miRNA Unimodal SYBR qPCR Master Mix

MQ102

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Instruction for Use

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01/Product Description

This product is a specialized premix designed for miRNA quantification using the SYBR Green I fluorescence method. The premix is dyed blue for convenient and accurate pipetting. Due to the short length of miRNA sequences and the high similarity often found within miRNA families, achieving high specificity is critical for accurate quantification. The core enzyme of this product has been selectively developed through the BioSmart platform. It features exceptional specificity, strong 3'-end mismatch recognition, and the ability to extend the shortest possible base sequences. Combined with high-sealing dual-species antibodies, the enzyme forms a hot-start Taq polymerase that ensures strict activity suppression at 55°C. Paired with an optimized Buffer specifically designed for qPCR, this product delivers high sensitivity and specificity for miRNA quantification. This premix also includes a specially formulated ROX Passive Reference Dye, compatible with a wide range of qPCR instruments. No additional adjustments to ROX concentration are required across different platforms. Simply add primers and templates to the reaction system to begin amplification. In addition, this product provides universal U6 internal reference primers for human, rat, and mouse samples, enabling the generation of excellent standard curves over a broad dynamic range.

For optimal performance, we recommend using this premix with the **miRNA 1st Strand cDNA Synthesis Kit (by stem-loop) (Vazyme #MR101)** or the **miRNA 1st Strand cDNA Synthesis Kit (by tailing A) (Vazyme #MR201)**.

02/Components

	Components	MQ102-01 125 rxns (20 μl/rxn)	MQ102-02 500 rxns (20 μl/rxn)
□	2 × miRNA Unimodal SYBR qPCR Master Mix[a]	1.25 ml	4 × 1.25 ml
■	mQ Primer R (10 μM)[b]	50 μl	200 μl
■	U6 Forward Primer (10 μM)[c]	50 μl	200 μl
■	U6 Reverse Primer (10 μM)[c]	75 μl	300 μl

a. It contains dNTP, Mg^{2+}, SYBR Green I, Specific ROX Reference Dye, etc.

b. The universal reverse primer for miRNA sequences, compatible with reverse transcription primers designed using Vazyme's miRNA Design software. The primer sequence is AGTGCAGGGTCCGAGGTATT.

c. Provides U6 reference gene primers (forward and reverse) suitable for human, rat, and mouse; for other species, custom-designed reference gene primers are required.

03/Storage

Store at -30 ~ -15°C and protect from light. Ship at ≤0°C. After thawing, Master Mix can be stably stored for 6 months at 2 ~ 8°C protected from light.

04/Applications

It is applicable for dye-based fluorescent quantitative PCR of miRNA from animals, plants, and other sources.

05/Notes

1. Avoid repeated freeze-thaw cycles to prevent a decrease in enzyme activity. If small amounts are used each time, it is recommended to aliquot the product for convenience.
2. Before use, gently invert the Master Mix to mix. Do not vortex to avoid bubble formation, which may affect quantification results. After briefly centrifuging to ensure uniform mixing, the Master Mix is ready for use. During sample loading, avoid forceful pipetting. If bubbles form, a brief centrifugation is required before use.
3. As the product contains the fluorescent dye SYBR Green I, it should be stored away from light. When preparing the reaction mixture, minimize exposure to strong light.
4. Due to the high sensitivity of this product, it is easily contaminated by aerosol particles in the air. Therefore, it is recommended to prepare the reaction mix in a laminar flow hood. Use sterile tips, reaction tubes, and, if possible, dedicated pipettes and filter tips.
5. The blue dye in this product has been tested and shown not to interfere with the fluorescence signal collection of SYBR Green I.

06/Experiment Process

06-1/Prepare the mixture in the qPCR tube

1. Stem Loop Method

miRNA Gene System Preparation

Components	Volume (Single Reaction)	
2 × miRNA Unimodal SYBR qPCR Master Mix	10.0 μl	☐
Specific Primer (10 μM)[a]	0.4 μl	
mQ Primer R (10 μM)[b]	0.4 μl	■
Template cDNA	x μl	
ddH_2O	To 20.0 μl	

Reference Gene System Preparation

Components	Volume (Single Reaction)	
2 × miRNA Unimodal SYBR qPCR Master Mix	10.0 μl	☐
U6 Forward Primer (10 μM)	0.4 μl	■
U6 Reverse Primer (10 μM)	0.4 μl	■
Template cDNA	x μl	
ddH_2O	To 20.0 μl	

a. The forward primers for miRNA genes need to be designed by the user.

b. This product includes a universal reverse primer for miRNA sequences, which is compatible with the reverse transcription primers designed using Vazyme's miRNA Primer Design Tool. If using different stem-loop sequences, users are required to design and synthesize their own qPCR downstream primers.

