Ver. HB230112

Hieff™ miRNA Universal qPCR SYBR Master Mix (Tailing)

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 most 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 (Tailing) (Cat# 11148) to obtain optimal experimental results.

Components

Components No.	Name	11171ES03	11171ES08
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

Specifications

Hot Start	Built-in hot start
Detection method	SYBR
PCR method	qPCR
Polymerase	Taq DNA polymerase
Type of sample	DNA
Application equipment	Most qPCR instruments
Product type	SYBR premix for real-time fluorescence quantitative PCR
Apply to (application)	Gene Expression

Storage

The product should be stored at -25℃~-15℃ for 1 year.

Instructions

1. Reaction System

Components	Volume (μL)	Volume (μL)	The final concentration

2×Hieff™ miRNA Universal qPCR SYBR Master Mix	10	25	1×
Forward Primer	X	X	200 nmol/L
Reverse Primer	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.

2.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°C	10 min	1
Denaturation	95°C	15 s	35-40
Annealing/extension	60°C	20 s	
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°C	10 s	1
Denaturation	95°C	5 s	40
Annealing/extension	60°C	20 s	
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 QuantStudio™ 5, while quick program Settings are not available for instruments such as Bio-RAD CFX96, requiring regular program Settings.

3.Primer design guidelines

Based on mature miRNA sequences, U is usually replaced with T. The Tm value is recommended to be around 65°C. 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.

Notes

1. Dissolve at room temperature, store in ice box or on an ice bath after dissolution, and store at -20°C immediately after use.
2. For your safety and health, please wear lab coats and disposable gloves for operation.
3. This product is for research use ONLY!