miRNA 1st Strand cDNA Synthesis Kit (by stem-loop)

MR101

Version 22.1

Product Description

miRNA 1st Strand cDNA Synthesis Kit (by stem-loop) is a specific kit for stem-loop miRNA cDNA one-strand synthesis. It includes a genomic DNA removal step, which can quickly remove the contamination of genomic DNA at 42°C for 2 min. This ensures that subsequent results are more reliable. The HiScript II Reverse Transcriptase on which the Kit is based has extremely high thermal stability, and with an optimized buffer system, it is beneficial to the generation of miRNA-specific reverse transcriptase products. For subsequent quantification of cDNA products, Vazyme's miRNA Universal SYBR qPCR Master Mix (Vazyme #MQ101) is recommended to obtain optimal experimental results.

Components

Components	MR101-01 (50 rxns/20 μl reaction)	MR101-02 (100 rxns/20 μl reaction)
☐ RNase-free ddH_2O	1 ml	1 ml
■ 5 × gDNA Wiper Mix	100 μl	200 μl
■ 10 × RT Mix[a]	100 μl	200 μl
■ HiScript II Enzyme Mix[b]	100 μl	200 μl

a. It contains dNTPs.
b. It contains RNase inhibitor.

Storage

Store at -30 ~ -15℃ and transport at ≤0℃.

Applications

This kit is suitable for one-strand synthesis of miRNA and cDNA by stem-loop method.

Notes

1. Prevent RNase contamination

Keep the experimental area clean; wear clean gloves and masks during operation; consumables such as centrifuge tubes and pipette tips used in the experiment must be RNase-free.

2. Use of 5 × gDNA Wiper Mix

The 5 × gDNA Wiper Mix contains a high concentration of glycerol. Before use, please briefly centrifuge to collect it at the bottom of the reaction tube, and mix by gently pipetting with a pipette. The pipette tip should not be inserted too far into the liquid surface, otherwise the amount of enzyme will be lost due to sticking of the pipette tip wall.

3. The preparation of the reaction solution should be completed on ice.

Experiment Process

1. Genomic DNA Removal

a. Prepare the following mixture in an RNase-free centrifuge tube:

RNase-free ddH_2O	to 10 μl	☐
5 × gDNA Wiper Mix	2 μl	■
Total RNA	10 pg - 1 μg	

Gently pipette up and down several times to mix thoroughly.

b. Perform the genomic DNA removal reaction under the following conditions:

Incubate at 42℃ for 2 min.

2. First-strand cDNA synthesis

a. Prepare the following mixture in an RNase-free centrifuge tube:

RNase-free ddH_2O	to 20 μl	☐
Mixture from the last step	10 μl	
Stem-loop primer (2 μM)*	1 μl	
10 × RT Mix	2 μl	■
HiScript II Enzyme Mix	2 μl	■

* Stem-loop primers are recommended to be designed using our company's miRNA Design software. In this way, the matched reverse qPCR primers in the miRNA Universal SYBR qPCR Master Mix (Vazyme #MQ101), a subsequent quantification product of cDNA products, can be used directly without additional design and synthesis.

Gently pipette up and down several times to mix thoroughly.

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

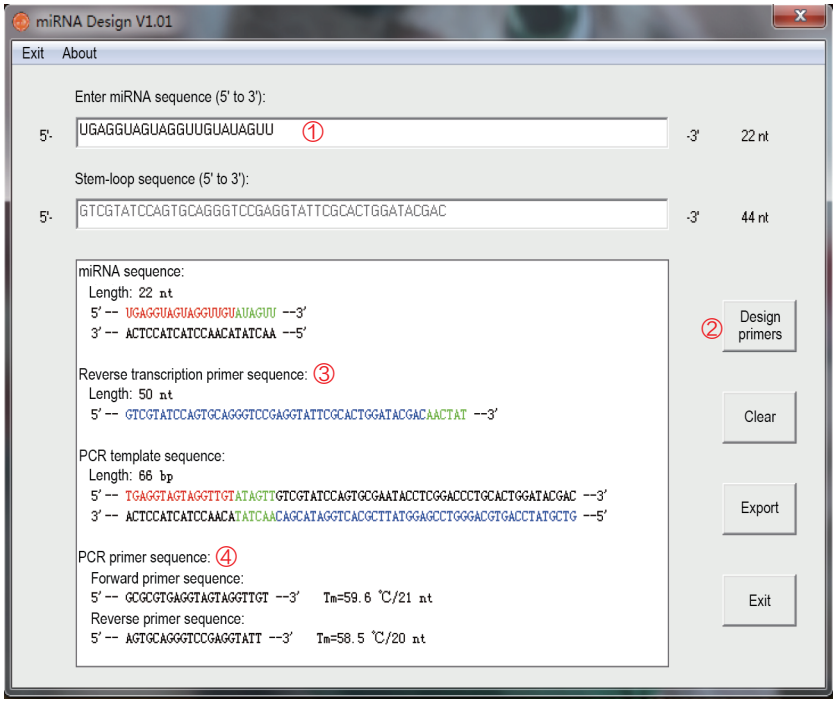
25℃	5 min
50℃*	15 min
85℃	5 min

* If the template has complex secondary structure or high GC region, the reaction temperature can be increased to 55℃ to help increase the yield.

The products can be used for PCR immediately or be stored at -20℃ for 6 months. However, it is recommended to store in aliquots at -70℃ for long term storage, and cDNA should be avoided repeated freezing and thawing.

Primer Design

This product is suitable for stem-loop reverse transcription, and the recommended general stem-loop sequence is GTCGTATCCAGTGC AGGGTCCGAGGTATTCGCACTGGATACGAC. Usually, the reverse transcription primer only needs to add 6 bases to the stem-loop sequence according to the miRNA sequence. The primer design software miRNA Design (free download address: www.vazyme.com) launched by our company can also be used for reverse transcription and qPCR primer design. When using this software, primer sequences can be obtained by simply inputting miRNA sequences.



1. Enter the miRNA sequence at ① (the miRNA sequence can be queried from the miRBase database);
2. Click ② to design primers;
3. Obtain reverse transcription primers and qPCR primers at ③ and ④ respectively.