



## miAnalysis™ TaqMan PCR System

### User's Instruction

#### Description

As the gold standard for microRNA quantitative research, TaqMan method has the characteristics of high specificity and sensitivity. miAnalysis™ TaqMan PCR System is designed for miRNA detection and quantification. cDNA generated by the miAnalysis™ TaqMan RT Kit is used as the template for real-time PCR with the miAnalysis™ TaqMan PCR Kit.

#### Kit Contents

##### miAnalysis™ TaqMan RT kit

The miAnalysis™ TaqMan RT Kit makes miRNA reverse transcription as easy as the reverse transcription of mRNA. Simply mixing the extracted miRNA with the reaction buffer and putting it on the thermocycler, you can obtain a high concentration of cDNA products containing all miRNA molecules. The obtained cDNA product is suitable for quantitative detection by the miAnalysis™ TaqMan qPCR Kit.

	100 preps
1. 5 × TaqMan miRNA RT Solution A	100 μl
2. 10 × TaqMan miRNA RT Solution B	200 μl
3. 10 × TaqMan miRNA RT Primer	200 μl
4. RNase Free H <sub>2</sub> O	1 ml × 2

##### miAnalysis™ TaqMan PCR Kit

The miAnalysis™ TaqMan PCR Kit can greatly improve the detection specificity of miRNA, which can distinguish between highly similar miRNA molecules and reduce non-specific amplification. We have optimized primers for more than 10,000 miRNAs, which ensures amplification efficiency and specificity. Please check this [excel file](#) to see whether the miRNA you are studying have already been optimized. If not, please contact us and we can optimize for you.

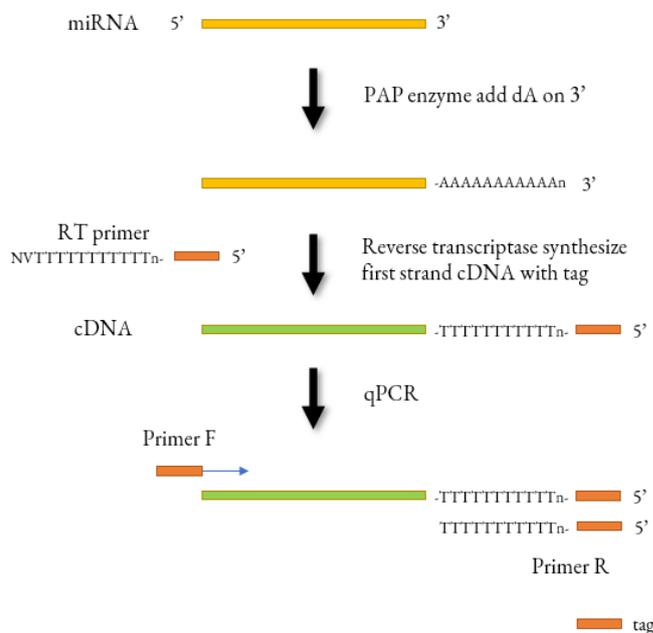
	100 preps
1. 5 × TaqMan miRNA qPCR Mix	400 μl
2. 50 × ROX Reference Dye	200 μl



### 3. 20 × miRNA TaqMan Assay

100 μl

#### Detection Principle



#### Protocol

##### miAnalysis™ TaqMan RT

- Mix the reagents required for the reverse transcription as the following table.

Reagent	Volume
miRNA (5-100 ng/ μl)	4 μl
5 × TaqMan miRNA RT Solution A	1 μl

- Run thermocycler for the reaction (recommended)
  - 37°C for 30 min
  - 85°C for 5 min
- After completion of the above reaction, add the following reagents to the above 5 μl reaction system and mix evenly.



Reagent	Volume
10 × TaqMan miRNA RT Primer	2 μl
10 × TaqMan miRNA RT Solution B	2 μl
RNase Free H <sub>2</sub> O	11 μl

4. Run thermocycler for the reaction (recommended)
  - a) 30°C for 5 min
  - b) 55°C for 60 min
  - c) 95°C for 5 min
5. cDNA generated by the miAnalysis™ TaqMan RT Kit can be used as the template for real-time PCR with the miAnalysis™ TaqMan PCR Kit. Usually you can make 5 times dilution by adding 80 μl RNase Free H<sub>2</sub>O, and take 2 μl for RT-qPCR detection.

#### miAnalysis™ SYBR Green PCR

1. According to the instrument, carry out Step A or Step B.

**Step A:** For instruments which need ROX reference, please set up the reaction as the following table.

Reagent	Volume	Final Concentration
5 × TaqMan miRNA qPCR Mix	4 μl	1 X
50 × ROX Reference Dye	0.4 μl	1 X
20 × miRNA TaqMan Assay	1 μl	1 X
cDNA	1-2.5 μl	
RNase-Free Water	Up to 20 μl	

**Step B:** For instruments which do not need ROX reference, please set up the reaction as the following table.

Reagent	Volume	Final Concentration
5 × TaqMan miRNA qPCR Mix	4 μl	1 X



20 × miRNA TaqMan Assay	1 $\mu$ l	1 X
cDNA	1-2.5 $\mu$ l	
RNase-Free Water	Up to 20 $\mu$ l	

2. Run thermocycler for the reaction (recommended)

a) 95°C for 15 min

b) 95°C for 10 sec

60°C for 60 sec      40 cycles

3. FAM channel is used to collect signals