TransScript® miRNA First-Strand cDNA Synthesis SuperMix

Cat.No. AT351

Storage: at -20°C for two years

Description

TransScript® miRNA First-Strand cDNA Synthesis SuperMix provides all the necessary components for cDNA synthesis from miRNA template. High efficient poly(A) tail addition and first-strand cDNA synthesis are performed by *TransScript*® miRNA RT Enzyme Mix (containing tailing enzyme and RT enzyme) and 2×TS miRNA Reaction Mix.

Highlights

- Optimized enzyme and buffer system for high efficient cDNA synthesis.
- One-step Poly(A) tailing and cDNA synthesis.

Application

miRNA synthesis

Kit Contents

Component	AT351-01 (20 rxns)
TransScript® miRNA RT Enzyme Mix	20 µl
2×TS miRNA Reaction Mix	200 µl
Universal miRNA qPCR Primer (10 µM)	200 µl
RNase-free Water	1 ml

Prior to use, please centrifuge each component

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 Volume	20 µl

* Total RNA ≤ 5 µg. Since miRNA cannot be directly quantified by spectrophotometer, we suggest to use 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 ┐

50-60°C 15 sec★ ├ 40-45 cycles

72°C 10 sec★ ┘

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 Prism7700/7900, the time is 30 seconds.
* For ABI Prism7000/7300, the time is 31 seconds.
* For ABI Prism7500, the time is 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.

Passive Reference Dye

- Passive Reference Dye I (50×)
  ABI Prism7000/7300/7700/7900, Eppendorf, ABI Step One, ABI Step One Plus
- Passive Reference Dye II (50×)
  ABI Prism7500, ABI Prism7500 Fast, Stratagene Mx3000/Mx3005P, Corbett Rotor Gene 3000, ABI ViiA7
- No Passive Reference Dye
  Roche LightCycler480, Roche Light Cycler96, MJ Research Chromo4, Opticon (II),
  Bio-Rad iCycler iQ, iQ5, Bio-Rad CFX96, Corbett Rotor Gene 6000

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