艾科瑞生物
Accurate Biology

Instruction for Use

miRNA 1st Strand cDNA Synthesis Kit with gDNA Clean (Stem-loop)

AG11745
Version.V1E1

Research Use Only
Not For Diagnosis Procedures

1. Description

This product is a reverse transcription kit designed for the synthesis of first-strand miRNA cDNA using the stem-loop method. The included 5X gDNA Clean Reaction Mix II effectively removes residual genomic DNA (gDNA) from RNA samples under the condition of 42°C for 2 minutes, thereby improving result accuracy. This product is suitable for reverse transcription of samples containing miRNA, such as Total RNA or Small RNA. The resulting cDNA can be used for qPCR analysis with the TaqMan probe method. It is recommended to use this kit in combination with our SYBR Green Pro Taq HS qPCR Premix II (Code: AG11702); for applications requiring reduced primer-dimer formation, SYBR Green Pro Taq HS qPCR Premix IV (Code: AG11746) is recommended for optimal performance.

2. Kit Information

Kit Name	Cat. No	Specification
miRNA 1st Strand cDNA Synthesis Kit with gDNA Clean (Stem-loop)	AG 11745	50 rxns / 20 µl

3. Transportation and Storage

Storage	Store at −20°C
Transportation	Transport at −20°C Dry Ice or Blue Ice Condition

4. Kit Components

Kit Components	Volume
5X gDNA Clean Reaction Mix II	100 µl
5X miRNA RT Buffer (Stem-loop) *1	200 µl
miRNA RT enzyme mix (Stem-loop) *2	100 µl
RNase Free Water	1 ml

*1: This solution contains dNTPs.

*2: This solution contains Evo M-MLV RTase, RNase Inhibitor.

5. Protocol

5.1 Removal of Genomic DNA

Prepare the reaction mix on ice according to the table below, then perform the reaction in a PCR instrument:

Components	Reaction System
5X gDNA Clean Reaction Mix II	2 µl
Total RNA *1	≤1 µg
RNase free water	Up to 10 µl *3

Reaction Program:

Temperature	Time
42°C	2 min
70°C *2	10 min *2
4°C	-

*1: In the 10 µl gDNA removal reaction system, it is recommended that the amount of Total RNA does not exceed 1 µg.

*2: The 70 °C for 10 min step is to inactivate the 5X gDNA Clean Reaction Mix II. If amplification results are suboptimal, it may be due to incomplete inactivation of the reagent-consider increasing the temperature to 75 °C for 10 min.It is recommended to first prepare the reagents, excluding RNA, into a premix solution, mix thoroughly, and then add the RNA template.

5.2 Reverse Transcription

Prepare the RT mix as below,then perform the reaction in a PCR instrument*2*3:

Components	Reaction System
Reaction Mix from Step 5.1 gDNA removal	10 µl
5X miRNA RT Buffer (Stem-loop)	4 µl
miRNA RT enzyme mix (Stem-loop)	2 µl
Stem-loop RT Primer*1 (10 µM)	0.5 µl
RNase free water	Up to 20 µl

Reaction Program:

Temperature	Time
25°C	5 min
42°C	15 min
85°C	5 sec
4°C	-

*1: The Stem-loop RT Primer is designed based on the miRNA sequence. The recommended concentration is 0.25 µM, but it can be adjusted within the range of 0.1- 0.4 µM as needed.

*2: When preparing the reverse transcription reaction mix, components can be premixed and dispensed as 10 µl aliquots into the reaction solution from the previous step (Experimental Procedure - gDNA Removal). If not using a premix, add reagents to the previous step's reaction in the following order: RNase-free water, 5X miRNA RT Buffer (Stem-loop), mix well, then add the Stem-loop RT Primer and miRNA RT Enzyme Mix (Stem-loop). Gently mix to ensure maximum reverse transcriptase activity.

*3: The resulting cDNA can be used immediately for qPCR or stored at –20 °C for short-term storage.
For long-term storage, keep at - 80 °C.

6. Quantitative PCR Reaction Analysis

The cDNA obtained from the above reverse transcription reaction can be directly used for quantitative PCR analysis.
It is recommended to use it in combination with our SYBR Green Pro Taq HS Premixed qPCR Kit II (Code: AG11702).
For detailed instructions, please refer to the full manual available for download at www.agbio.com.cn.

Result Analysis

After the reaction, confirm the amplification curve and melting curve, and perform standard curve analysis.
(For analysis methods, please refer to the instrument's user manual.)

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