4× microRNA Reverse Transcription Mix

Catalog No.: EZB-miRT3

Description

The *EZ*Bioscience® 4× microRNA Reverse Transcription Mix is based on the Stem-loop method to synthesis the first strand of cDNA for miRNA. This Kit contains a genomic DNA removal step, which can remove the contamination of genomic DNA by reacting at room temperature for 5 min, which ensures the subsequent experimental results are more accurate.

This Kit contains two tubes of reagents: gDNA Remover and 4× miRNA RT Mix. gDNA Remover mainly includes concentrated DNase and buffer; 4× miRNA RT Mix mainly contains reverse transcriptase, buffer, RNase Inhibitor, dNTPs required for reverse transcription except reverse primer. The first strand of cDNA for miRNA can be rapidly synthesized by designing a specific reverse transcription primer for the target miRNA based on the sequence of the target miRNA and the Stem- loop sequence provided by this kit.

The Reverse transcriptase in this Mix is a genetic engineered enzyme based on M-MLV (RNase H-) reverse transcriptase. The reverse transcriptase lacking RNase H activity is suitable for preparing cDNA. And the multiple site-mutations of Reverse transcriptase can obviously increase its affinity to miRNA template and its strand extending ability, which make reverse transcription reaction more efficient. Moreover, this transcriptase is rather resistant to common reverse transcriptase inhibitors. This product is also very suitable for reverse transcription using plant tissue miRNA.

Components

Components	EZB-miRT3-S (50 Rxns)	EZB-miRT3-L (100 Rxns)
gDNA Remover	55 µl	110 µl
4× miRNA RT Mix	275 µl	550 µl
Nuclease free ddH_2O	1 ml	1 ml × 2 tubes

Storage

Store at -20℃.

Caution

Avoid RNase contamination

Please keep the environment of experiment clean. Clean gloves and mask should be worn during the experiment. Centrifuge tubes, tips and other supplies used in the experiment must be RNase free.

Protocol

miRNA RT Primer Design

A Specific Stem-loop RT primer is recommended for each miRNA by using this Kit. One suggested universal stem-loop primer sequence is as follows: 5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACT-GGATACGAC-3'. Thus, for each miRNA, the RT primer sequence should be like this: 5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACT-GGATACGAC **+ miRNA RCS** (6 ~ 8 nt of reverse complementary sequence for each miRNA, from 3' end of the mature miRNA sequence)-3'. [The mature miRNA sequence could be got from the NCBI gene database, or miRbase.]

And the general principle for forward qPCR primer designing is as follows:

1) Copy the target microRNA sequence, change the U in the sequence to T, then delete the last 6 nucleotides at the 3' end.

2) Add 3 ~ 6 nucleotides to the 5' end of the primer, to justify the Tm value of the primer to 55 ~ 60°C (the added sequence mainly contains G&C, such as CGGGC, GCGGGC, or A/TGCCCG).

3) Synthesize the primer and exam the quality of the primer by qPCR.

The reverse qPCR primer could be as follows: 5'-GTCGTATCC- AGTGCAGGGTC-3' (just choose 18 ~ 23 nt from the universal Stem-loop RT primer according to the Tm value of the forward qPCR primer).

gDNA Remover Treatment of RNA

1. Determine the concentration of RNA, and then add 0.5 µg total RNA containing miRNA to a new RNase free centrifuge tube. Add 1 µl gDNA Remover and mix (if the volume of the mixture is less than 5 µl, add ddH_2O to the volume of 5 µl). Incubate for 5 minutes at room temperature (19 ~ 27°C).

Reverse Transcription

1. Set up the following mixture according to the table below. Pipette up and down for 10 times to mix thoroughly.

Components	20 µl Reaction
gDNA Remover treated RNA	X µl (≥5 µl)
4× miRNA RT Mix	5 µl
Stem-loop primer（2 µM ）	1 µl
Nuclease free ddH_2O	up to 20 µl

2. Perform reverse transcription at 42°C for 15 minutes, 95°C for 30 seconds.

The cDNA products can be used in qPCR reactions immediately, or stored at -80°C for long-term storage. Avoid repeated freeze-thaw cycles.