2. Tail Addition Method

miRNA Gene System Preparation

Components	Volume (Single Reaction)	
2 × miRNA Unimodal SYBR qPCR Master Mix	10.0 μl	☐
Specific Primer (10 μM)[a]	0.4 μl	
Universal reverse Q primer (10 μM)[b]	0.4 μl	
Template cDNA	x μl	
ddH_2O	To 20.0 μl	

Reference Gene System Preparation

Components	Volume (Single Reaction)	
2 × miRNA Unimodal SYBR qPCR Master Mix	10.0 μl	☐
U6 Forward Primer (10 μM)	0.4 μl	■
U6 Reverse Primer (10 μM)	0.4 μl	■
Template cDNA	x μl	
ddH_2O	To 20.0 μl	

a. The forward primers for miRNA genes need to be designed by the user.

b. The reverse primers provided in Vazyme #MR201 for qPCR detection are ready to use and do not require additional synthesis. However, when using different reverse transcription reagents, users need to design and synthesize their own reverse primers for qPCR.

The amount of each component in the reaction mix can be adjusted based on the following guidelines:

▲ Generally, a final concentration of 0.2 μM for the primers yields optimal amplification. If reaction performance is suboptimal, the primer concentration can be adjusted within the range of 0.1 - 1.0 μM.

▲ For undiluted cDNA as the template, the volume used should not exceed 1/10 of the total qPCR reaction volume.

06-2/ Perform qPCR reaction under the following conditions

Stage 1	Initial Denaturation[a]	Rep: 1	95°C	30 sec
Stage 2	Cycles	Reps: 40	95°C	3 - 10 sec[b]
			60°C*	10 - 30 sec[c]
Stage 3	Melting Curve[d]		Default instrument settings	

a. The standard Initial Denaturation condition is suitable for most amplification reactions. For complex template structures, the denaturation time can be extended to 5 min to improve its effectiveness.

b. For standard programs, select 10 sec; for fast programs, the minimum time can be set to 3 sec.

c. For the extension step, standard programs typically use 30 sec. In fast programs, the extension time can be set to as short as 10 sec for amplicons smaller than 200 bp. For amplicons exceeding 200 bp, an extension time of 30 sec is recommended.

d. Melting curve acquisition programs vary with instrument type. The instrument's default melting curve acquisition program is recommended.

* Fluorescence Signal Acquisition.

06-3/Optimization Plan for the Reaction System

An ideal reaction system should exhibit the following characteristics: a single peak in the melting curve (amplification specificity), amplification efficiency close to 100% (amplification efficiency), and reasonable C_T values (amplification sensitivity). If the default reaction conditions do not perform well, optimization can be carried out based on the following guidelines.

1. Relationship Between Primer Concentration and Reaction Performance:

When the final primer concentration ranges from 0.1 to 1.0 μM, higher primer concentrations generally lead to higher amplification efficiency, but may reduce amplification specificity.

2. Relationship Between Amplification Program and Reaction Performance:

To improve amplification specificity, the annealing temperature can be increased:

Two-step Protocol	Increase the annealing temperature (increase by 3°C each time):
95°C/10 sec 60°C/30 sec	95°C/10 sec 63°C/30 sec

To improve amplification efficiency, extend the extension time in the two-step protocol or use the three-step protocol:

Two-step Protocol	Extend the extension time:	Three-step Protocol	Extend the extension time:
95°C/10 sec 60°C/30 sec	95°C/10 sec 60°C/60 sec	95°C/10 sec 56°C/30 sec 72°C/30 sec	95°C/10 sec 56°C/30 sec 72°C/60 sec

07/FAQ & Troubleshooting

◇ Template in relative quantification:

① **Stem-loop method**: miRNA genes and reference genes need to be reverse transcribed separately to obtain the corresponding templates, and then each template undergoes qPCR quantification. The reverse transcription primers for miRNA genes can be designed using the Vazyme's miRNA Primer Design Tool provided by Vazyme. The reverse transcription primers for reference genes can be the downstream primers of the reference gene. For example, if the reference gene is U6, you can use the U6 downstream primers provided in this product for reverse transcription without the need for additional synthesis.

② **Tailing method**: After reverse transcription, the obtained templates can be amplified using the specific quantitative primers for both the miRNA gene and the reference gene.

◇ Quantitative Primer Design Guidance

1. Stem-loop Method

√ Forward Primer:

When designing forward-specific primers for miRNA, we recommend using the remaining portion of the complete miRNA sequence after removing the last 6 nucleotides at the 3' end as the core sequence for the primer. This design approach helps improve primer specificity while eliminating potentially unstable regions at the 3' end that may affect primer efficiency. By replacing uracil (U) with thymine (T) in the sequence, the primer can be optimized for use in qPCR and other experiments, avoiding errors caused by the presence of U during the PCR process. To simplify the design process, Vazyme offers the miRNA Design tool, which can automatically design the most suitable primers based on the complete miRNA sequence. This online tool optimizes primer characteristics such as length and GC content, ensuring primer specificity and stability, thereby improving the accuracy of experimental results. [Vazyme's official website - Resource - Tools - miRNA Primer Design].

√ Reverse Primer:

The reverse universal primer for stem-loop qPCR is part of the stem-loop structure sequence in the stem-loop reverse transcription primer. The stem-loop sequence used is GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGAC. This product provides the reverse universal primer mQ Primer R for qPCR detection, which is designed to pair with the reverse transcription primer designed using Vazyme's miRNA Design software, so no additional synthesis is required. When using a different stem-loop sequence, the downstream qPCR primers must be designed and synthesized independently.

