ELK Biotechnology

For research use only.

EntiLink™ 1st Strand cDNA Synthesis Kit

Catalog No.	Specification	Storage/Shelf life
EQ003-01	50 rxn	-20℃/three year
EQ003-02	200 rxn	-20℃/three year

Introduction

The EntiLink™ 1st Strand cDNA Synthesis Kit is a complete system for the efficient synthesis of first-strand cDNA that can synthesize cDNA up to 13 kb. This product contains gDNA Eraser, which can quickly and completely remove genomic contamination.

The reverse transcription primer provided along with EntiLink 1st Strand cDNA Synthesis Kit is pd(N)9 and oligo $dT_{(18)}$.Reverse transcription of various cDNAs in the sample.Suitable for reverse transcription of various RNAs such as mRNA, lncRNA and circRNA.

The kit can also be used for gene-specific reverse transcription, such as miRNA reverse transcription.

Kit Components

Component	EQ003-01	EQ003-02
gDNA Eraser	50 μL	200 μL
10×gDNA Eraser Buffer	50 μL	200 μL
EntiLink™ Reverse Transcriptase	10000U	40000U
5×RT Buffer	0.5 mL	1.0 mL
RNase-Free ddH2O	1.5 mL	1.5 mL
pd(N)9	50 μL	200 μL
oligo dT (18)	50 μL	200 μL
User Manual	1 copy	1 copy

Advantage

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1. Can efficiently synthesize full-length first-strand cDNA up to 13kb
2. Can withstand reaction temperatures up to 55 ° C
3. Fully provide all the components needed for the RT reaction

Kit application

1. cDNA library construction.
2. RT-qPCR reaction and RT-PCR reaction.
3. Primer extension.
3. RNA sequencing.

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.RNase-free 200μL microcentrifuge tube

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

3. Disposable gloves, masks and other protective equipment

4. Constant temperature water bath

5. In RNase-free laboratory operations: Because of the RNase in saliva and skin, wear latex gloves and a mask during the whole process of RNA extraction.

* may require RNase Inhibitor

Operation steps

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1．Remove genomic DNA response (on ice):

Reagent	Usage amount
10×gDNA Eraser Buffer	1.0 μL
gDNA Eraser	1.0 μL
Template RNA	0.5-5 μg
RNaser-Free Water	to 10.0 μL

*Incubate at 42 ° C for 2 min (or room temperature for 5 min)

Store at 4 ° C

2．Reverse transcription reaction (on ice)

Reagent	Usage amount
Step 1 reaction solution	10.0 μL
oligodT (10 μM) or pd(N)9 (10 μM) or Gene Specific Primers (10 μM) *1	1.0 μL or 1.0 μL or 1.0 μL
RNase-Free ddH2O	to 15.0 μL

After heating the mixture at 70 ° C for 5 min, it was quickly placed on ice for cooling. After a brief centrifugation, add the following components:

Reagent	Usage amount
Step 1 reaction solution	
5×RT Buffer	4.0 μL
EntiLink™ Reverse Transcriptase*2	1.0 μL
RNase Inhibitor*3	1.0 μL

*1The kit provide oligodT and pd(N)9, which can be selected according to experimental needs; Gene Specific Primers need to be prepared by customers

*2The amount of EntiLink™ Reverse Transcriptase should be reduced to 0.05-0.5 μl when less than 0.5 μg of Total RNA (such as reverse transcription of viral RNA). Otherwise, subsequent PCR amplification may result in non-specific amplification products.

*3 When adding less than 0.5 μg of Total RNA, it is recommended to add 1 μl of RNase Inhibitor (Cat. No. EQ010).

3、Reverse transcription program settings

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

*1 When using pd(N)9, it takes 25℃, 5min. This step can be omitted if oligdT or Gene Specific Primers are used.

*2 To increase the cDNA yield, the reverse transcription time can be extended to 60min.