mi-RNA FIRST STRAND-SYNTHESIS KIT FS-RT-1034

Introduction

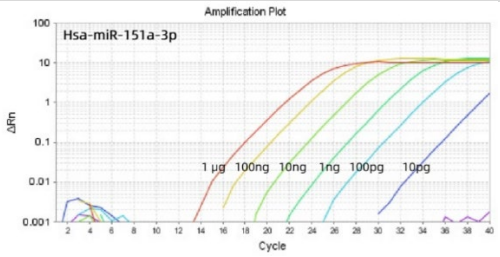
This kit is suitable for cDNA first strand synthesis using microRNA as template through the tail addition method, where the Poly (A) tail addition reaction and reverse transcription reaction at the 3 'end of miRNA can be efficiently carried out simultaneously.

miRNA-A Enzyme Mix contains Poly (A) Polymerase (PAP) and reverse transcriptase. PAP is mainly used to add Poly (A) tails at the 3 'end of RNA molecules, and can also specifically recognize single stranded RNA, effectively avoiding RT reactions of pre-miRNA with double stranded or stem-loop structures.

The modified reverse transcriptase lacks of RNase H activity and increases its affinity with RNA, resulting in a significant improvement in the efficiency and sensitivity of miRNA reverse transcription. The obtained cDNA can be directly used for qPCR detection using either SYBR Green dye-base or Taqman probe-base reagent.

Kit Components

Components	FS-RT-1034
miRNA-A Enzyme Mix (20X)	20 µl
miRNA-A Reaction Buffer (2X)	200 µl
Universal RT Primer	60 µl
Universal miRNA-A qPCR Primer R (10 µM)*	200 µL
U6 qPCR Primer F (10 µM)**	100 µL
Nuclease-free ddH2O	1 mL



***Universal miRNA-A qPCR Primer R (10µM) can be used together with designed qPCR forward primers for qPCR detection.**
****U6 qPCR Primer F, a universal reference forward primer for human, mouse and rat U6, can be used together with Universal miRNA-A qPCR Primer R for qPCR detection.**

HIGHLIGHTS

- . High specificity: The kit only performs Poly (A) tail addition reaction and reverse transcription reaction on single-stranded miRNAs, avoiding interference from pre-miRNAs with secondary structure;
- Convenient and fast: Poly (A) tail addition reaction and reverse transcription reaction can be completed in one preparation.
- High sensitivity: Total RNA as low as 10 pg can be detected

Ordering Information

Cat.#	Description	Size
FS-RT-1034	miRNA First Strand cDNA Synthesis Kit	20 Reactions (20 µl)

Operation Description

1. Add the following components to the RNase-free PCR tube on ice, mix well and centrifuge briefly.

	20 μL
RNA	10 pg-1 μg Total RNA or 200 ng miRNA
Universal RT Primer	3 μL
miRNA-A Reaction Buffer (2X)	10 μL
miRNA-A Enzyme Mix (20X)	1 μL
Nuclease-free ddH2O	Up to 20 μL

2. **Reverse transcription reaction procedure.**

Temp.	**Time**
37 ℃	50 min
85 ℃	5 min

The product can be immediately applied to subsequent qPCR detection, or stored at -20°C. It is recommended to store at -80°C for storage longer than six months. Avoid repeated freezing and thawing.

Primer design for qPCR detection

Forward primer:It is recommended to design forward primers based on the complete miRNA sequence and replace U with T.

Storage: -20°C

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