TIANGEN®

miRcute Plus miRNA qPCR Kit (SYBR Green)

For detection of miRNA using real-time RT-PCR (SYBR Green)

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miRcute Plus miRNA qPCR Kit (SYBR Green)

Cat. no. GFP411

Kit Contents

Contents	GFP411-01 (20 µl×125 rxn)	GFP411-02 (20 µl×500 rxn)
2×miRcute Plus miRNA PreMix (SYBR&ROX)	1.25 ml	4×1.25 ml
Reverse Primer(10 µM)	55 µl	220 µl
50× ROX Reference Dye	250 µl	1 ml
RNase-Free ddH_2O	2×1 ml	5×1 ml
Handbook	1	1

Storage

The miRcute Plus miRNA qPCR Kit (SYBR Green) should be stored immediately upon receipt at -30~-15°C, protected from light. Thaw each components and mix thoroughly before use. If the 2× miRcute Plus miRNA Premix (SYBR&ROX) are thawed but not used, it is important to thoroughly mix them prior to re-freezing (The layering of salts during the thawing process and subsequent crystallization during freezing will damage the enzyme and decrease product performance). This kit could be stored at -30~-15°C for 1 year.

Introduction

miRcute Plus miRNA qPCR Kit is designed for miRNA real-time PCR by using SYBR Green I. The kit is composed of 2× miRcute plus miRNA Premix, 50× ROX Reference Dye and Reverse Primer (10 μM).

2× miRcute plus miRNA premix (with SYBR and ROX) was developed specifically for quantitative detection of miRNA. The DNA polymerase in this kit is the antibody modified hot-start DNA polymerase. Combined with the unique buffer, the hot-start DNA polymerase ensures high specificity, sensitivity and accurate quantitative detection in a wide range.

Note: The kit has to be used with miRcute Plus miRNA First-Strand cDNA Kit (Cat. no. GKR211-01/02).

Important Notes

1. The kit contains SYBR Green I. Store and prepare PCR reaction in dark condition.
2. Pipet the reaction solution and aliquot with sterile tips and consumable items to avoid contamination.

Reagents and Materials Not Supplied

1. RNase-Free ddH_2O
2. Forward primer

Forward Primer Design Principles

1. Follow the most common design principles of primers.
2. Based on mature miRNA sequence, change U to T to design forward prime.
3. Tm value of Reverse Primer (10μM) in the kit is about 65°C. Ensure that Tm value of forward prime is basically consistent with supplied Reverse Primer (10 μM).
4. If Tm value of the designed forward prime is too low according to principle 2, it is recommended to add several nucleotides (G or C is optional) to 5'-terminal, or add one or several adenines to 3'-terminal. Optionally, modify both 5' terminal and 3' terminal at the same time
5. If Tm value of the designed forward prime is too high according to principle 2, remove several nucleotides from 5' or 3' terminal.

Protocol

1. Thaw 2× miRcute Plus miRNA PreMix (SYBR&ROX) and Reverse Primer (10 μM) at room temperature.
2. Mix the 2× miRcute Plus miRNA PreMix (SYBR&ROX) thoroughly by inverting gently. Avoid bubbling in the process and centrifuge gently before use.

   Note: The performance will decrease unless the reagents are mixed thoroughly. Do not vortex.
3. Place all the reagents on ice, and prepare the reaction according to Table A.

   Note: If using PRISM 7000/7300/7700/7900HT, Step one/ Step one plus PCR system from ABI, prepare the reaction according to Table B.

   Table A

Components	Volume (50 μl)	Volume (20 μl)	Final Conc.
2× miRcute plus miRNA Premix (SYBR&ROX)	25 μl	10 μl	1×
Forward Primer (not supplied)	-	-	200 nM
Reverse Primer (10 μM)	1 μl	0.4 μl	200 nM
miRNA first-strand cDNA	-	-	-
ddH_2O	Up to 50 μl	Up to 20 μl	

Note: Ensure addition of miRNA first-strand cDNA is less than 1/10 volume of real-time PCR reaction. Dilute the cDNA according to actual concentration (Dilution ratio may be 1/10 or 1/100) since high concentration of cDNA will lead to non-specific amplification.

Table B

Components	Volume (50 µl)	Volume (20 µl)	Final Conc.
2× miRcute plus miRNA Premix (SYBR&ROX)	25 µl	10 µl	1×
Forward Primer (not supplied)	-	-	200 nM
Reverse Primer (10 µM)	1 µl	0.4 µl	200 nM
miRNA first-strand cDNA	-	-	-
50× ROX Reference Dye	4 µl	1.6 µl	5×
ddH_2O	Up to 50 µl	Up to 20 µl	-

Note: Ensure addition of miRNA first-strand cDNA is less than 1/10 volume of real-time PCR reaction. Dilute the cDNA according to actual concentration (Dilution ratio may be 1/10 or 1/100) since high concentration of cDNA will lead to non-specific amplification.

Compatible Real-time Instruments

PRISM 7000/7300/7500/7700/7900HT Real-Time PCR System, Step one/ Step one plus PCR System (Applied Biosystems)

LightCycler (Roche)

Mx3000P, Mx3005P and Mx 4000 (Stratagene)

Mastercycler ep realplex (Eppendorf)

Line-Gene (Bioer)

Other Real-time instruments

PCR program outlined in the following table

1. In general, a real-time PCR reaction can be set up as follows:

Cycle numbers	Temperature	Time	Comments
1×	95°C	15 min	Initial PCR denaturation
40-45×	94°C	20 sec	Denaturation
	60°C	34 sec	Annealing and extension
Melting/Dissociation Curve Stage			

2. To improve the detection specificity and detection rate of low abundance miRNA, the following procedure can be used:

Cycle numbers	Temperature	Time	Comments
1×	95°C	15 min	Initial PCR denaturation
5×	94°C	20 sec	Enrichment of low abundance target miRNA. Do not collect the fluorescent signal.
	63-65°C	30 sec	
	72°C	34 sec	
40-45×	94°C	20 sec	Denaturation
	60°C	34 sec	Annealing and extension
Melting/Dissociation Curve Stage			