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

NB-54-0036-01
NB-54-0036-02

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#Cat: NB-54-0036-01 Size: 50rxns
#Cat: NB-54-0036-02 Size: 100rxns

Introduction:

miRNA 1st Strand CDNA Synthesis Kit (through stem loop) is a special kit designed for miRNA cDNA 1st strand synthesis using Stem-loop method. The kit contains genomic DNA removal step, which can quickly remove genomic DNA contamination at 42℃ for 2 min, thus making subsequent results more reliable. The Hiscript II Reverse Transcriptase in Kit has high thermal stability, and it is beneficial to the generation of miRNA-specific reverse transcription products in combination with the optimized buffer system. For the subsequent quantification of cDNA products, it is recommended to use miRNA Universal SYBR GPCR Master Mix (Neo Biotech #NB-54-0035) for optimal experimental results.

Components

Components	NB-54-0036-01 (50 rxn/20 µl reaction)	NB-54-0036-02 (100 rxn/20 µl reaction)
RNase-free ddH₂O	1 ml	1 ml
5x gDNA Wiper Mix	100 µl	200 µl
10x RT Mix [a]	100 µl	200 µl
HiScript II Enzyme Mix [b]	100 µl	200 µl

a. Contains dNTP
b. Contains RNase inhibitor

Storage Store

at -20℃.

Protocol

1. Removal of Genomic DNA

a. Prepare the reaction mix in an RNase-free tube:

RNase-free ddH₂O	To 10 µl
5x gDNA Wiper Mix	2 µl
Total RNA	10 pg - 1 µg

Mix the solution by pipetting.
b. Place the reaction mix in 42℃ for 2 min to remove the genomic DNA.

2. Synthesis of 1st strand cDNA

a. Prepare the reaction mix in an RNase-free tube:

RNase-free ddH₂O	To 20 µl
Previous mixture	10 µl
Stem-loop Primer (2 µM)	1 µl
10x RT Mix	2 µl
HiScript II Enzyme Mix	2 µl

The stem-loop primers are recommended to be designed using our miRNA design software. And the reverse qPCR primers in the miRNA Universal SYBR QPCR Master Mix (Neo Biotech # NB-54-0035) can be used directly, do not need to design and synthesis separately.
Mix gently with a pipette.

b. Perform the 1st strand cDNA synthesis reaction according to the following conditions:

25℃	5 min
50℃	15 min
85℃	5 min

If the template has a complex secondary structure or a high GC region, the reaction temperature can be increased to 55°C, which helps to increasethe yield.
The product can be used immediately in the qPCR reaction, or stored at -20°C, and used within half a year; long-term storage is recommended to be stored at -80°C after packaging. Please avoid repeated freezing and thawing.

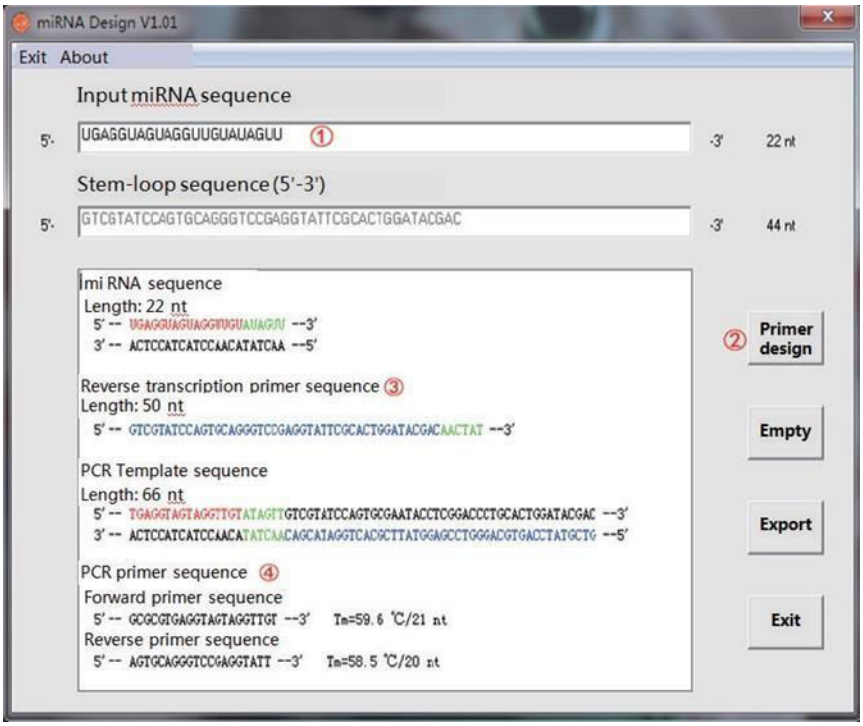
Notes

Prevent Rnase contamination
Keep the test area clean; wear clean gloves and masks during operation; the centrifuge tubes and tips used in the experimentmust be guaranteed Rnase-free.

Usage of 5 x gDNA Wiper M ix
The 5x gDNA Wiper Mix contains a high concentration of glycerol. Briefly centrifuge to collect it to the bottom of the reaction tube and mix gently by pipetting. The tip of the pipette should not be inserted too deep into the liquid, otherwise the loss of enzyme amount will be caused by the adhesion of the wall of the pipette tip.

The preparation of the reaction solution should be completed

on ice.Primer Design
This product is suitable for reverse transcription by stem-loop method. The recommended stem-loop sequence is
GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGAC.
In general, the reverse transcription primer only needs to add 6 bases to the stem loop sequence based on the miRNA sequence. Reverse transcription and qPCR primer design can also be performed using a primer design software miRNA Design (contact us for more details), which can be used to obtain primer sequences by simply inputting the
MIRNAL sequence.
1. Enter the miRNA sequence at æ (the miRNA sequence can be obtained from the miRBase database);
2. Click on ç to design primers;
3. Reverse transcription primers and qPCR primers were obtained at è and é respectively.


miRNA Design V1.01
Exit About
Input miRNA sequence
5'- UGAGGUAGUAGGUUGUAUAGUU ① -3' 22 nt
Stem-loop sequence (5'-3')
5'- GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGAC -3' 44 nt
Imi RNA sequence
Length: 22 nt
5' -- UGAGGUAGUAGGUUGUAUAGUU --3'
3' -- ACTCCATCATCCAACATATCAA --5'
Reverse transcription primer sequence ③
Length: 50 nt
5' -- GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAACTAT --3'
PCR Template sequence
Length: 66 nt
5' -- TGAGGTAGTAGGTTGTATAGTTGTCGTATCCAGTGCGAATACCTCGGACCCTGCACTGGATACGAC --3'
3' -- ACTCCATCATCCAACATATCAACAGCATAGGTCACGCTTATGGAGCCTGGGACGTGACCTATGCTG --5'
PCR primer sequence ④
Forward primer sequence
5' -- GCGCGTGAGGTAGTAGGTTGT --3' Tm=59.6 'C/21 nt
Reverse primer sequence
5' -- AGTGCAGGGTCCGAGGTATT --3' Tm=58.5 'C/20 nt
② Primer design
Empty
Export
Exit