TransScript® Green miRNA Two-Step qRT-PCR SuperMix

Please read the manual carefully before use

Cat. No. AQ202

Version No. Version 1.0

Storage: at -18°C or below in the dark 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).

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

Mix and centrifuge each component before use.

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.

Recommended qPCR System and Conditions (taking 20 μl reaction system as an example)

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
50-60°C 15 sec★
72°C 10 sec★
40-45 cycles
Dissociation Stage

Thermal cycling conditions (two-step)

94°C 30 sec
94°C 5 sec
60°C 30 sec★
40-45 cycles
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 amplification efficiency assay.

Passive Reference Dye

- Passive Reference Dye I (50×)
  ABI Prism 7000/7300/7700/7900, ABI Step One, ABI Step One Plus, ABI 7900HT, ABI 7900HT Fast
- Passive Reference Dye II (50×)
  ABI Prism 7500, ABI Prism 7500 Fast, ABI QuantStudio Dx/3/5, ABI QuantStudio 6/7/12K Flex, ABI ViiA 7, Stratagene Mx3000P/Mx3005P/Mx4000
- 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, Qiagen Corbett Rotor-Gene 3000, Mastercycler ep realplex

For research use only, not for clinical diagnosis.

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