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EntiLink™ 1st Strand cDNA Synthesis Kit

Catalog No.	Specification	Storage/Shelf life
EQ003	100 rxn	-20℃/1.5 years

Introduction

The EntiLink™ 1st Strand cDNA Synthesis Kit is a complete system for the efficient synthesis of first-strand cDNA .

The EntiLink™ Reverse Transcriptase has dramatically improved thermal stability and can withstand reaction temperatures up to 50°C, making it suitable for reverse transcription of RNA templates with complex secondary structures. The EntiLink™ Reverse Transcriptase also has an increased affinity for templates and is suitable for reverse transcription of small amounts of templates and low-copy genes. The EntiLink™ Reverse Transcriptase also has an improved ability to synthesize full-length cDNAs, which can be amplified up to 10 kb in length.

The kit contains all the components needed to synthesize high-quality first-strand cDNA from total RNA or mRNA, and provides two primers for cDNA synthesis: Random Primers N6 and oligo (dT)18. The synthesized single-stranded cDNA product can be used directly in subsequent PCR or qPCR reactions.

Kit Components

Component	Quantity
RNase-Free ddH2O	2×1 mL
5× Buffer	400 µL
EntiLink™ Enzyme Mix	200 µL
Oligo (dT)18 (50 µM)	100 µL
Random Primers N6 (50 µM)	100 µL
User Manual	1 copy

Note: 1) 5× Buffer contains dNTPs. 2) EntiLink™ Enzyme Mix contains RNase inhibitor

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Advantage

1. Can efficiently synthesize full-length first-strand cDNA up to 10kb.
2. Can withstand reaction temperatures up to 50 ° 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.

Notes

1.All operations should be performed on ice, and RNase contamination should be avoided.
2.For your safety and health, please wear lab coat and disposable gloves to operate.
3.This product is for research use ONLY!

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.

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Operation steps

1. Reverse transcription reaction system preparation (20 μL system)

Components	Volume
Total RNA	1 ng -5 μg*
or mRNA	1 ng-500 ng*
5× Buffer	4 μL
Random Primers N6 (50 μM)	1 μL
or Oligo (dT)18 (50 μM)	or 1 μL
or Gene Specific Primers (2 μM)	or 1 μL
EntiLink™ Enzyme Mix	2 μL
RNase-free H2O	To 20 μL

[Note]: *If the subsequent experiment is qPCR, it is recommended that the amount of Total RNA or mRNA input does not exceed 1 μg or 100 ng, and if the expression abundance of the target gene is very low, up to 5 μg of Total RNA or 500 ng of mRNA can be input.

** If the subsequent experiment is PCR, for complex templates, the RNA, H_2O, and reverse transcription primers can be incubated at 65°C for 5 min and then quickly cooled on ice before adding the EntiLink™ Enzyme Mix; if the subsequent experiment is qPCR, the incubation step at 65°C can be omitted and the EntiLink™ Enzyme Mix can be added directly into the system.

2. Reaction program

Reaction Temperature	Reaction Time
25℃	5 min
42℃	30 min
85℃	5 min

[Note]: 1) Fluorescence quantification experiments can be performed using only Random Primers N6; they can also be mixed 1:1 with Oligo $(dT)_{18}$ for better results.

2) Reverse transcription temperature: 42°C is recommended. For high GC content templates or complex templates, the reverse transcription temperature can be increased to 50℃.

3) Reverse transcription products can be stored at -20℃ for a short period of time, if long-term storage is required, it is recommended to store them at -80℃ after dispensing to avoid repeated freezing and thawing.

3. Primer selection

1) If the template is of eukaryotic origin, it is recommended to choose Oligo $(dT)_{18}$, which pairs with the 3' Poly A tail of the eukaryotic mRNA for the highest yield of full-length cDNA.

2) For reverse transcription of prokaryotic RNA, use Random Primers N6 or gene-specific primers.

3) Random Primers N6 is widely applicable. mRNA, rRNA, tRNA, small RNA and LncRNA templates can be reverse transcribed with Random Primers N6.

4) For cDNA synthesis of less than 2 kb, use 1-2 μL of Random primers N6; for cDNA synthesis of more than 2 kb, use 0.4-1 μL of Random primers N6.