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microRNA Real-Time PCR Assay kit

Product Number: PCK51

Storage condition

Store at -20°C in the dark for at least 12 months, thoroughly dissolve and mix before use. 2×microRNA qPCR Mix (with Sybr Green) can be stored at 4°C for short-term use to avoid repeated freeze-thaw cycles. Reverse primer (10μM) should be stored at -20° C after each use.

Component

Component	125T×20μL
2 ×microRNA qPCR Mix(With Sybr Green)	1.25μL
Reverse primer(10μM)	55μL

Description

The enhanced microRNA fluorescence quantitative PCR detection kit adopts SYBR The principle of Green I chimeric fluorescence method for microRNA fluorescence quantitative detection. This kit contains all the reagents for microRNA fluorescence quantitative detection, including 2×microRNA qPCR Mix and Reverse Primer. 2×microRNA qPCR Mix (including Sybr Green) is a new generation of pre mixed fluorescent quantitative PCR detection reagent specially developed for microRNA quantitative detection. The DNA polymerase used in it is an antibody modified hot start form, combined with a special buffer system, making the reaction more specific, sensitive, and able to accurately quantify over a wider range.

Note: This kit must be used in conjunction with the Enhanced microRNA Real-Time PCR Assay kit(PCK50).

Self provided reagents

1. Molecular biology experimental grade water (without nucleases)
2. QPCR upstream primer corresponding to microRNA to be detected (Forward primer)

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

1. Melt 2 x microRNA qPCR Mix and Reverse Primer (10 μ M) at room temperature.
2. 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.
3. 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) 35-45 cycles
72°C 20 sec
Dissociation Stage

PCR cycle (two-step method)

94°C 2-3 min
94°C 10 sec) 35-45 cycles sec
60°C 30-34 sec
Dissociation Stage

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