Tel : 86-10-56953015
Email : info@cwbio.com

2. Reverse primer
   This product provides Universal Reverse Primer for qPCR detection, with an annealing temperature of approximately 63.6 ℃.

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SuperStar miRNA First-Strand cDNA Synthesis Kit (by tailing A)

Cat. No. : CW2151

Introduction

This kit uses the A-tailing method to synthesize the first-strand cDNA of miRNA. The miRNA Enzyme Mix contains *E. coli* Poly(A) polymerase, RTase, and RNase inhibitor. *E. coli* Poly(A) Polymerase not only has a high efficiency of A-tailing, but also specifically recognizes single-stranded miRNAs, thus avoiding further reverse transcription of miRNA precursors with double-stranded structures. The 2×miRNA RT Mix in this product contains all the ingredients and primers required for miRNA tailing and reverse transcription reactions, and has been optimized to ensure that the Poly(A) modification and reverse transcription processes at the 3' end of miRNA are performed efficiently at the same time. Combined with the universal reverse primers in the kit, subsequent qPCR only requires the design of specific forward primers to detect the miRNA in the sample.

Storage Condition: -20 ℃.
Product Shelf Life: 1 year

Components

Component	CW2151S 10 rxns	CW2151M 50 rxns
2×miRNA RT Mix	100 μL	500 μL
miRNA Enzyme Mix	15 μL	75 μL
RNase-free ddH_2O	1 mL	1 mL
Universal Reverse Primer	250 μL	1.25 mL

This product is for scientific research only, which shall not be used for clinical diagnosis or other purposes.

Features

1. Easy to use
   Tailing and reverse transcription can be completed in one tube.
2. High sensitivity/high cDNA yield
   This kit has very high Poly(A) modification and reverse transcription efficiency, and can efficiently obtain the first strand of cDNA corresponding to miRNA from 10 pg-2 μg of total RNA and > 100 copies of miRNA.
3. Good specificity
   It can distinguish the single-base difference between miRNAs of the same family, and can detect multiple miRNAs from cDNA synthesized in one reaction at the same time, thereby reducing errors and saving samples.
4. Wide range of applications
   It can perform reverse transcription reactions on miRNAs extracted from a variety of different types of samples.

Notes

To prevent RNase contamination, the following aspects should be noted:

1. Use RNase-free plastics and tips to avoid cross-contamination.
2. Glassware should be hot-air sterilized at 180 ℃ for 4 hours before use. Plasticware can be soaked in 0.5M NaOH for 10 minutes, rinsed thoroughly with water and autoclaved.
3. RNase-free water should be used to prepare the solution.
4. Operators should wear disposable masks and gloves, and change gloves frequently during the experiment.

Protocol

1. Reverse transcription system
   Prepare the following reaction solution in RNase-free centrifuge tubes on ice:

Reagent	20 μL System	Final Concentration
2×miRNA RT Mix	10 μL	1×
miRNA Enzyme Mix	1.5 μL	-
Total RNA/miRNA*	X μL	Up to 2 μg
RNase-free ddH_2O	To 20 μL	

*** The recommended dosage is 2-5 μL. Please determine the amount according to the abundance of the target miRNA.**

2. Reverse transcription program
   Gently mix the above reaction solution and collect the liquid to the bottom of the tube by a short centrifugation. Perform the reverse transcription reaction of miRNAs as follows:

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

- Repeated freeze-thaw should be avoided for reverse transcription products, and it is recommended to store at -20 ℃ for short-term storage and -70 ℃ for long-term storage.
- In order to avoid inhibition of the quantitative PCR reaction by the reverse transcription system, the cDNA reaction can be diluted 10-1000 times depending on the specific Ct value.

Quantitative Primer Design

1. Forward primer

- It is recommended to design miRNA forward-specific primer based on the complete miRNA sequence and replace the U with the T.
- If the annealing temperature of the designed forward-specific primer is too low, it is recommended to add a few bases at the 5' end of the primer (mainly G and C), and verify the primer specificity after adding the base to avoid non-specific amplification. If the annealing temperature of the primer is too high, it is recommended to delete a few bases at the 5' end.
- To avoid non-specific amplification of isometric fragments of miRNA precursors, it is recommended to add 1-3 A bases at the 3' end of the forward-specific primer.
- For miRNAs with similar sequences, it is recommended that the 3' end of the forward-specific primer terminates at the differential base. If the annealing temperature is too low due to the short length of the primer, a few bases can be added to the 5' end of the primer to match the Tm values of the upstream and downstream primers.