ABScript miRNA First-Strand Synthesis Kit (by tailing A)

Cat. No. : RK30170

Product components

Components	Component number	Size 20 RXN
ABScript miRNA-A Enzyme Mix (20X)	RM30271	20 μL
ABScript miRNA-A Reaction Buffer (2X)	RM30272	200 μL
Universal RT Primer	RM30273	60 μL
Universal miRNA-A qPCR Primer R (10 μM)*	RM30274	200 μL
U6 qPCR Primer F (10 μM)**	RM30275	100 μL
Nuclease-free ddH_2O	RM30276	1 mL

*Universal miRNA-A qPCR Primer R (10 μM) 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.

Product Description

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. ABScript 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.

Storage

-20℃

Operation Description

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

Components	20 μL
RNA	10 pg-1 μg Total RNA or 200 ng miRNA
Universal RT Primer	3 μL
ABScript miRNA-A Reaction Buffer (2X)	10 μL
ABScript miRNA-A Enzyme Mix (20X)	1 μL
Nuclease-free ddH_2O	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

Foward primer

It is recommended to design foward primers based on the complete miRNA sequence and replace U with T.

1. If the annealing temperature of the foward primer is too low, it is recommended to add 2-3 bases (mainly G and C) at the 5' end of the primer, and verify the specificity of the primer to avoid non-specific amplification. If the annealing temperature of the primer is too high, it is recommended to delete 2 to 3 bases at the 5' end.
2. In order to avoid non-specific amplification of equal length fragments of pre-miRNA, it is recommended to add 1-3 A bases to the 3' end of the primer.
3. For miRNAs with similar sequences, most of the differential bases that determine their specificity are located at the 3 'end. It is recommended that forward specific primers terminate at the 3' end of the differential base. If the annealing temperature is too low due to the excessively short length of primer, 2-3 bases can be added to the 5' end of primer to match the Tm values of forward and reverse primers.

Reverse primer

This kit provides a reverse prImer Universal miRNA-A qPCR Prime R for qPCR detection. Its annealing temperature is approximately 60°C. **2X Universal SYBR Green Fast qPCR Mix (RK21203)** is recommended for subsequent qPCR detection.