1st Strand cDNA Synthesis Kit (+gDNA Eraser)

Description

1st Strand cDNA Synthesis Kit

Catalog No.	Specification	Storage/Shelf life
10-300EQ	50 rxn	-20â„ƒ/three year
20-300EQ	200 rxn	-20â„ƒ/three year

Introduction

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 Î¼L	200 Î¼L
10Ã—gDNA Eraser Buffer	50 Î¼L	200 Î¼L
EntiLinkâ„¢ Reverse Transcriptase	10000U	40000U
5Ã—RT Buffer	0.5 mL	1.0 mL
RNase-Free ddH2O	1.5 mL	1.5 mL
pd(N)9	50 Î¼L	200 Î¼L
oligo dTï¼ˆ18ï¼‰	50 Î¼L	200 Î¼L
User Manual	1 copy	1 copy

Advantage

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

Kit application

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

Active unit

The product concentration is 200U/Î¼L.

A unit of activity (U) is defined as: Poly (A) as the template and Oligo (dT) as the primer,Reaction at 37Â°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 200Î¼L 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

Remove genomic DNA response (on ice):

Reagent	Usage amount
10Ã—gDNA Eraser Buffer	1.0 Î¼L
gDNA Eraser	1.0 Î¼L
Template RNA	0.5-5 Î¼g
RNaser-Free Water	to 10.0 Î¼L

Incubate at 42 Â° C for 2 min (or room temperature for 5 min) Store at 4 Â° C

Reverse transcription reaction (on ice)

Reagent	Usage amount

Step 1 reaction solution	10.0 Î¼L
oligodTï¼ˆ10 Î¼Mï¼‰ or pd(N)9ï¼ˆ10 Î¼Mï¼‰	1.0 Î¼L
or Gene Specific Primers ï¼ˆ10 Î¼Mï¼‰*1	or 1.0 Î¼L or 1.0 Î¼L
RNase-Free ddH2O	to 15.0 Î¼L

After heating the mixture at 70 Â° 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	
5Ã—RT Buffer	4.0 Î¼L
EntiLinkâ„¢ Reverse Transcriptase*2	1.0 Î¼L
RNase Inhibitor*3	1.0 Î¼L

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

The amount ofÂ Reverse Transcriptase should be reduced to 0.05-0.5 Î¼l when less than 0.5 Î¼g of Total RNA (such as reverse transcription of viral RNA). Otherwise, subsequent PCR amplification may result in non-specific amplification products.

When adding less than 0.5 Î¼g of Total RNA, it is recommended to add 1 Î¼l of RNase Inhibitor

(Cat. No. 010EQ).

Reverse transcription program settings

Temperature	**Time**
25â„ƒ***1**	**5min**
42â„ƒ	**30min*2**
85â„ƒ	**5min**

1 When using pd(N)9, it takes 25â„ƒ, 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.

Date Created
2024/07/03