25 APR 2024

RScript™ miRNA cDNA Synthesis Kit (Poly-A)

Catalog Number	Size
RK005-0100	100 rxns

Storage Conditions

Stable for up to 24 months at -20°C

Description

This kit is a special kit for the first-strand cDNA synthesis by poly(A) microRNA (miRNA), which contains all the raw materials for the miRNA -poly A-tail and reverse transcription reactions. Through careful optimization, it ensures that the poly(A) modification process at the 3' end of miRNA and reverse transcription process can be carried out efficiently at the same time. The RScript II miRNA-A enzyme mix contains the poly(A) polymerase (PAP) and RScript II Reverse Transcriptase. The high efficiency rendered by the PAP-poly A tail can specifically recognize the single-stranded RNA and effectively avoid the reverse transcription reaction of miRNA precursors with the double-stranded or stem-loop structures. RScript II Reverse Transcriptase has been engineered to lose the RNase H activity and increase the affinity with RNA, resulting in a significant increase in the reverse transcription efficiency and sensitivity of miRNAs. The reverse transcription products obtained with this product can be directly used for subsequent quantitative detection by dye or probe methods.

Kit Content(s)

RK005-0050	RScript II miRNA-A Enzyme Mix	100 µl x 1 vial
	2X Sharp miRNA-A Reaction Mix	1 ml x 1 vial
	Universal RT Primer	300 µl x 1 vial
	Universal miRNA-A qPCR-R (10 µM) [a]	1 ml x 1 vial
	U6-A qPCR-F (10 µM) [b]	500 µl x 1 vial
	Nuclease-free water	1.5 ml x 1 vial

[a] Universal miRNA-A qPCR-R, a universal downstream poly-tailing primer, is designed to be used with the qPCR upstream primers for the detection of target miRNAs.

[b] U6-A qPCR-F, a universal upstream internal control primer applicable to humans, mice, and rats, can be used in conjunction with the universal downstream primer, Universal miRNA-A qPCR-R, in qPCR detection.

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.

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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 a PCR tube. Keep it on ice just prior to use.

Component	Volume	Final conc.
RNA template	X µl	10 pg-1µg Total RNA or 200 ng miRNA
Universal RT Primer	3 µl	
2X Sharp miRNA-A Reaction Mix	10 µl	
RScript miRNA-A Enzyme Mix	1 µl	
Nuclease-Free Water	Add to 20 µl	
Total volume	20 µl	

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

Temperature	Time
37°C	50 min
85°C	5 min

4. The cDNA obtained at the end of the reaction can be used immediately for qPCR detection. The highly expressed miRNAs, can be diluted 10-1,000-fold, depending on the CT value, before use. The synthetic cDNA is recommended for direct use in downstream experiments to avoid non-specific amplification. Try to avoid repeated freeze-thaw cycles, and store at -20°C or -70°C for short-term storage (<2 days).

Addition information

qPCR Assay Primer Design

1. Design of Upstream Primers: It is recommended to design the upstream miRNA-specific primers based on the intact miRNA sequences and replace U with T.
   1.1 If the annealing temperature of the upstream primer is too low, it is recommended to add 2-3 bases (mainly G and C) to the 5' end of the primer and verify the primer specificity after adding the base to avoid non-specific amplification. If the primer annealing temperature is too high, it is recommended to delete 2-3 bases at the 5' end.
   1.2 To avoid non-specific amplification of isometric fragments of miRNA precursors, it is recommended to add 1-3 A bases to the 3' end of the primer.

1.3 For miRNAs with similar sequences, it is recommended that the 3' end of the primer to terminate at the differential base. If the annealing temperature is too low due to the short length of the primer, 2-3 bases can be added to the 5' end of the primer to match the Tm values of the upstream and downstream primers.

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