

Hifair™ miRNA 1st Strand cDNA Synthesis Kit (Poly(A) tailing)

Product Information

Product name	Cat#	Size
Hifair™ miRNA 1st Strand cDNA Synthesis Kit (With Dye)	11148ES50	50 T

Product Description

MicroRNAs are a class of non-coding RNAs with a length of about 22 nt, which play an important role in the regulation of gene expression in plants and animals. This kit uses Poly(A)-tailing method to perform reverse transcription from miRNA first-strand to cDNA. The 2 × Hifair™ miRNA RT buffer in the product contains all the raw materials and primers for miRNA Poly(A)-tailing reaction and reverse transcription reaction. Careful optimization ensures that Poly(A) modification process and reverse transcription process can be performed simultaneously and efficiently in miRNA 3' end.

This product is recommended to be used in conjunction with our company's Hieff™ miRNA Universal qPCR SYBR Master Mix (Cat#11171) for optimal experimental results.

Product Components

Component Number	Components	11148ES50 (50 T)
11148-A	Hifair™ miRNA RT enzyme mix	87.5 μL
11148-B	2×Hifair™ miRNA RT buffer	250 μL
11148-C	RNase-free H ₂ O	10 mL
11148-D	Universal Reverse Primer (10 μM)	4 mL
11148-E	U6 Forward Primer (10 μM)	500 μL
11148-F	U6 Reverse Primer (10 μM)	500 μL

Shipping and Storage

The components are shipped with ice packs and stored at -20°C for 1 year.

Operating Steps

System preparation

Melt the Hifair™ miRNA RT enzyme mix and 2×Hifair™ miRNA RT buffer at room temperature, invert and mix, place on ice and configure the reaction system according to the following table:

Component	Volume (μL)	Final Concentration
2×Hifair™ miRNA RT buffer	5	1×
Hifair™ miRNA RT enzyme mix	1.75	-
RNA*	-	X
RNase-free H ₂ O	Up to 10	-

[Note]: *The concentration of total RNA and extracted miRNA in range of 10 pg-2 μg, the minimum copy number of synthesized miRNA can reach 60 copies, and the input volume does not exceed 3.25 μL.

Reverse transcription

Gently mix the above-prepared reaction solution with a pipette, and perform the reverse transcription reaction of miRNA according to the following procedure:

Reaction Temperature	Reaction Time	Remark
37°C	50 min	miRNA Poly(A)-tailing and reverse transcription
85°C	5 sec	Enzyme inactivation process

[Note]: The reverse transcript can be directly detected by qPCR. In order to avoid the inhibition of the qPCR reaction by the reverse transcription system, the product can be diluted 10-1000 times before use. If downstream experiments are not performed in a short time, it can be stored at -20°C. For long-term storage, it is recommended to store at -80°C after aliquoting to avoid repeated freezing and thawing.

Cautions

1. Fully thaw the frozen components and mix gently before use.
2. It is recommended to amplify the fragment within 1 kb in length for the best amplification efficiency.
3. When experimenting, please use RNase-free consumables to avoid unnecessary losses affecting the experimental results.
4. Avoid repeated freezing and thawing of this product and avoid strong light exposure during preparation.
5. For your safety and health, please wear lab coats and disposable gloves for operation.
6. For research use only!