

# 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* <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>®</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.

#### Thermal cycling conditions (three-step)

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

Dissociation Stage

#### Thermal cycling conditions (two-step)

94°C	30 sec	} 40-45 cycles
94°C	5 sec	
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 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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