



## miAnalysis™ SYBR Green PCR System

### User's Instruction

#### Description

miAnalysis™ SYBR Green PCR System is designed for miRNA detection and quantification. cDNA generated by the miAnalysis™ SYBR Green RT Kit is used as the template for real-time PCR with the miAnalysis™ SYBR Green PCR Kit.

#### Kit Contents

##### miAnalysis™ SYBR Green RT kit

The miAnalysis™ SYBR Green 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 for 1 hour, 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™ SYBR Green qPCR Kit.

	100 preps
1. 4 × SYBR Green RT mix	500 μl
2. 10 × miRNA RT primers	200 μl
3. RNase Free H <sub>2</sub> O	1 ml × 2

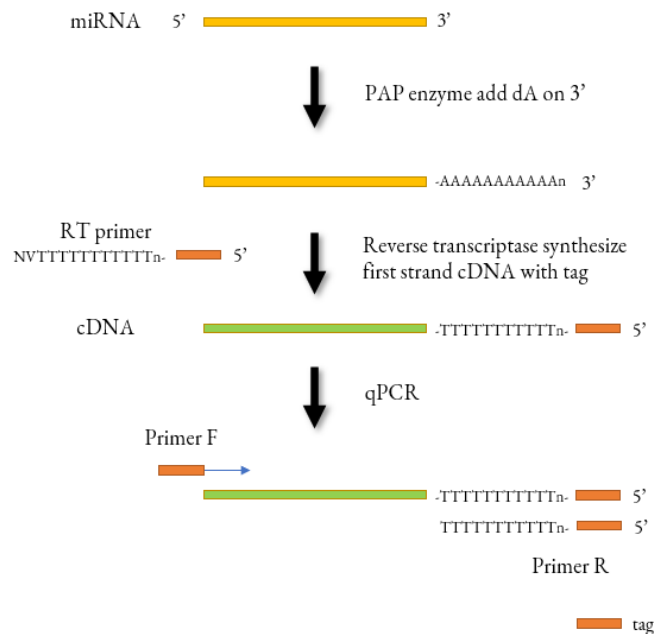
##### miAnalysis™ SYBR Green PCR Kit

The miAnalysis™ SYBR Green 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. 2 × miAnalysis SYBR Green PCR mix	1 ml
2. 50 × ROX Reference Dye	200 μl
3. miRNA Primer F (10μM)	100 μl
4. miRNA Primer R (10μM)	100 μl
5. miRNA standard (1pM)	100 μl



## Detection Principle



## Protocol

### miAnalysis™ SYBR Green RT

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

Reagent	Volume
miRNA (0.01-0.1 µg) or Total RNA	0.1-0.2 µg
4 × miAnalysis SYBR Green RT mix	5 µl
10 × miRNA RT primers	2 µl
RNase-Free Water	Up to 20 µl

- Run thermocycler for the reaction (recommended)
  - 37°C for 60 min
  - 95°C for 5 min
- cDNA generated by the miAnalysis™ SYBR Green RT Kit is used as the template for real-time PCR with the miAnalysis™ SYBR Green PCR Kit.



## 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
2 × miAnalysis SYBR Green PCR mix	10 µl	1 X
50 × ROX Reference Dye	0.4 µl	1 X
miRNA Primer F (10 Mm)	0,4 µl	
miRNA Primer R (10 Mm)	0.4 µl	
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
2 × miAnalysis SYBR Green PCR mix	10 µl	1 X
miRNA Primer F (10 Mm)	0,4 µl	
miRNA Primer R (10 Mm)	0.4 µl	
cDNA	1-2.5 µl	
RNase-Free Water	Up to 20 µl	

2. Run thermocycler for the reaction (recommended)

- a) 95°C for 15 min
- b) 95°C for 10 sec  
60°C for 30 sec      30-35 cycles
- c) Dissociation analysis