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RScript™ miRNA cDNA Synthesis Kit (Stem-loop)

Catalog Number	Size
RK004-0100	100 rxns

Storage Conditions

Stable for up to 24 months at -20°C

Description

This kit is developed based on RScript II reverse transcriptase, which uses a reverse transcription primer with a stem-loop structure binding to the 3' end of the miRNA molecule. Under the action of RScript II reverse transcriptase, the artificially elongated miRNA first-strand cDNA is obtained. The recommended general stem-loop sequence is 5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGAC-3'; other stem-loop sequences can also be selected according to experimental needs. Typically, reverse transcription primers only need to add 6 reverse, complementary bases at the 3' end of the miRNA to the stem-loop sequence. This method is highly specific and only conducts reverse transcription on the mature miRNAs without being interfered by its precursors. The miRNAs with highly homologous sequences can be accurately distinguished. The reverse transcription products obtained with this product can be directly used for subsequent quantitative detection by dye or probe methods.

Kit Content(s)

RK004-0050	RScript II miRNA Enzyme Mix	100 µl x 1 vial
	2X Sharp miRNA Reaction Mix	1 ml x 1 vial
	U6 Stem-loop RT Primer (5 µM) [a]	100 µl x 1 vial
	U6 qPCR-F (10 µM) [b]	100 µl x 1 vial
	Universal miRNA qPCR-R (10 µM) [c]	150 µl x 1 vial
	Nuclease-free water	1.5 ml x 1 vial

[a] U6 Stem-loop RT Primer is a general reverse transcription primer for humans, rats, and mice as an U6 internal control. For other species or other internal controls, they need to be redesigned according to the species sequence.

[b] U6 qPCR-F is a universal U6 upstream internal control primer for humans, rat, and mice and can be used in conjunction with the universal miRNA qPCR-R downstream primer for qPCR detection.

[c] Universal miRNA qPCR-R is a universal stem-loop downstream primer, which can be used in conjunction with the designed qPCR upstream primer for the detection of target miRNAs. For more primers, refer to Supplementary Note 3 for self-synthesis.

Required materials but not provided

- Vortex or equivalent
- Microcentrifuge
- PCR tubes for your instruments
- Ice water bath
- Temperature-controlled water bath or heat blocks; the thermal cycler can also be used.

Template

Total RNA or miRNA should be avoided for cross-contamination with DNA.

Reaction Setup

cDNA Synthesis

1. For each 20 ul cDNA synthesis reaction, assemble the following in an RNase-free tube. Keep it on ice just prior to use.

Component	Volume	Final conc.
RNA template	X µl	10 pg-1µg Total RNA or 100 ng miRNA
miRNA with U6 Stem-loop RT primer *	1 µl	0.1-0.25 µM
2X Sharp miRNA Reaction Mix	10 µl	
RScript II miRNA Enzyme Mix	1 µl	
Nuclease-Free Water	Add to 20 µl	
Total volume	20 µl	

* The amount of Stem-loop RT primer can be adjusted between 2-5 µM depending on the needs of the experiment.

2. Mix the reaction solution gently by pipetting.
3. Cap the tubes and centrifuge briefly. Place them in the temperature-controlled water bath or heat blocks. Incubate the tubes as follows:

Temperature	Time
25°C	5 min
50°C	15 min
85°C	5 min

4. At the end of the reaction, the resulting cDNA should be placed on ice for the subsequent experiment(s) or cryopreservation.

Addition information

1. Reverse Transcription Primer Design: Reverse transcription primers only require the addition of 6 reverse, complementary bases at the 3' end of the miRNA to be tested to the stem-loop sequence. It is recommended to use the general stem-loop sequence as 5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGAC-3'. Taking mature hsa miR15-5p(5'-TAGCAGCACGTAAATATTGGCG-3') as an example, the 6 basesat the 3' end are "TTGGCG", and the reverse complementary sequence is "CGCCAA", as shown in the following table.

2. Design of qPCR Upstream Primers: The upstream primers of qPCR are the "protective base" and 13-16 bases at the 5' end of the miRNA" (removal of the 6 bases at the 3' end of the miRNA). Please note to replace the original U with T. If the GC content in the miRNA molecule is relatively high, the 3-4 bases can be reduced at the 3' end of the miRNA sequence, thus reducing the primer's Tm value. Conversely, when the GC content in the miRNA molecule is relatively low, a few GC bases can be added at the 5' end of the miRNA sequence to increase the Tm value of the primer. Take miR15-5p as an example, the first 16 bases are TAGCAGCACGTAAATA, with a GC content of 37.5%, so the Tm value is 38.5°C. Therefore, when designing the primer, the protective bases "CGCG" are added at its 5' end, as shown in the following table.
3. Design of qPCR Downstream Primers: For a segment in the stem-loop sequence, this kit recommends the use of a universal stem-loop sequence and a universal downstream primer(s) (Universal miRNA qPCR-R, with the sequences as the following table). When using other stem-loop sequences, you will need to design and synthesize your qPCR downstream primers.
4. After the design of primers, it is recommended to perform a pre-experiment to test the specificity of the primers. In general, a melting curve is required to check the specificity of the primers, and the PCR product can also be subjected to agarose gel electrophoresis to detect whether the product is a single product (for a short product length, 2% gel is recommended).

Primer	Sequence (5'-3')
Stem-loop RT Primer	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGAC**CGCCAA**
miR15-5p qPCR-F	**CGCG**TAGCAGCACGTAAATA
Universal miRNA qPCR-R	AGTGCAGGGTCCGAGGTATT