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microRNA RT & qPCR Detection kit

Product Number: PCK52

Storage condition

Store at -20°C .

Component

Component	PCK52
microRNA RT Enzyme Mix	50μL
2 × microRNA RT Reaction Mix	250μL
Reverse primer(10μM)	200μL
2 ×microRNA qPCR Mix(With Sybr Green)	5mL
ROX Reference Dye	100μL
RNase free H_2O	1mL

Description

The enhanced microRNA reverse transcription/fluorescence quantitative detection kit contains all the reagents for microRNA detection. This product adopts the Poly (A) tail addition method, using microRNA as a template, and using a specially optimized pre mixed microRNA RT Enzyme mix (including Poly (A) tail addition enzyme and reverse transcription enzyme) to efficiently complete cDNA synthesis through a one-step method of Poly (A) tail addition and reverse transcription; MicroRNA detection was performed using a 2 x microRNA qPCR mix. Suitable for samples containing microRNAs such as Total RNA or Small RNA.

Features

1. The optimal ratio of Poly (A) tail enzyme and reverse transcriptase, as well as the optimized reaction buffer, ensure the reverse transcription efficiency of microRNA.
2. PolyA tail addition and reverse transcription cDNA synthesis are completed in one step in the same tube.
3. 2×microRNA qPCR Mix has high amplification efficiency, strong specificity, and sensitivity.
4. Equipped with ROX Reference Dye, it can be used for various models that require high and low ROX reference dyes.

Protocol

1. MicroRNA 3 'end undergoes Poly (A) tail addition and reverse transcription reaction (first strand synthesis)

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 20 μ L (finally add microRNA RT Enzyme Mix).

Component	Volume	Final concentration
Total RNA	xμL	Up to 2μg
2 ×microRNA RT Reaction Mix	10μL	1 ×
microRNA RT Enzyme Mix	2μL(See precautions)	-
RNase free H2O 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.

1.3. 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 fluorescence quantitative PCR detection

Forward Primer design principles

1. Follow the most common principles of primer design.
2. Based on mature microRNA sequences, replacing U with T is the most basic and simplest design method.
3. The Tm value of downstream primers provided in the kit is 65°C, and the Tm value of upstream primers should be designed to be around 65°C.
4. If the Tm value of the primer designed directly according to principle 2 is too low, several bases (preferably G or C bases) can be added to the 5 'end of the primer; One or several A bases can also be added at the 3 'end; Or add both the 5 'end and the 3' end simultaneously.
5. If the Tm value of a primer designed directly according to principle 2 is too high, a few bases can be removed from the 5 'or 3' end of the primer.

Note

1. The amount of microRNA first strand cDNA added should not exceed 1/10 of the real-time PCR volume.
2. For special detection systems, high levels of cDNA templates can easily lead to non-specific amplification. Therefore, cDNA should be appropriately diluted (5-10 times or 100 times) based on the abundance of the detected microRNA. Using enriched microRNAs as starting templates can reduce non-specific amplification and enhance sensitivity.
3. This product contains the fluorescent dye Sybr Green I. When storing this product or preparing PCR reaction solutions, avoid exposure to strong light.
4. The 2 x microRNA qPCR Mix does not contain the reference dye ROX. The customer will decide whether to add ROX reference dye based on the qPCR instrument technical guidance to eliminate signal background and correct fluorescence signal errors between wells.

Protocol

3. Melt 2 x microRNA qPCR Mix and Reverse Primer (10 μ M) at room temperature.
4. When using, please invert the 2 × microRNA qPCR Mix upside down and gently mix it evenly to avoid foaming. After slight centrifugation, use it. If the reagents are not mixed evenly, their reaction performance will decrease. Note: Please do not mix with an oscillator.
5. Prepare the reaction solution on ice according to the components listed in the table below

Components	Volume		Final Concentration
2 ×microRNA qPCR Mix(With Sybr Green)	25μL	10μL	1×
Forward primer(10μM)	1μL	0.4μL	0.2μM
Reverse primer(10μM)	1μL	0.4μL	0.2μM
MicroRNA first strand cDNA	xμL	xμL	-
ddH2O to final volume	50μL	20μL	-

PCR cycle (three-step method)

94°C 2-3 min

94°C 10-20 sec

60°C 10-20 sec

72°C 20 sec

35-45 cycles

Dissociation Stage

PCR cycle (two-step method)

94°C 2-3 min

94°C 10 sec

60°C 30-34 sec

35-45 cycles

Dissociation Stage

Note: Improve specificity by selecting a two-step method. Choose the three-step method to improve amplification efficiency.