艾科瑞生物
Accurate Biology

Instruction for Use

miRNA 1st Strand cDNA Synthesis Kit

AG11717
Version.V3E1

Research Use Only
Not For Diagnosis Procedures

1. Description

This Product is optimized for miRNA 1st strand cDNA synthesis, applying poly (A) tailing method. Firstly added with poly (A) tail on the 3' end of miRNA, the first strand cDNA is then synthesized via reverse transcription using the general reverse transcription primers (with Oligo(dT) primer specifically modified).

It is optimized that the poly (A) tailing and reverse transcription reaction being completed in one tube reaction system, simplifying operation procedures, save time cost and reducing operational errors. This kit is suitable for templates including Total RNA, Small RNA and other miRNA containing templates. The miRNA qPCR 3' primer required for downstream qPCR analysis is included in this kit.

2. Kit Information

Kit Name	Cat. No	Specification
miRNA 1st Strand cDNA Synthesis Kit	AG 11717	50 rxns / 10 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
miRNA RT Enzyme Mix*1	62.5 µl
2X miRNA RT Reaction Solution*2	250 µl
miRNA qPCR 3' primer (10 µM)*3	625 µl x 2 pcs
RNase Free Water	1 ml

*1: The Component contains Poly(A) Polymerase, M-MLV RTase, RNase Inhibitor;

*2: Consists of universal Reverse Transcription primers;

*3: Tm value is 59°C, for qPCR reaction.

5. Protocol

5.1 Poly(A) Tailing and 1st Strand cDNA Synthesis

(1) Prepare the reaction mix as below.

Components	Input Volume
miRNA RT Enzyme Mix*1	1.25 µl
2X miRNA RT Reaction Solution	5 µl
Total RNA *2	20 ng ~ 1 µg
RNase free water	Up to 10 µl

*1: Before use, thaw the reagent tube, and vigorously vortex for 30–60 seconds to ensure homogeneity. Before use, briefly centrifuge to collect contents at the bottom of the tube. Avoid frequent freeze-thaw.

*2: Recommended Total RNA Input volume is 20 ng ~ 1 µg, upmost to 8 µg.

*3: Recommend to prepare all reaction mix on ice.

(2) Run Thermal Cycling Program as below.

The cycling parameters below are offered as a guideline and may be modified as necessary for optimal results.

Temperature	Time
37°C *1	60 min
85°C	5 min
4°C	-

* For complex structure template, tune the Reverse Transcription temperature to 42°C, to optimize results.

Note: After reverse transcription, the 1st strand cDNA product shall be stored at -20°C; or directly processed for downstream qPCR analysis.

Note: Recommend to dilute the cDNA product by 1:10 before used for qPCR analysis; or adjust the dilution ratio in the range of 1:5-1:100, upon the actual amplification result.

5.2 Quantitative PCR (qPCR) Amplification and Analysis

Use the retrieved cDNA product from step 5.1 above for downstream qPCR analysis.

Use AG SYBR Green Premix *Pro Taq* HS qPCR Kit II (Product Code. AG 11702) for technical example.

Prepare the qPCR reaction mix as below.

Components	Final Concentration	Volume
2X SYBR Green *Pro Taq* HS Premix II*1	1 X	10 µl
Template	-	2 µl
miRNA-specific primer (10 µM)*2	0.4 µM	0.8 µl
miRNA qPCR 3' primer (10 µM)	0.4 µM	0.8 µl
ROX Reference Dye (4 µM) *3	0.08 uM	0.4 µl
RNase free water	-	Up to 20 µl

*1: Recommend to dilute the cDNA product by 1:10 before used for qPCR analysis;

*2: The miRNA specific primer is designed and prepared by user self. It shall be designed specifically upon target miRNA;

*3: If ROX required by the qPCR instrument, please check the appendix of qPCR instrument compatibility table for required Rox Reference Dye. [Rox Reference Dye (20uM) AG 11703; Rox Reference Dye (4uM) AG 11710]

The cycling parameters below are offered as a guideline and may be modified as necessary for optimal results.

2 Step Thermal Cycling Setup*1

Step	Temperature	Time	Number of Cycles
Step 1	95°C	30 sec*2	1
Step 2	95°C	5 sec	40
	60°C	20 sec*3	
Step 3	Dissociation Stage		

*1: Recommend to run the 2 Step program firstly; if result is not satisfactory or primer Tm value is low, recommend to try again using the 3 Step program below, to optimize the result;

*2: Pre-denaturation time is recommended to be 30 secs. If denaturation efficiency is not satisfactoyr, prolong the time to 1 ~ 5 min;

*3: To increase amplification specificity, increase the extention temperature accordingly; if to increase amplification efficiency and sensitivity, extend the cycling time for extension stage. Or recommend to run using the 3 Step program below.

3 Step Thermal Cycling Setup

Step	Temperature	Time	Number of Cycles
Step 1	95°C	30 sec	1
Step 2	95°C	5 sec	40
	55°C*1	20 sec	
	72°C	20 sec*2	
Step 3	Dissociation Stage		

*1: Annealing temperature could be altered based on the Tm values of Forward and Reverse primers;

*2: Extend the cycling time for extension stage, if the amplification efficiency is not satisfactory.

Appendix of qPCR Instrument Compatibility Table

Brand	Instrument Model	Rox
Analytik Jena	qTOWER3	-
Agilent	Mx3000P™, Mx3005P™, MX4000™	4 μM
Bioer	Line-Gene	-
Bio-Rad	IQ5, CFX96™, CFX384™, CFX Connect™, MJOpticon, Opticon 2	-
Cepheid	SmartCycler® System, Smart Cycler II System	-
Eppendorf	Mastercycler ep realplex	-
Qiagen	Rotor-Gene® Q, 3000, 6000	-
Roche	LightCycler® 2.0, 480, 96	-
TaKaRa	Thermal Cycler Dice™ TP950	-
Thermo (Life/ABI)	ABI 7500, 7500 Fast, ViiA™7, QuantStudio™ 3/5, QuantStudio™ 6/7/12K Flex, QuantStudio™ Dx	4 μM
Thermo (Life/ABI)	ABI 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne, StepOnePlus	20 μM

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