HB220429

Hieff™ miRNA Universal qPCR SYBR Master Mix (Plus A method)

Product Information

Product Name	Cat#	Size
Hieff™ miRNA Universal qPCR SYBR Master Mix (Plus A method)	11171ES03	1 mL
	11171ES08	5×1 mL

Product Description

MicroRNA, a class of non-coding Rnas with a length of about 22 nt, is widely concerned and plays a very important role in regulating gene expression in plants and animals. This kit uses SYBR Green I chimeric fluorescence method for quantitative detection of miRNA fluorescence. The 2 × Hieff™ miRNA Universal qPCR SYBR Master Mix is a new generation of premix fluorescent quantitative PCR detection reagent specially developed for the quantitative detection of miRNA. It contains special ROX Passive Reference Dye. Suitable for all qPCR instruments, there is no need to adjust the concentration of ROX on different instruments, just add primers and templates in the preparation of reaction system can be amplified. The DNA Polymerase uses chemically modified heat-activated Polymerase, combined with a special Buffer system, to make the reaction more specific, more sensitive, and can be accurately quantified in a wider range.

This product is recommended for use with our Hifair™ miRNA 1st Strand cDNA Synthesis Kit (plus A method) (Cat#11148) to obtain optimal experimental results.

Product Components

Component Number	Components	Cat#/Size	
		11171ES03 (20 μL×100 rxn)	11171ES08 (20 μL×500 rxn)
11171-A	2×Hieff™ miRNA Universal qPCR SYBR Master Mix	1 mL	5×1 mL
11171-B	RNase-free H_2O	2×1 mL	5×1 mL

Shipping and Storage

The product is shipped with ice packs and can be stored at -20°C for 1 year.

Reagents and Consumables to Be Prepared by Yourself

1) No nuclease contamination of the head and centrifuge tube.
2) Forward Primer for PCR.

Forward Primer Design Principles

Based on mature miRNA sequences, U is usually replaced with T. The Tm value is recommended to be around 65℃. Several G or C bases can be appropriately added to the 5 'end of the primer to increase Tm value, or several bases can be appropriately removed from the 5' or 3 'end of the primer to reduce Tm value, and the introduction of secondary structure should be avoided.

Cautions

1. Before use, fully melt the frozen components and gently mix them before use.
2. Keep the experimental area clean and ensure all the supplies is RNase free.
3. Please avoid repeated freezing and thawing and avoid strong light when preparing.

4. For your safety and health, please wear lab coats and disposable gloves for operation.
5. This product is for research use **ONLY**!

Operating Steps

System configuration

1. Melt the reagent at room temperature, upside down before use, mix gently, and use after slightly centrifugation to avoid foaming.
2. Place reagents on ice and prepare reagents according to the following table. RNase-free H2O can be replaced with other nuclease-free water for molecular biology experiments.

Note: No mixing reagent, use of oscillator mixing, no reagent on ice will lead to the degradation of reaction performance.

3. Preparation of the reaction mixture.

Components	Volume (μL)	Volume (μL)	The final concentration
2×Hieff™ miRNA Universal qPCR SYBR Master Mix	10	25	1×
Forward Primer（Bring your own）	X	X	200 nmol/L
Reverse Primer (10 μmol/L)	0.8	2	200 nmol/L
cDNA	X	X	-
RNase-free H2O	up to 20	up to 50	-

[Note]: The amount of miRNA first-strand cDNA should not exceed 1/10 of the RT-qPCR volume. High concentrations of cDNA lead to nonspecific amplification and can be appropriately diluted 10-1000 times.

Reaction Program

The following two procedures can be referred to for quantitative PCR reaction.

a. Routine fluorescence quantitative PCR amplification procedure (two-step method)

Cycle step	Temp.	Time	Cycles
Initial denaturation	95℃	10 min	1
Denaturation	95℃	15 sec	35-40
Annealing/extension	60℃	20 sec	
Melting curve stage	Instrument Default Settings		1

b. Fluorescence quantitative PCR rapid amplification program (two-step method)

Cycle step	Temp.	Time	Cycles
Initial denaturation	95℃	10 sec	1
Denaturation	95℃	5 sec	40
Annealing/extension	60℃	20 sec	
Melting curve stage	Instrument Default Settings		1

[Note]: a) Annealing/extension temperature and time can be adjusted according to experimental requirements.

b)The routine procedure and the rapid procedure are selected according to the experimental instrument.

For example, quick program Settings are available for instruments such as ABI QuantStudio 5, while quick program Settings are not available for instruments such as Bio-RAD CFX96, requiring regular program Settings.