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microRNA 1st Strand cDNA synthesis kit

Product Number: PCK50

Storage condition

-20°C

Component

Component	25T
microRNA RT Enzyme Mix	50μL
2 × miRT Reaction Mix	250μL
RNase free H_2O	1mL

Description

Enhanced microRNA 1st Strand cDNA synthesis kit This kit uses the Poly (A) tail addition principle. Firstly, a Poly (A) tail is added to the 3 'end of microRNA, and then an Anchored oligo (dT) - universal tag universal reverse transcription primer is used for reverse transcription reaction to generate the corresponding cDNA first chain of microRNA. This kit uses a specially optimized pre mixed microRNA RT Enzyme Mix to combine Poly (A) tailing and reverse transcription into one step, simplifying the operation steps and improving the efficiency of Poly (A) tailing and reverse transcription. The kit has the ability to effectively prepare microRNA corresponding cDNA first strands from 20pg-2μg Total RNA. A single synthesized cDNA can detect multiple microRNAs, saving samples and costs.

Note: This kit must be used in conjunction with the Enhanced microRNA Real-Time PCR Assay kit(PCK51).

Protocol

1. Poly (A) tail addition and reverse transcription reaction (first strand synthesis) at the 3 'end of microRNA:

1.1. Thaw 2 x microRNA RT Reaction Mix and mix well. Place the miRT Enzyme Mix in ice for later use. Add the following reagents to a total volume of 20μL (finally add microRNA RT Enzyme Mix).

Components	Volume	Final Concentration
Total RNA	xμL	Up to 2μg
2 ×microRNA RT Reaction Mix	10μL	1×
microRNA RT Enzyme Mix	2μL	-
RNase free H_2O to final volume	20μL	-

Note: microRNA RT Enzyme Mix is very viscous, and the solution is prone to adsorption on the tube wall and the outside of the suction head, resulting in loss. Before use, please spin centrifuge and avoid adhesion and loss on the outer wall of the suction head. The enzymes in Enzyme Mix are all excessive, and even if used at 1.8μL each time, it does not affect the effectiveness of use.

*The total RNA used in the reaction must contain small molecule RNA (microRNA). This process can also use enriched microRNAs. Simple microRNAs cannot be directly quantified using a spectrophotometer. It is recommended to add 2μL to 5μL directly. The amount of microRNA added can be determined based on the abundance of the target microRNA, but for low abundance microRNA samples (such as serum plasma extracts), a maximum volume of 8μL can be directly added.

1.2. Gently mix the prepared reaction solution with a pipette, centrifuge briefly, and react at 42°C for 60 minutes.

Heat at 85°C for 5 seconds to inactivate microRNA RT Enzyme Mix. The synthesized cDNA reaction solution can be stored at -20°C; Downstream PCR or fluorescence quantitative PCR detection can also be directly performed.

2. Perform quantitative PCR using our company's microRNA Real-Time PCR Assay kit.

Note: When using the cDNA template obtained by following the above steps for downstream PCR or fluorescence quantitative PCR detection, the amount used can be selected according to the actual situation. For special detection systems, high content of

cDNA templates can easily lead to non-specific amplification. cDNA can be appropriately diluted (5-10 times or 100 times) according to the abundance of detected microRNA before use. If non-specific amplification bands are found or the melting curve shows non-specific amplification, it often indicates an excess of cDNA template. You can try diluting the above cDNA template by tens to hundreds or even thousands of times before use.