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miRNA cDNA Synthesis Kit

Product Number: PCK41

Shipping and Storage

-20°C.

Components

Component	PCK41 25rxn
Tris-Hcl,1mM,PH8.0	1ml
E. coli Poly(A) Polymerase,5U/µl	15µl
10×Poly(A) Polymerase Buffer	80µl
ATP,10 mM	15µl
RT Primer,25 µM	90µl
5×SuperRT Buffer	120µl
UltraPure dNTP Mix,10 mM each	30µl
SuperRT	15µl
RNase-Free Water	1ml

Description

This kit uses the method of adding a poly (A) tail at the 3 'end of miRNA to make miRNA have a Poly (A) tail, and then uses Oligo (dT) - Universal tag universal reverse transcription primer for reverse transcription reaction to finally synthesize the first strand cDNA corresponding to miRNA.

The miRNA cDNA first strand synthesis kit contains all the reagents required for the miRNA 3 'end Poly (A) tail modification process and post modification reverse transcription process.This kit has very high Poly (A) modification and reverse transcription efficiency, and can effectively obtain the first strand of cDNA corresponding to miRNA from 1ng-2µg of total RNA.And the operation is simple and fast, which can be used to simultaneously detect multiple miRNAs from a single reaction synthesized cDNA. This not only reduces errors and saves samples, but also achieves high throughput detection.

Note

This kit must be used in conjunction with the miRNA fluorescence quantitative detection kit.

Self prepared experimental material

1ng-2µg of Total RNA , or 0.1ng-1µg of Small molecule RNA.

Note

To prevent RNase pollution, attention should be paid to the following aspects:

1. Use plastic products and gun heads without RNase to avoid cross contamination.
2. Glassware should be dried at a high temperature of 180℃ for 4 hours before use, while plastic utensils can be soaked in 0.5M NaOH for 10 minutes, thoroughly rinsed with water, and then sterilized under high pressure.
3. The solution should be prepared using water without RNase.
4. Operators should wear disposable masks and gloves, and change gloves frequently during the experiment.

Protocol

1. The process of miRNA adding Poly (A) tail:

1.1. Firstly, based on the amount of RNA used, dilute 10mM ATP with 1mM Tris (PH8.0) according to the following formula: ATP dilution coefficient=5000/__ng of Total RNA .

Example: If the initial dosage of total RNA is 100ng, then the ATP dilution coefficient is 5000/100=50. Dilute ATP by 50 times (1μl 10mM ATP plus 49μl 1mM Tris, pH 8.0).

1.2. Add the following reagents to the pre cooled RNase free reaction tube in the ice bath to a total volume of 25μl.

Reagent	25μl Reaction	Final Conc.
total RNA*	Xμl	Up to 2μg
10×Poly(A) Polymerase Buffer	2.5μl	1×
Diluted ATP in step '1'	1μl	—
E. coli Poly(A) Polymerase, 5U/μl	0.5μl	2.5 U
RNase-Free Water	up to 25μl	—

***The total RNA used in the reaction must contain small molecule RNA**

This process can also directly use small molecule RNA (recommended dosage is 2-5μl. Please determine the addition amount based on the abundance of the target miRNA.

1.3. Gently mix the above reaction solution and briefly centrifuge to collect the liquid at the bottom of the tube. Incubate at 37℃ for 15 minutes. After this process is completed, the synthesis of the first strand cDNA is immediately carried out or temporarily stored at -20℃. If long-term storage is required, it is recommended to store at -80℃.

2. The process of synthesizing the first strand of modified miRNA cDNA

2.1. Add the reagents in the table below to the pre cooled RNase free reaction tube in the ice bath until the final volume is 20μl:

Reagent	20μl Reaction
The above Poly (A) reaction solution	4μl
UltraPure dNTP Mix,10mM each	1μl
RT Primer,25μM	3μl
5×SuperRT Buffer	4μl
SuperRT	0.5μl
RNase-Free Water	7.5μl

2.2. Gently mix the above reaction solution and briefly centrifuge to collect the liquid at the bottom of the tube. Incubate at 42℃ for 50 minutes.

2.3. Incubate at 85℃ for 5 minutes and terminate the reaction. The synthesized cDNA reaction solution can be directly used for fluorescence quantitative detection experiments or stored at -20℃ for future use.