microRNA Reverse Transcription Kit PLUS

Catalog No.: EZB-miRT2-plus

Description

The ***EZ*Bioscience**® microRNA Reverse Transcription Kit PLUS uses the method of adding poly A tail to the 3' end of miRNA by the *E. coli* Poly(A) Polymerase to synthesize the first strand cDNA of the miRNA. This kit contains a forward U6 primer, and a universal qPCR reverse primer (Universal 3'qPCR Primer) in it, which can be used for the quantitative detection of all miRNAs. So only a *specific forward primer for each target miRNA* is needed for qPCR reactions. This Kit includes two steps: first, The RNAs are treated with gDNA Remover to eliminate the genomic DNA. Then, poly(A) tail is added to the 3'-end of miRNA molecules in the poly(A) tailing reaction. The reverse transcription reaction is performed using a specially designed Universal RT Primer to generate the first strand of the miRNA.

The Kit is a rapid reverse transcription kit containing high-efficiency DNase for the removal of genomic DNA. It contains four tubes of reagents: gDNA Remover, miRNA RT Enzyme Mix, 4× miRNA RT Buffer, and a Universal 3'qPCR Primer.

Among them, gDNA Remover mainly includes concentrated DNase and buffer. It only needs to react at room temperature (19 ~ 27°C) for 5 minutes to degrade more than 95% of residual genomic DNA, 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. The *E. coli* Poly(A) Polymerase in it not only has high efficiency of adding poly(A) tail, but also specifically recognizes single-stranded miRNA, thus avoiding further reverse transcription reaction of miRNA precursor with double-stranded structure; The mutant M-MLV reverse transcriptase has strong anti-interference ability and amplification ability, and the amplification efficiency is particularly good.

The 4× miRNA RT Buffer contains all the raw materials and primers for miRNA polyadenylation and reverse transcription reaction, including Oligo (dT)-universal tag primers, buffer and dNTPs, and has been carefully optimized to ensure polyadenylation and the reverse transcription are simultaneously performed efficiently.

Components

Components	EZB-miRT2-plus-S (20 Rxns)	EZB-miRT2-plus-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
Universal 3' qPCR Primer (10 µM)	300 µl	750 µl
U6 Primer (10 µM)	60 µl	150 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 (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).

Polyadenylation and Reverse Transcription

2. 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)
miRNA RT Enzyme Mix	2 µl
4× miRNA RT Buffer	5 µl
Nuclease free ddH_2O	up to 20 µl

3. Perform the Polyadenylation and reverse transcription at 37°C for 15 minutes, 42°C for 10 minutes, 95°C for 3 minutes.

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

Suggestions for Primer Design

The reverse primer for microRNA quantification (a universal qPCR primer) is already provided in this Kit. The following general principals may be considered when designing primers for target microRNAs:

1. First, get the target microRNA sequence from microRNA data base (such as miRBase: http://www.mirbase.org/).

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

3. 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).

4. Synthesize the primer and exam the quality of the primer by qPCR. If the Ct value derived is between 15 ~ 32, the amplification curve displays representative S form curve, and the melt curve has a typical single peak, the primer could be approved. If there are apparently double peaks in the melt curve, optimize the primer by add or delete the specific nucleotides to the 5' or 3' end of the primer.