Green miRNA Two-Step qRT-PCR SuperMix

Catalogue No.:abx098036

Green miRNA Two-Step qRT-PCR SuperMix contains all the necessary components for the detection and quantification of miRNA from total RNA, small RNA and other miRNA-containing samples. The kit includes miRNA Enzyme Mix, containing Poly(A) polymerase and reverse transcriptase, and miRNA Reaction Mix to efficiently add Poly(A) tails and synthesize first-strand cDNA. Green qPCR SuperMix is provided for miRNA quantification. The Passive reference dye, which can be used to normalize fluorescent signals between ractions, is compatible with different qPCR instruments:

- **50X Passive Reference Dye 1:**ABI Prism® 7000/7300/7700/7900, ABI Step One Plus®
- **50X Passive Reference Dye 2:** ABI Prism® 7500/7500 Fast, ABI Q6, ABI QuantStudio® 6/7 Flex, ABI ViiA® 7, Stratagene Mx3000®/Mx3005P®, Qiagen Corbett Rotor-Gene® 3000.
- **No Passive Reference Dye:** Roche LightCycler® 480/96, MJ Research Chromo4®, MJ Research Opticon® 2, Takara TP-800®, Bio-Rad iCycler iQ®, Bio-Rad iCycler iQ5®, Bio-Rad CFX96®, Bio-Rad CI000® Thermal Cycler, Thermo Scientific Pikoreal® 96, Qiagen Corbett Rotor-Gene® 6000/G/Q

Target: Green miRNA Two-Step qRT-PCR SuperMix

Storage: Store at -20°C. Stable for 12 months from date of receipt.

Kit Components:
• miRNA RT Enzyme Mix: 20 µl
• 2X miRNA Reaction Mix: 200 µl
• Universal miRNA qPCR Primer (10 µM): 200 µl
• 2X Green qPCR SuperMix: 5 × 1 ml
• 50X Passive Reference Dye (Optional): 200 µl
• RNase-Free Water: 1 ml

Note: THIS PRODUCT IS FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC, THERAPEUTIC OR COSMETIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION.
This product is shipped with dry ice.

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Directions for use: Tail addition and first strand cDNA synthesis

Reaction Components:

Component	Volume
Total RNA/miRNA*	*x* µl
miRNA RT Enzyme Mix	1 µl
2X miRNA Reaction Mix	10 µl
RNase-Free Water	to 20 µl

* Total RNA should be ≤ 5 µg. Since miRNA cannot be directly quantified by the spectrophotometer, it is recommended to use 1-9 µl for a total reaction volume of 20 µl.

Mix gently, then incubate at 37 °C for 1 hour. Incubate at 85 °C for 5 seconds to inactivate the miRNA Enzyme Mix.

Suggested qPCR Conditions:

Component	Volume	Final Concentration
cDNA[1]	Variable	as required
Forward Primer (10 µM)[2]	0.4 µl	0.2 µM
Universal miRNA qPCR Primer (10 µM)	0.4 µl	0.2 µM
2X Green qPCR SuperMix	10 µl	1X
50X Passive Reference Dye (Optional)	0.4 µl	1X
Nuclease-Free Water	Variable	N/A
Total Volume	**20 µl**	N/A

[1] It is recommended to dilute synthesized cDNA 5-10 fold.

[2] The upstream primer is the target miRNA specific primer, which should be designed by the end user according to the target miRNA.

Thermal Cycling Conditions (2-step):

The 2-step qPCR method is recommended for higher specificity.

Number of Cycles	Temperature	Time
1 cycle	94 °C	30 seconds
40-45 cycles	94 °C	5 seconds
	60 °C	30 seconds
	Dissociation Stage	

Thermal Cycling Conditions (3-step):

The 3-step qPCR method is recommended for higher sensitivity.

Number of Cycles	Temperature	Time
1 cycle	94 °C	30 seconds
40-45 cycles	94 °C	5 seconds
	50-60 °C	15 seconds
	72 °C	10 seconds
	Dissociation Stage	

Fluorescent signals can be collected during the annealing or extension stage. For ABI qPCR instruments, the following signal collection times are recommended:

- ABI Prism® 7700/7900: 30 seconds
- ABI Prism® 7000/7300: 31 seconds
- ABI Prism® 7500: 34 seconds
- ABI ViiA® 7: 19 seconds

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