TransScript® Green miRNA Two-Step qRT-PCR SuperMix

Cat.No. AQ202

Storage: at -20°C for two years

Description

TransScript® Green miRNA Two-Step qRT-PCR SuperMix provides all the necessary components for the detection and quantification of miRNA from total RNA, small RNA or other miRNA containing samples. *TransScript*® miRNA RT Enzyme Mix (Poly(A) polymerase and reverse transcriptase are included) and 2×TS miRNA Reaction Mix are supplied to efficiently add Poly(A) tails and synthesize first-strand cDNA. *PerfectStart*™ Green qPCR SuperMix is used for miRNA quantification.

Highlights

- Optimal ratio of Poly(A) polymerase to reverse transcriptase and optimized reaction buffer, so as to ensure high transcription efficiency of miRNA.
- One-step completion of Poly(A) tail addition and first-strand cDNA synthesis in a single tube.
- High amplification efficiency, specificity and sensitivity ensured by *PerfectStart*™ Green qPCR SuperMix, leading to accurate data.
- Passive reference dyes compatible with different qPCR instruments (normalize the fluorescent signals between reactions).

Application

Multiple copy and low copy gene detection

Passive Reference Dye

- Passive Reference Dye I (50×)
  ABI Prism® 7000/7300/7700/7900, ABI Step One®, ABI Step One Plus®
- Passive Reference Dye II (50×)
  ABI Prism® 7500, ABI Prism® 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, Roche Light Cycler® 96, MJ Research Chromo4®, MJ Research Opticon® 2, Takara TP-800®, Bio-Rad iCycler iQ®, Bio-Rad iCycler iQ5®, Bio-Rad CFX96®, Bio-Rad C1000® Thermal Cycler, Thermo Scientific Pikoreal® 96, Qiagen Corbett Rotor- Gene® 6000, Qiagen Corbett Rotor-Gene® G, Qiagen Corbett Rotor-Gene® Q

Kit Contents

Component	AQ202-01
TransScript® miRNA RT Enzyme Mix	20 µl
2×TS miRNA Reaction Mix	200 µl
Universal miRNA qPCR Primer (10 µM)	200 µl
2×*PerfectStart*™ Green qPCR SuperMix	5×1 ml
Passive Reference Dye (50×)	200 µl
RNase-free Water	1 ml

Tail addition and first-strand cDNA synthesis

1. Reaction Components

Component	Volume
Total RNA/ miRNA*	x μl
TransScript® miRNA RT Enzyme Mix	1 μl
2×TS miRNA Reaction Mix	10 μl
RNase-free Water	to 20 μl

* Total RNA ≤ 5 μg. Since miRNA cannot be directly quantified by spectrophotometer, we suggest using 1-9 μl for 20 μl reaction.

2. Mix gently, and incubate at 37°C for 1 hour.

3. Incubate at 85°C for 5 seconds to inactivate RT Enzyme Mix.

Suggested qPCR conditions (20 μl reaction volume)

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
2×*PerfectStart*™ Green qPCR SuperMix	10 μl	1×
Passive Reference Dye (50×) (optional)	0.4 μl	1×
Nuclease-free Water	Variable	-
Total Volume	20 μl	-

*1. We suggest diluting the synthesized cDNA 5-10 folds.

*2. Upstream primer is target miRNA specific primer, which will be designed by customers according to target miRNA.

Thermal cycling conditions (three-step)

94°C	30 sec	
94 °C	5 sec	40-45 cycles
50-60°C	15 sec★	
72°C	10 sec★	

Dissociation Stage

Thermal cycling conditions (two-step)

94°C	30 sec	
94°C	5 sec	40-45 cycles
60°C	30 sec★	

Dissociation Stage

Fluorescent signals can be collected during the annealing or extension stage. For ABI qPCR instrument, we suggest using the following signal collecting time:

* For ABI Prism® 7700/7900, the time to 30 seconds.
* For ABI Prism® 7000/7300, the time to 31 seconds.
* For ABI Prism® 7500, the time to 34 seconds.
* For ABI ViiA® 7, the time is at least 19 seconds.

Two-step qPCR is more suitable for higher specificity assay.

Three-step qPCR is more suitable for higher sensitivity assay.

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