MCLAB
Molecular Cloning Laboratories

User Manual

Version 3.0

Product name: miRNA cDNA Synthesis and qPCR Kit

Cat #: MCSQ-100, MCSQ-200

Description:

Small, non-coding miRNAs are widely present in eukaryotes. Many studies evidenced that miRNAs control many important physiological processes in cell development and differentiation. Therefore, the quantitative assay of miRNA is important in both basic and applied research.
miRNA cDNA Synthesis Kit provides a universal first-strand cDNA synthesis system which combines optimized polyadenylation with reverse transcription reactions based on our proprietary highly pure and active enzymes. The robust kit is ideal for most of the reliable first strand synthesis and with higher cDNA yields, sensitivity, and accurate quantitation from 10 pg to 1 µg of total RNA input.
The universal first-strand cDNA synthesis allows for detection of mRNA species, including Actin and GapDH, from the same cDNA sample. The miRNA-specific amplification could be done with target-specific PCR forward primers during the subsequent PCR reaction. When compared to the traditional hybridization-based detection methods, such as Northern blot analysis, the qRT-PCR method is faster, more specific and sensitive using less sample material.

Features:

- Efficient reverse transcription of miRNAs into cDNA in a single step.
- Precise quantification and accurate measurement of miRNA expression profiles with Universal qPCR Primer included in the kit gives you the flexibility to order your qPCR detection reagents separately.
- Differentiation between mature and precursor miRNA.
- Profiling of small RNAs, miRNAs, or mRNAs from a single cDNA synthesis reaction.
- Recommended Storage Condition: -20°C

List of Components

miRNA cDNA Synthesis Kit is supplied for 20 or 100 tailing reactions of 20 µL each.
20-rxn size:
10X Enzyme Mix 40 µl
5X Reaction Mix 80 µl
10 µM Universal qPCR Primer 100 µl
DEPC-Treated Water 1.5 ml

100-rxn size:
10X Enzyme Mix 200 µl
5X Reaction Mix 400 µl
10 µM Universal qPCR Primer 100 µl
DEPC-Treated Water 1.5 ml

Materials Supplied by the User

The following items are supplied by the user:

- Total RNA, isolated by a method that preserves low-molecular weight (LMW) RNA
- Micro centrifuge
- RNase-free pipette tips
- 1.5-ml RNase-free micro centrifuge tubes
- A forward qPCR primer specific for the miRNA of interest.

You may order specific forward primer for your miRNA of interest from MCLAB or design by yourself following the guidelines as below.

a) Start with the entire miRNA sequence (in DNA primer form, i.e., use T's to replace U's).

b) Use a primer design program to determine the primer Tm, 3' stability, self-complementarity, and end-self complementarity.

c) The optimal primer Tm is around 61°C. If the Tm for the entire miRNA sequence is too low, try adding non-templated bases G's and C's in random combinations to the 5' end of the primer. If the Tm is too high, truncate from the 5' end of the primer only. Adding one or more A's to the 3' end of the primer is acceptable and sometimes helps the primer overcome high 3' stability or self-complementarity issues. To achieve an optimal Tm, you can truncate bases from the 5' end while at the same time adding A's to the 3' end.

- qPCR MasterMix
- qPCR instrument
- Appropriate PCR plates/tubes for instrument

General Guidelines

1. Choose total RNA isolation method preserves the low-molecular-weight (LMW) RNA in the sample. Enrichment for small RNAs is typically not necessary.
2. The optimal amount of starting material is 100 pg to 1 µg of total RNA.
3. High-quality, intact RNA is essential for accurate quantification.
4. Genomic DNA contamination could be eliminated by using DNase I.
5. Follow necessary requirements for handling of RNA.

Protocol

miRNA cDNA Synthesis

Component	Quantity *
Nuclease-free water	To 20 µL
5x Reaction Mix	4 µL
10x Enzyme Mix	2 µL
RNA Template	x µL

*: For multiple reactions, master mix should be made with 5% extra reagents to reduce pipette error.

1. Gently mix thoroughly and then centrifuge briefly.
2. Incubate the tube at 37°C for 60 minutes.
3. Terminate the reaction at 90°C for 5 minutes.
4. Hold the reaction at 4°C until the next use or store at –20°C for long-term storage.

miRNA qPCR Assay

1. For instrument-specific type of qPCR SuperMix, 2X HotSybr Real-time PCR Kits are available form MCLAB separately.
2. Use up to 10% of undiluted cDNA in qPCR reaction, such as 2 µL cDNA to total 20 µL PCR reaction.
3. Use 0.5 µL the Universal qPCR Primer (Tm = 61.8, GC% = 50%) provided as a separate tube in the kit as the reverse primer in the total 25 µL qPCR reaction.
4. Add equal molecule of miRNA Specific Forward Primer ordered separately from MCLAB or synthesized by yourself.
5. Refer to the instruction of qPCR Mix and instrument. Typically set annealing temperature around 58° C and run a standard 3-step qPCR reaction are recommended.

For more information, please contact MCLAB directly.

References

1. Drummond, D.R. et al. (1985) J. Cell. Biol. 100, 1148.
2. Galili, G. et al. (1988) J. Biol. Chem. 263, 5764.
3. Lingner, J. and Keller, W. (1993) Nucleic Acids Res. 21, 2917.