

## EvaGreen miRNA qPCR Mastermix

Cat. No.	Description	Quantity Store at -20°C
Mastermix-mR	EvaGreen miRNA qPCR Mastermix- ROX	500 X 20 µl reactions (5 ml)
Mastermix-mL	EvaGreen miRNA qPCR Mastermix- low ROX	500 X 20 µl reactions (5 ml)
Mastermix-mC	EvaGreen miRNA qPCR Mastermix- iCycler	500 X 20 µl reactions (5 ml)
Mastermix-mS	EvaGreen miRNA qPCR Mastermix	500 X 20 µl reactions (5 ml)

### Product Description

EvaGreen miRNA qPCR Mastermix is designed for quantitative real-time analysis of first strand of miRNA obtained from RNA samples (Cat. No.: G269 or G270, ABM). Instrument-specific EvaGreen miRNA qPCR Mastermix is specially formulated to ensure optimal performance, including high sensitivity, reproducibility and primer specificity. The mastermix is also designed to eliminate primer dimer formation and amplification of premature miRNAs. The chemically modified Hotstart Taq polymerase included in our Mastermix significantly reduces non-specific PCR amplification observed with regular Taq polymerase.

Due to variations in qPCR instrument performance, we offer different EvaGreen miRNA qPCR Mastermix formulations optimized for different machines. Please refer to the following table as a guideline for the appropriate qPCR Mastermix for your particular instrument model.

Cat. No.	Product Name	qPCR Instruments
Mastermix-mR	2X EvaGreen miRNA qPCR Mastermix- ROX	-ABI® 7000,7300,7700,7900, StepOne-Plus™, StepOne™ -Eppendorf® Realplex 4
Mastermix-mL	2X EvaGreen miRNA qPCR Mastermix- low ROX	-ABI® 7500 -Stratagene® Mx3000, Mx3005, Mx4000
Mastermix-mC	2X EvaGreen miRNA qPCR Mastermix- iCycler	-BioRad® iCycler®, iQ™5, MyiQ™  -BioRad® CFX96 -Roche LightCycler® 480 -MJ Research Opticon™ and Opticon™ 2
Mastermix-mS	2X EvaGreen miRNA qPCR Mastermix	-MJ Research Chromo® 4 -Corbett Rotor-gene® 600,3000 -Eppendorf® Realplex 2

### Product Application

EvaGreen miRNA qPCR Mastermix is specially formulated for qPCR analysis of miRNA obtained from RNA samples.

## Kit Components

EvaGreen miRNA qPCR Mastermix is a 2X mix of dNTPs, Hotstart Taq polymerase, MgCl<sub>2</sub>, fluorescent detection dye, reference dye, and proprietary buffer components.

## Shipping and Storage

Upon arrival, EvaGreen miRNA qPCR Mastermix should be stored at -20°C and protected from light. After each experiment, the remaining mixture can be stored at 4°C if it is to be used within the next 7 days; However, freshly thawed Mastermix is highly recommended for optimal results. Avoid repeated freeze-thaw cycles to retain maximum performance. EvaGreen miRNA qPCR Mastermix is stable for 1 year from the date of shipping when stored and handled properly.

## Reaction Setup

Thaw EvaGreen miRNA qPCR Mastermix, template DNA, primers and RNase-free water on ice. Mix each solution well.

### Prepare a reaction Mastermix as follows:

Components	Volume/Reaction	Final Concentration
EvaGreen miRNA Mastermix	10 µl	1X
Forward Primer	Varies	300 nM
Reverse Primer	Varies	300 nM
Template DNA	Varies	≤ 500 ng/reaction
Sterile water	Varies	-
Total Volume	20 µl	-

### Perform qPCR reactions using the following 3-step cycling program.

Step	Temperature	Duration (Standard)	Cycles
Enzyme activation	95°C	10 min	Hold
Denature	95°C	10 sec	40
Anneal	63°C	15 sec	
Extension	72°C	5 sec	
Melting curve	According to the instrument guidelines		

### Recommendations for Optimal Results

- Aliquot reagents to avoid contamination and repeated freeze-thaw cycles
- EvaGreen miRNA qPCR Mastermix components are light sensitive; avoid exposure to light
- Start PCR as soon as the reaction mixture is prepared and always keep the reaction mixture chilled in an ice box prior to PCR reactions