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RT Master Mix for qPCR III (poly A)

1 Packing list

Components	HY-K0512-10 rxns	HY-K0512-50 rxns
miRNA RT Mix	17.5 μL	87.5 μL
2 × miRNA RT Buffer	50 μL	250 μL
Universal Reverse Primer (10 μM)	800 μL	4 mL
U6 Forward Primer (10 μM)	100 μL	500 μL
U6 Reverse Primer (10 μM)	100 μL	500 μL
RNase-Free H_2O	2 mL	10 mL

2 Introduction

MicroRNAs are a class of non-coding RNAs with a length of about 22 nt, which play an important role in the regulation of gene expression in plants and animals.

MCE RT Master Mix for qPCR III (poly A) uses poly(A)-tailing method to perform reverse transcription from miRNA first-strand to cDNA. The kit contains poly(A) polymerase (PAP) for the miRNA 3′ end poly(A) reaction and all the raw materials and primers for miRNA reverse transcription reaction. It ensures that poly(A) modification process and reverse transcription process can be performed simultaneously and efficiently in miRNA 3′ end. Only design miRNA-specific forward primers and use them together with the universal reverse primers included in the kit for subsequent qPCR experiments. Additionally, this kit provides universal U6 internal forward/reverse primers applicable for human, rat, and mouse that can be used for plotting standard curves.

Upon completion of the first-strand cDNA synthesis, the cDNA product can be directly applied as a template in a standard PCR and qPCR. MCE SYBR Green qPCR Master Mix (HY-K0501、HY-K0501A) is highly recommended for detection of the expression levels of interested genes

Features of MCE RT Master Mix for qPCR III (poly A)

- High sensitivity: 10 pg RNA can be detected.
- Time-saving: The combination of poly(A) tailing and cDNA synthesis in one step within the same reaction system simplifies the operation.

3 General Protocol

1. Preparation of Reverse Transcription Reaction System

Thaw miRNA RT Mix and the 2 × miRNA RT Buffer at room temperature. Mix solutions gently but thoroughly.

Components	Volume/μL
miRNA RT Mix	1.75 μL
2 × miRNA RT Buffer	5 μL
Total RNA	X
RNase-Free H_2O	To 10

Note: The concentration of total miRNA in range of 10 pg-2 ug, the minimum copy number of synthesized miRNA can reach 60 copies, and the input volume of RNA should not exceed 3.25 μL.

2. Setting up the Reverse Transcription Program

Mix the components well and collect by brief centrifugation. Incubate the mixture in a PCR instrument or water bath as follows:

Temperature	Time	Remark
37ºC	50 min	miRNA Poly(A)-tailing and reverse transcription
85ºC	5 s	Enzyme inactivation

3. Product Collection

The newly synthesized first-strand cDNA is ready for immediate downstream applications (PCR, qPCR). If downstream experiments are performed in a short time, it can be stored at -20°C. For long term storage, it is recommended to store at-80°C after aliquoting to avoid repeated freezing and thawing.

Note: For qPCR, in order to avoid the inhibition of the qPCR reaction by the reverse transcription system, the product can be diluted 10-1000 times before use.

4 Storage

-20ºC，1 year

Avoid repetitive freeze-thaw cycles.

5 Precautions

1. Dissolve at room temperature, store in ice box or on an ice bath after dissolution, and store at-20°C immediately after use.
2. When preparing reaction mixture, avoid strong light and repeated freeze-thaw cycles.
3. Please use RNase-free laboratory consumables to prevent unnecessary losses that could impact experimental results.
4. This product is for R&D use only, not for drug, household, or other uses.
5. For your safety and health, please wear a lab coat and disposable gloves to operate.