HB220922

Hieff™ miRNA Universal qPCR SYBR Master Mix (Stem loop method)

Product Information

Product name	Cat#	Size
Hieff™ miRNA Universal qPCR SYBR Master Mix (Stem loop method)	11170ES03	1 mL
	11170ES08	5×1 mL
	11170ES25	5×5 mL

Product Description

Hieff™ miRNA Universal qPCR SYBR Master Mix is a premix for 2×real-time quantitative PCR amplification with high sensitivity and specificity, and quantitative PCR can obtain good linear relationship in a wide quantitative area. The Hieff™ miRNA Taq DNA polymerase, a core component, was thermally initiated by antibody method and combined with an optimized buffer to effectively inhibit non-specific amplification caused by primer annealing during sample preparation. In addition, ROX Reference Dye is added to the premixed liquid system, which can be applied to different qPCR instruments. When configuring the reaction system, only the primer and template can be added for amplification.

Shipping and Storage

The components are shipped with ice packs and can be stored at -20°C for 18 months.
Please avoid repeated freeze-thaw. The product contains fluorescent dye SYBR Green I, which should be avoided when storing or preparing the reaction system.

Cautions

1. Recommended for use with our Hifair™ III 1st Strand cDNA Synthesis Kit (gDNA digester plus) (Cat#11139).
2. Master Mix components should be fully shaken before use, centrifugal at low speed.
3. For your safety and health, please wear lab coats and disposable gloves for operation.
4. This product is for research use **ONLY**!

Reaction system

Components	Volume (μL)
Hieff™ miRNA Universal qPCR SYBR Master Mix	10
Forward Primer (10 μmol/L)	0.5
Reverse Primer (10 μmol/L)	0.5
Template cDNA	X
RNase free ddH_2O	up to 20

[Note]: Be sure to mix thoroughly before use to avoid excessive bubbles caused by violent shock.

a) Primer concentration: the final primer concentration is usually 0.25 μmol/L, and can also be adjusted between 0.1 and 1.0 μmol/L according to the situation.

b) Template concentration: if the template is undiluted cDNA stock, the volume used should not exceed 1/10 of the total volume of qPCR reaction.

c) Template dilution: 5-10 times of dilution is recommended for the original cDNA solution, and the best amount of template is 20-30 cycles of Ct value obtained by amplification.

d) Reaction system: 20 μL reaction system is recommended to ensure the effectiveness and repeatability of target gene amplification.

e) System preparation: please prepare it in the ultra-clean workbench, and use the gun head and reaction tube without nuclease residue; It is recommended to use the head with filter element. Avoid cross contamination and aerosol contamination.

Reaction Program

Cycle step	Temp.	Time	Cycles
Initial denaturation	95℃	5 min	1
Denaturation	95℃	10 sec	40
Annealing/extension	60℃	30 sec*	
Melting curve stage	Instrument Default Settings		1

[Note]:a) Annealing temperature and time: please adjust according to the length of primer and target gene.

b) Collection of fluorescence signal (*) : Please set the experimental program according to the instructions of the instrument. The time settings of several common instruments are as follows:

20 sec：Applied Biosystems 7700, 7900HT, 7500 Fast

31 sec：Applied Biosystems 7300

32 sec：Applied Biosystems 7500

30 sec：BIO-Rad CFX 96

c) Melting curve: The acquisition program of melting curve varies with the type of instrument, and the default melting program can be used.

Results analysis

Quantitative detection requires at least three biological repeats. After the reaction, the linear shape and smoothness of the amplification curve and the peak shape of the melting curve should be confirmed.

1) Amplification curve: The standard amplification curve is S-type.

When Ct value falls between 20 and 32, quantitative analysis is more accurate;

If the Ct value is less than 10, it is necessary to dilute the template and conduct the experiment again;

When the Ct value is between 30 and 35, it is necessary to increase the template concentration or increase the volume of the reaction system to improve the amplification efficiency and ensure the accuracy of the result analysis Indeed;

When Ct value was greater than 35, the detection results could not be used for quantitative analysis of gene expression, but could be used for qualitative analysis.

2) Melting curve:

The melting curve is single peak, indicating that the reaction specificity is good and quantitative results can be analyzed. If the melting curve has double or multiple peaks, it cannot be quantitatively analyzed.

If the melting curve has double peaks, DNA agarose gel electrophoresis can further determine whether the non-target peak is primer dimer or non-specific amplification.

If it is a primer dimer, it is recommended to reduce the primer concentration or redesign the primer with high amplification efficiency.

If non-specific amplification, increase annealing temperature or redesign primer with higher specificity.