miRNA 1st Strand cDNA Synthesis Kit (by tailing A)

MR201

Version 23.1

Product Description

miRNA 1st strand cDNA Synthesis Kit (by tailing A) is a kit for microRNA (miRNA) reverse transcription (RT) by tailing A. Poly A polymerase (PAP) adds a Poly(A) tail to the 3' end of miRNA or total RNA, and then universal reverse transcription primers initiate RT reaction with reverse transcriptase. HiScript miRNA Enzyme Mix contains bioengineered reverse transcriptase and PAP. Combined with the optimized buffer, the reverse transcription of non-miRNAs is effectively inhibited, achieving highly specific qPCR results. 2 × miRNA RT Mix and HiScript miRNA Enzyme Mix contains all components required for miRNA tailing and reverse transcription. Two reactions can be performed simultaneously in one tube.

Components

	Components	MR201-01 (20 rxns)	MR201-02 (50 rxns)
☐	RNase-free ddH_2O	1 ml	1 ml
■	2 × miRNA RT Mix[a]	200 μl	500 μl
■	HiScript miRNA Enzyme Mix[b]	40 μl	100 μl
■	Universal reverse Q primer[c] (10 μM)	500 μl	1.25 ml

a. It contains dNTPs, reverse transcription primers, etc.

b. It contains RNase inhibitor.

c. It is the universal primer for qPCR detection.

Storage

Store at -30 ~ -15°C and transport at ≤0°C.

Applications

It is applicable for A-tailed reverse transcription using total RNA or miRNA as a template.

Notes

For research use only. Not for use in diagnostic procedures.

1. Prevent RNase contamination

   Please keep the experiment area clean; Wear disposable gloves and masks; Use RNase-free consumables such as centrifuge tubes and pipette tips.

2. Prepare the mixture on ice.

Experiment Process

1. Mix the following components in an RNase-free centrifuge tube:

Components	Volume	
2 × miRNA RT Mix	10 μl	■
HiScript miRNA Enzyme Mix	2 μl	■
Total RNA/miRNA*	100 ng - 2 μg	
RNase-free ddH_2O	To 20 μl	☐

*Total RNA used in the reaction must contain miRNA.

Gently pipette up and down several times to mix thoroughly.

2. Carry out the first-strand cDNA synthesis reaction under the following conditions:

Temperature	Time
37℃	60 min
85℃	5 min

▲ The cDNA obtained from the reaction can be used for qPCR immediately or after dilution. For highly expressed miRNAs, dilute 10 - 1,000 times according to the C_T value.

▲ cDNA should avoid repeated freezing and thawing. For short-term storage, it is recommended to store at -20°C; for long-term storage, it is recommended to store at -70°C.

qPCR Primer Design

1. Forward Primer

① Design forward primers based on the complete miRNA sequence and replace U with T.

② If the annealing temperature of the forward primer is too low, it is recommended to add a few bases (mainly G and C) at the 5' end of the primer. Verify the specificity of the primers to avoid nonspecific amplification. If the annealing temperature of the primers is too high, it is recommended to delete a few bases at the 5' end.

③ It is recommended to add 1 - 3 Adenine bases at the 3' end of the forward primer to prevent nonspecific amplification of the miRNA precursor during qPCR.

④ For miRNA with similar sequence, it is recommended that forward primers be designed to terminate at the different base at the 3' end of miRNA to distinguish similar miRNAs. If the annealing temperature is too low due to the short length of the primer, a few bases can be added to the 5' end of the primer to match the Tm values of the forward and reverse primers.

2. Reverse Primer

Universal reverse Q primer (10 μM) is included in the kit, which is the universal primer for qPCR detection. The annealing temperature is around 66°C.