RNase is present in saliva and skin, please wear latex gloves and a mask during the whole experiments.

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Protocol

1. Add the following reagents to a RNase-free sterilized microcentrifuge tube.

1) 2 µl oligo(dT)$_{12-18}$(25 µM) or 2 µl random primers (25 µM) or 2 µl gene specific primers (1 µM).

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

* When total RNA is less than 0.5 µg, it was recommended to add 1 µl RNase Inhibitor (Cat. No. 8008125).

* If the RNA template needs to be incubated at 70℃ for 5 min to destroy the secondary structure, the addition of RNase Inhibitor should not be omitted.

3) 1 µl dNTPs (10 mM each).

4) Add RNase-free Water to 15 µl.

* If the RNA template is GC-rich or has a complex secondary structure, add the following steps: Incubated at 70℃ for 5 min to destroy the RNA secondary structure, then quickly place on ice to prevent the secondary structure from re-forming, then briefly centrifuge.

2. Add the reagents according to the table below:

The liquid mixture in step 1	15 µl
5×RT Buffer	4 µl
Hypersensitive M-MLV Reverse Transcriptase	1 µl *
Total	20 µl

* When total RNA is less than 0.5 µg (such as viral RNA reverse transcription), the amount of Hypersensitive M-MLV Reverse Transcriptase should be reduced to 0.05~0.5 µl, otherwise it may lead to subsequent PCR amplification to produce non-specific amplification products.

3. Mix gently, if using random primers as primers, hold at 25℃ for 10 min.

4. Incubate at 50℃ for 30 min.

5. Incubate at 95℃ for 5 min, then cool on ice or store below -20℃ for later use.

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