Tinzyme Co., Limited

Email: sales@tinzyme.com Website: www.tinzyme.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

miRNA qPCR Assay Kit

Product Number: PCK42

Shipping and Storage

-20°C.

Components

Component	PCK42 125rxns
2×miRNA qPCR Mixture（ROX）	2×750μl
Reverse Primer，10μM	60μl
ddH_2O	1.5mL

Description

This kit uses the principle of SYBR Green I chimeric fluorescent dye method for miRNA fluorescence quantitative PCR detection. The kit contains 2×MiRNA qPCR Mixture and Reverse Primer.

2×MiRNA qPCR Mixture is a new generation of pre mixed form fluorescent quantitative PCR detection reagent developed specifically for miRNA quantitative detection. The fluorescent dye SYBR Green I contained in it can bind to all double stranded DNA, making it suitable for detecting different target sequences without the need to synthesize specific labeled probes.The Golden Star Taq DNA Polymerase is a highly efficient chemically modified hot start enzyme, combined with a unique buffer system, which enhances reaction specificity, sensitivity, and enables accurate quantification of miRNA over a wider range.2×MiRNA qPCR Mixture contains ROX dye, suitable for fluorescence quantitative PCR instruments that require ROX as a calibration dye.

Note

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

Self prepared experimental material

qPCR upstream primer(Forward primer)

Forward Primer Design Principles

1. Follow the most common principles of primer design.
2. Replacing U with T based on mature miRNA sequences is the most fundamental and simplest design method.
3. The Tm value of the downstream primer provided in the kit is 63.6℃, and the Tm value of the upstream primer should be designed to be around 63.6℃ as much as possible.
4. If the Tm value of the primer directly designed 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; Alternatively, both the 5'and 3' ends can be modified simultaneously.
5. If the Tm value of a primer designed directly according to principle "2" is too high, several bases can be removed from the 5'or 3' end of the primer.

Note

1. Before using the reagent, please gently mix it upside down and avoid foaming as much as possible, and use it after brief centrifugation.
2. The addition amount of miRNA first strand cDNA should not exceed 10% of the volume of Real time PCR.
3. For special detection systems, high content of cDNA templates can easily lead to non-specific amplification, and cDNA should

be appropriately diluted (10 or 100 times diluted) based on the abundance of the detected miRNA.

4. 2× miRNA qPCR mixture in this product contains SYBR Green I and ROX dyes. When storing this product or preparing PCR reaction solution, strong light exposure should be avoided.
5. Avoid repeated freezing and thawing of this product. Repeated freezing and thawing may cause a decrease in product performance. This product can be stored at -20℃ for a long time. If frequent use is required in the short term, 2×miRNA qPCR mixture can be stored at 2-8℃. The Reverse Primer still needs to be stored at -20℃.

Protocol:

1. Room temperature melting 2×MiRNA qPCR Mixture and Reverse Primer (10μM).
2. When using, please insert 2×Mix miRNA qPCR mixture upside down gently and evenly to avoid foaming, and use after brief centrifugation. If the reagent is not well mixed, its reaction performance will decrease.
3. Place the reagent on ice and prepare the reaction system according to the following table:

Reagent	volume	Final Conc.
2×miRNA qPCR Mixture（ROX）	10μl	1×
Forward Primer，10μM	0.4μl	0.2μM
Reverse Primer，10μM	0.4μl	0.2μM
MiRNA first strand cDNA	Xμl	-
ddH_2O	Up to 20μl	-

4. The reaction program is set as follows:

Attention! The pre denaturation reaction of this product must be completed at 95 ℃ for 10 minutes!

Step	Temperature	Time
Pre denaturation	95℃	10min[1)]
Denaturation	95℃	15s ┐ 40-45cycles
Annealing/Extension	60℃	1min ┘
Dissolution curve analysis	Set according to PCR instrument requirements	

Note: 1) The hot start enzyme used in this product must be activated under pre denaturation conditions of 95℃ and 10 minutes.

2) The annealing temperature should be set at 60-64℃ as a reference range. When non-specific reactions occur, the annealing temperature can be increased.