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ODR51

Micro -RNA (miRNA) cDNA Synthesis Kit

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Product Introduction

This miRNA First-Strand cDNA Synthesis Kit employs the polyadenylation (A-tailing) method. The miRNA Enzyme Mix contains E. coli Poly(A) Polymerase, RTase, and RNase inhibitor. The E. coli Poly (A) Polymerase not only has high A-tailing efficiency but also can specifically recognize single-stranded miRNA, avoiding reverse transcription of the double-stranded miRNA precursor. The 2× miRNA RT Mix in this kit contains all the components and primers needed for miRNA polyadenylation and reverse transcription. It is optimized to enable efficient simultaneous Poly (A) modification of the miRNA 3' end and reverse transcription. For subsequent qPCR, simply design specific forward primers and use the universal reverse primer provided in the kit to detect miRNA in the sample.

Components

No.	Components	Size (50T)
Reagent 1	2× miRNA RT Mix	500μL
Reagent 2	miRNA Enzyme Mix	75μL
Reagent 3	RNase-Free Water	1mL
Reagent 4	Universal Reverse Primer(10μM)	1.25mL

Storage

The unopened kit can be stored at -20℃ for 12 months.

Product Features

1. Simple Operation
   Polyadenylation and reverse transcription are completed simultaneously in one tube.
2. High Sensitivity/ High cDNA Yield
   The kit enables efficient Poly (A) modification and reverse transcription. It can effectively generate the first-strand cDNA corresponding to miRNA from 10 pg - 2 μg of Total RNA and $>10^3$ copies of miRNA.
3. Good Specificity
   It can distinguish single-base differences between miRNAs of the same family, allowing the detection of multiple miRNAs from cDNA synthesized in one reaction. This reduces errors and saves samples.
4. Wide Application Range
   It can perform reverse transcription reactions on miRNAs extracted from various types of samples.

Precautions

To prevent RNase contamination, the following should be observed:

1. Use RNase-free plasticware and pipette tips to avoid cross-contamination.
2. Glassware should be dry-heated at 180℃ for 4 hours before use. Plasticware can be soaked in 0.5M NaOH for 10 minutes, then thoroughly rinsed with water and autoclaved.
3. Solutions should be prepared using RNase-free water.
4. Operators should wear disposable masks and gloves, and change gloves frequently during the experiment.

Instructions for Use

1. Reverse Transcription Reaction Setup
   Prepare the reaction mixture in an RNase-free centrifuge tube on ice as follows:

Components	Total (20μL)	Final Con.
2× miRNA RT Mix	10μL	1×
miRNA Enzyme Mix	1.5μL	-
Total RNA/miRNA*	XμL	About 2μg
RNase Free Water	To 20μL	

***Note: Add 2-5μL (adjust the amount according to the abundance of the target miRNA).**

2. Reverse Transcription Program
   Gently mix the reaction mixture and briefly centrifuge to collect the liquid at the bottom of the tube. Perform the reverse transcription reaction as per the table below:

Reaction Temperature	Reaction Time
37℃	60 min
85℃	5 min

- The synthesized cDNA reaction mixture should avoid repeated freeze-thaw cycles. It is recommended to store it short-term at -20℃ and long-term at -70℃.
- Fluorescent quantitative detection can be performed directly. To prevent inhibition of the qPCR reaction by the reverse transcription system, the cDNA reaction mixture can be diluted 10-1000 times based on the specific Ct value before use.

Quantitative Primer Design

1. Forward Primer

- It is recommended to design miRNA-specific forward primers based on the complete miRNA sequence, replacing U with T.
- If the annealing temperature of the designed forward primer is too low, add a few bases (mainly G and C) to the 5' end of the primer. After adding bases, verify primer specificity to avoid non-specific amplification. If the annealing temperature is too high, remove a few bases from the 5' end.
- For non-specific amplification of long miRNA precursors, add 1-3A bases to the 3'end of the forward primer.
- For miRNAs with similar sequences, terminate the 3' end of the forward primer at the differential base. If the primer is too short, leading to a low annealing temperature, add a few bases to the 5'end to match the Tm values of the upstream and downstream primers.

2. Reverse Primer
   The product provides a universal reverse primer (Universal Reverse Primer) for qPCR detection, with an annealing temperature of approximately 63.6℃.