miRNAcDNASynthesisKit

Project number: M665754

Storage conditions: -20° C.

Products content:

Component	M6657542 5rxns
Tris-HCl, 1 mM, pH 8.0	1ml
*E.*coliPoly(A) Polymerase, 5U/μl	15 μl
10×Poly(A)PolymeraseBuffer	80 μl
ATP, 10 mM	15 μl
RTPrimer, 25 μM	90 μl
5×SuperRTBuffer	120 μl
UltraPuredNTPMix, 10mMeach	30 μl
SuperRT, 200U/μl	15 μl
RNase-FreeWater	1ml

Product Introduction:

This kit adopts the method of adding poly(A) tail at the 3' end of miRNA to make miRNA with Poly(A) tail, followed by reverse transcription reaction using Oligo(dT)-Universaltag universal reverse transcription primer, and finally synthesize the first strand cDNA corresponding to miRNA.

The miRNAcDNA First Strand Synthesis Kit contains all the reagents needed for the miRNA 3' end Poly(A) tail modification process and the reverse transcription process after modification. The kit has a very high Poly(A) modification and reverse transcription efficiency, and can effectively obtain the corresponding cDNA first strand of miRNA from 1ng-2μg of totalRNA. Moreover, it is easy and fast to operate, and can be used for simultaneous detection of multiple miRNAs from cDNA synthesized by one reaction, which not only reduces errors and saves samples, but also realizes high throughput of detection.

Note: **This kit must be used in conjunction with the miRNA Fluorescence Quantitative Detection Kit.**

Self-contained lab materials: 1ng-2μg of total RNA, or 0.1ng-1μg of small molecule RNA.

caveat

To prevent RNase contamination, the following should be noted:

1. Use RNase-free plastics and tips to avoid cross-contamination.

2. Glassware should be dry-roasted at 180° C for 4 hours before use, and plasticware can be soaked in 0.5MNaOH for 10 minutes, rinsed thoroughly with water and autoclaved.

3. RNase-free water should be used to prepare the solution.

4. Operators wear disposable masks and gloves, and change gloves frequently during the experiment.

Usage

A. The process of miRNA plus Poly(A) tail:

1. First, according to the amount of RNA used, dilute 10 mM ATP with 1 mMTris (PH8.0) according to the following formula: ATP dilution factor = 5000/___ng of total RNA

Example: If the starting amount of total RNA is 100ng, then the ATP dilution factor = 5000/100 = 50. i.e., dilute the ATP 50-fold (1 μl of 10 mM ATP plus 49 μl of 1 mMTris, pH 8.0).

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

reagents	25 μl reaction system	final concentration
totalRNA*	X μl	Up to 2 μg
10 Poly(A)PolymeraseBuffer	×2.5 μl	1×
Diluted ATP in step "1"	1 μl	
E.coliPoly(A) Polymerase, 5U/μl	0.5 μl	2.5U
RNase-FreeWater	upto25 μl	—

*TotalRNA used in the reaction must contain small molecule RNA.

This procedure can also be done using small molecule RNA directly (recommended addition amount is 2-5 μl. Please determine the amount to be added based on the abundance of the target miRNA).

3. Gently mix the above reaction solution and collect the liquid at the bottom of the tube by brief centrifugation. incubate at 37° C for 15 minutes. At the end of the process, synthesize the first strand cDNA immediately or store it at -20° C. For long term storage, it is recommended to store it at -80° C. For long-term storage, it is recommended to store at -80℃.

B. The process of synthesizing the first strand of modified miRNAcDNA:

1. Add the reagents in the table below to a pre-cooled RNase-free reaction tube in an ice bath to a final volume of 20 μl:

reagents	20 μl reaction system
Poly(A) reaction solution above	4 μl
UltraPuredNTPMix, 10mMeach	1μl
RTPrimer, 25 μM	3μl
5×SuperRTBuffer	4 μl
SuperRT, 200U/μl	0.5μl
RNase-FreeWater	7.5μl

2. Gently mix the above reaction solution and collect the liquid at the bottom of the tube by centrifugation briefly. 42° C, incubate for 50 minutes.

3. Incubate at 85℃ for 5 minutes to terminate the reaction. The synthesized cDNA reaction solution can be used directly for quantitative fluorescence detection or stored at -20℃ for spare parts.