

miRNA qPCR Assay Kit

Project number: M665794

Storage conditions: -20° C.

Product Content:

Component	M665794
	125 rxns
2×miRNA qPCR Mixture (ROX)	2×750 μl
Reverse Primer, 10 μM	60 μl
ddH ₂ O	1.5 ml

Product Introduction:

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

The 2×miRNA qPCR Mixture is a new generation of pre-mixed fluorescent PCR reagents specially developed for miRNA quantitative detection. The fluorescent dye SYBR Green I can bind to all double-stranded DNAs, which enables the product to be used for the detection of different target sequences without the need to synthesize specific labeling probes. The GoldStarTaq DNA polymerase is a chemically modified hot-start enzyme, which, together with the unique buffer system, enables better specificity and sensitivity of the reaction and accurate quantification of miRNAs in a wider range. The 2×miRNA qPCR Mixture contains ROX dye and is suitable for fluorescent PCR instruments that require ROX as a calibration dye.

Note: This kit must be used in conjunction with the miRNA cDNA First Strand Synthesis Kit.

Self-contained experimental materials: qPCR upstream primer (Forward primer).

Forward Primer Design Principles

1. Follow the most general principles of primer design.
2. The most basic and simple design method is based on mature miRNA sequences, replacing U with T.
3. The T_m value of the downstream primer provided in the kit is 63.6°C, and the T_m value of the upstream primer should be designed to ensure that the T_m value is around 63.6°C as much as possible.
4. If the T_m value of a primer designed directly according to principle "2" is too low, a few bases (preferably G or C bases) can be added to the 5' end of the primer;

one or more A bases can be added to the 3' end; or both the 5' and 3' ends can be modified. The 5' and 3' ends can be modified at the same time.

5. If the T_m 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.

caveat

1. Before using the reagents, please mix them gently by turning them up and down, avoid foaming as much as possible, and use them after brief centrifugation.
2. Do not add more than 10% of the Real time PCR volume of the miRNA first strand cDNA.
3. For special assay systems where high levels of cDNA template tend to lead to non-specific amplification, dilute the cDNA appropriately according to the abundance of the miRNA being detected (10-fold or 100-fold dilution).
4. The 2×miRNA qPCR Mixture in this product contains SYBR Green I and ROX dyes, so avoid strong light when storing this product or preparing PCR reaction solution.
5. Avoid repeated freezing and thawing of the product, repeated freezing and thawing may degrade the performance of the product, the product can be stored at -20°C for long-term storage. If the product needs to be used frequently in the short term, the 2×miRNA qPCR Mixture can be stored at $2-8^{\circ}\text{C}$. Reverse primer should be stored at -20°C .

Operational Steps:

1. Melt 2× miRNA qPCR Mixture and Reverse primer ($10\ \mu\text{M}$) at room temperature.
2. When using, please mix the 2× miRNA qPCR Mixture gently and evenly by turning it up and down to avoid foaming, and after a short period of time away from the Use after centering. If the reagents are not mixed well, their reactivity will be reduced.
3. Place the reagents on ice and prepare the reaction system according to the table below:

reagents	volumetric	final concent ration
2×miRNA qPCR Mixture (ROX)	$10\ \mu\text{l}$	$1\times$
Forward primer ($10\ \mu\text{M}$)	$0.4\ \mu\text{l}$	$0.2\ \mu\text{M}$
Reverse primer ($10\ \mu\text{M}$)	$0.4\ \mu\text{l}$	$0.2\ \mu\text{M}$
miRNA first strand cDNA	$X\ \mu\text{l}$	-
ddH ₂ O	up to $20\ \mu\text{l}$	-

4. The reaction program is set up as follows:

Caution! The pre-denaturation reaction of this product must be completed at 95°C for 10 minutes!

步骤	温度	时间	
预变性	95℃	10 min ¹⁾	
变性	95℃	15 s	} 40-45 个循环
退火/延伸	60℃	1 min	
溶解曲线分析	根据PCR仪要求设定		

Attention:

(1) The hot-start enzyme used in this product should be activated under the condition of pre-denaturation at 95 °C for 10 min.

(2) Please take 60-64 °C as the reference for the setting range of annealing temperature, and the annealing temperature can be increased when non-specific reaction occurs.