2. Tailing A method

√ Forward Primer:

① It is recommended to design the forward-specific miRNA primer based on the complete miRNA sequence and replace uracil (U) with thymine (T) in the sequence.

② If the annealing temperature of the forward-specific primer designed from the miRNA sequence is too low, it is suggested to add a few nucleotides (preferably G and C) at the 5' end of the primer. After adding nucleotides, verify the primer specificity to avoid non-specific amplification. If the annealing temperature is too high, it is recommended to remove a few nucleotides from the 5' end.

③ For non-specific amplification of miRNA precursor fragments of equal length, it is suggested to add 1 to 3 A nucleotides at the 3' end of the forward-specific primer.

④ For miRNAs with similar sequences, it is recommended to terminate the 3' end of the forward-specific primer at the differential base. If the primer is too short and causes a low annealing temperature, a few nucleotides can be added at the 5' end to match the Tm

values of the upstream and downstream primers.

√ Reverse Primer:

Vazyme #MR201 provides the Universal Reverse Q Primer for qPCR detection, with an annealing temperature of approximately 55 ~ 66°C, and no additional synthesis is required. When using different reverse transcription reagents, the qPCR reverse primer must be designed and synthesized independently.

◇Abnormal Amplification Curve

① **Amplification curve is not smooth**: This may occur due to weak signals, which are corrected by the system. Increase the template concentration and repeat the experiment.

② **Amplification curve breaks or declines**: This happens when the template concentration is too high, causing the baseline endpoint to exceed the C_T value. Reduce the baseline endpoint (C_T value - 4) and reanalyze the data.

③ **Sudden drop in individual amplification curves**: This is caused by air bubbles in the reaction tubes. Ensure proper centrifugation of samples and carefully check for any air bubbles before starting the amplification reaction.

◇ No Amplification Curve After Reaction Completion

① **Insufficient number of cycles**: Typically, 40 cycles are set, but be cautious as too many cycles can increase background signal and reduce data reliability.

② **Check if signal collection is set in the program**: In the two-step protocol, signal collection is generally set during the annealing-extension phase; in the three-step protocol, signal collection should be set during the 72°C extension phase.

③ **Check if primers have degraded**: Primers that have not been used for a long time should be tested for integrity using PAGE electrophoresis to rule out degradation.

④ **Template concentration is too low**: Reduce dilution and repeat the experiment, starting with the highest concentration for unknown samples.

⑤ **Template degradation**: Reprepare the template and repeat the experiment.

◇ C_T Value Appears Too Late

① **Extremely low amplification efficiency**: Optimize reaction conditions, try the three-step amplification protocol, or redesign and synthesize new primers.

② **Template concentration is too low**: Reduce dilution and repeat the experiment, generally starting with the highest concentration for unknown samples.

③ **Template degradation**: Reprepare the template and repeat the experiment.

④ **PCR inhibitors present in the system**: These are usually introduced by the template. Increase template dilution or reprepare the template and repeat the experiment.

◇ Melt Curve Shows Multiple Peaks

① **Poor primer design**: Redesign and synthesize new primers following design principles. This may involve optimizing primer length, GC content, or ensuring specificity to avoid nonspecific binding or secondary structures.

② **High primer concentration**: Reduce the primer concentration to prevent primer-dimer formation or non-specific binding, which can lead to additional peaks.

③ **Genomic contamination in cDNA template**: Reprepare the cDNA template, ensuring thorough removal of genomic DNA. Using a gDNA removal step during RNA preparation or a specific DNase treatment during cDNA synthesis can help eliminate this issue.

◇ Significant Amplification in Negative Control

① **Contamination of the reaction system:** Replace with fresh Mix, ddH_2O, and primers, and repeat the experiment. Prepare the reaction system in a laminar flow hood to reduce aerosol contamination.

② **Primer dimers:** Analyzing using the Melting Curve.

◇ Poor Reproducibility of Experiment

① **Pipetting volume inaccuracies**: Use a high-quality pipette and dilute the template to a higher concentration for more accurate volume addition to the reaction mixture.

② **Inconsistent temperature control at different positions in the qPCR instrument**: Regularly calibrate the instrument to ensure uniform temperature distribution.

③ **Low template concentration**: The lower the template concentration, the worse the reproducibility. Reduce template dilution or increase the volume added to the reaction.

◇ Poor Linearity of the Standard Curve in Absolute Quantification

① **Dilution errors:** Use a stepwise dilution method for template dilution. When preparing the reaction system, try to use larger volume pipettes and high-performance pipettors with low-adsorption consumables.

② **Inappropriate dilution range:** Select an appropriate dilution range in a preliminary experiment, using 5 to 7 gradient points for standard curve linearity calculation.

③ **Poor primer amplification efficiency:** Design and synthesize new primers based on design principles.

④ **Degradation of standards:** Reprepare the standards and repeat the experiment.

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