

# TransScript® miRNA First-Strand cDNA Synthesis SuperMix

Please read the manual carefully before use

**Cat. No.** AT351

**Storage:** at -18°C or below 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. This product enables the reverse transcription of miRNA from samples such as total RNA or small RNA, and converts other RNA types present, including mRNA and snRNA, into cDNA.

## 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 )	AT351-02 (50 rxns )
TransScript® miRNA RT Enzyme Mix	20 µl	50 µl
2×TS miRNA Reaction Mix	200 µl	500 µl
Universal miRNA qPCR Primer (10 µM)	200 µl	500 µl
RNase-free Water	1 ml	2×1 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	Variable
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* <sup>1</sup>	Variable	as required
Forward Primer (10 µM)* <sup>2</sup>	0.4 µl	0.2 µM
Universal miRNA qPCR Primer (10 µM)	0.4 µl	0.2 µM
2× <i>PerfectStart</i> <sup>TM</sup> 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.

**qPCR (three-step)**

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

} 40-45 cycles

Dissociation Stage

**qPCR (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 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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