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EntiLink™ Reverse Transcriptase

M-MLV

Catalog No.	Specification	Storage/Shelf life
EQ002-01	10000U	-20℃/three year
EQ002-02	40000U	-20℃/three year

Introduction

EntiLinkTM Reverse Transcriptase is a reverse transcriptase (MMLV RT) 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.

Kit Components

Component	EQ002-01	EQ002-02
EntiLink™ Reverse Transcriptase	10000U	40000U
5×RT Buffer	0.5 mL	1mL
RNase-Free Water	1.5 mL	1.5 mL
User Manual	1 copy	1 copy

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

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Advantage

1. No RNaseH 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 product 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.

Self supplied Reagents and items

1.oligo$(dT)_{12-18}$ (10 uM) or random primer (10 mM) or 2 p mole gene-specific primers

2. RNase Inhibitor may be required (when the amount of starting RNA is less than 0.5 μg, it is recommended to add RNase Inhibitor)

3.RNase-free 1.5ml centrifuge tube

4. Pipettes and tips (to avoid RNase contamination, RNase-free pipette tips with filter cartridges must be used)

5. Disposable gloves, masks and other protective equipment

6. Constant temperature water bath

7. 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

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

Reagent	Usage amount
oligo(dT)$_{18}$ (10 uM)	1.0 μL
or Random Primers 9 (10 uM)	or 1.0 μL
or Gene Specific Primers (10 uM)	or 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 0.5 μ g 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.

2，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
EntiLink™ 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.

3，Reverse Transcription Program Settings

25℃*1	**5min**
42℃	**30min*2**
85℃	**5min**

*1 When pd (N) 9 is used, 25 ℃ is required for 5min. If oligdT or Gene Specific Primers are used, this step can be omitted.

*2 If the production of cDNA needs to be increased, the reverse transcription time can be extended to 60 minutes.