microRNA Reverse Transcription Kit

Catalog No.: EZB-miRT4

Description

The ***EZ*Bioscience® microRNA Reverse Transcription Kit** uses the method of Poly (A) tailing reaction and reverse transcription reaction in a single step assay to synthesize the first strand cDNA of miRNAs. Mature miRNAs from total RNA are modified by extending the 3' end of the mature transcript through Poly(A) addition using E. coli Poly (A) Polymerase. The modified miRNAs then undergo universal reverse transcription using Oligo (dT)-universal tag primer to synthesize the first strand cDNA for all miRNAs. The kit can be used with microRNA probe qPCR Kits *(Catalog No.: EZB-miProbe/EZB-miProbe-R0/EZB-miProbe-R1/EZB-miProbe-R2).*

The kit contains three tubes of reagents: gDNA Remover, miRNA RT Enzyme Mix, and 4× miRNA RT Buffer.

Among them, gDNA Remover mainly includes concentrated DNase and buffer. It can degrade more than 95% of residual genomic DNA only needing to react at room temperature (19 ~ 27°C) for 5 minutes, which greatly reduces the interference to the results.

The miRNA RT Enzyme Mix mainly contains *E. coli* Poly(A) Polymerase, reverse transcriptase, and RNase Inhibitor. *E. coli* Poly (A) Polymerase not only has efficient Poly (A) tailing reaction efficiency, but also specifically recognizes mature single-stranded miRNA, thereby avoiding reverse transcription reaction of miRNA precursors with double-stranded structure The mutant M-MLV reverse transcriptase has strong anti-interference ability and amplification ability.

The 4 × miRNA RT Buffer reagent contains all raw materials and primers for Poly(A) tailing reaction and reverse transcription reaction, including Oligo(dT)-universal tag primer, buffer and dNTPs, and has been carefully optimized to ensure Poly (A) tailing reaction and reverse transcription reaction efficiently.

Components

Components	EZB-miRT4-S (20 Rxns)	EZB-miRT4-L (50 Rxns)
gDNA Remover	22 µl	55 µl
miRNA RT Enzyme Mix	44 µl	110 µl
4× miRNA RT Buffer	110 µl	275 µl
Nuclease free ddH_2O	1 ml	1 ml

Storage

Store at -20°C.

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

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 thoroughly (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).

Poly(A) tailing and reverse transcription Reaction

2. Add components to the gDNA Remover-treated RNA according to the following table, then mix gently with a pipette and centrifuge the mixture briefly to the bottom of the tube.

Components	20 µl Reaction
gDNA Remover treated RNA	X µl (⩾5 µl)
miRNA RT Enzyme Mix	2 µl
4× miRNA RT Buffer	5 µl
Nuclease free ddH_2O	up to 20 µl

3. Perform the Poly(A) tailing reaction and reverse transcription reaction at 37°C for 15 minutes, 42°C for 10 minutes and 95°C for 3 minutes.

4. The Poly(A) tailing and reverse transcription reaction product can be directly used in PCR applications, but the cDNA is recommended to dilute and mix thoroughly before use (The specific dilution factor depends on the abundance of gene expression. Generally, cDNA is diluted 5 ~ 10 times). If the qPCR experiment is not performed immediately, it is recommended to store at -80°C for long-term storage. Avoid repeated freeze-thaw cycles.