miRNA Universal SYBR® qPCR Master Mix

MQ101-01/02

Version 8.1

Vazyme biotech co., ltd.

Introduction

This product is a dedicated master mix for miRNA quantification using the SYBR® Green I method. Since the miRNA sequence is short and the miRNA sequences of the same family are often highly similar, the specificity is extremely required in quantification. This product is based on chemical hot start AceTaq® DNA Polymerase with optimized Buffer to greatly reduce non-specific amplification. At the same time, the special ROX Reference Dye makes the premixed solution suitable for all qPCR instruments. It is not necessary to adjust the ROX concentration on different instruments. It is only necessary to add primers and templates to prepare the reaction system for amplification. It is recommended to be used in conjunction with our miRNA 1st Strand cDNA Synthesis Kit (by stem-loop) (Vazyme #MR101).

Components

Components	MQ101-01 (125 rxn / 20 µl reaction)	MQ101-02 (500 rxn / 20 µl reaction)
2 × miRNA Universal SYBR qPCR Master Mix [a]	1.25 ml	4 × 1.25 ml
mQ Primer R (10 µM) [b]	70 µl	250 µl

a. Contain dNTP, Mg2+, AceTaq DNA Polymerase, SYBR Green I, Specific ROX Reference Dye and so on.

b. Sequence is AGTGCAGGGTCCGAGGTATT

Components

Applied Biosystems 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne™, StepOnePlus™, 7500, 7500 Fast, ViiA™7.

Bio-Rad CFX96™, CFX384™, iCycler iQ™, iQ™5, MyiQ™, MiniOpticon™, Opticon®, Opticon 2, Chromo4™.

Qiagen/Corbett Rotor-Gene® Q, Rotor-Gene® 3000, Rotor-Gene® 6000.

Stratagene MX4000™, MX3005P™, MX3000P™.

Eppendorf Mastercycler® ep realplex, realplex 2s.

Roche Applied Science LightCycler™ 480. and other qPCR instruments.

▲This product contains special ROX Reference Dye, applicable to all QPCR instruments, do not need to adjust the Rox concentration for different instruments.

Storage

Store at -20°C, and protected from light.

Protocol

1. Prepare a reaction solution in a qPCR tube as follows:

2 × miRNA Universal SYBR qPCR Master Mix	10.0 µl
Specific Primer (10 µM)	0.4 µl
mQ Primer R (10 µM)*	0.4 µl
Template DNA/cDNA	x µl
ddH_2O	To 20.0 µl

* mQ Primer R is matched with the reverse transcription primer designed by our miRNA design software. The stem loop sequence used is GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGAC. When different stem-loop sequences are used, the qPCR reverse primer should be self-designed and synthesized.

The amount of each component in the reaction solution can be adjusted according to the following principles:

▲ 0.2 µM of primer final concentration is applicable for most cases. The concentration can be adjusted within 0.1 - 1.0 µM when amplification efficiency is not satisfactory.

▲ The accuracy amount of template added has significant influence on the final quantitative results due to the extremely high sensitivity of qPCR reaction. It is recommended to dilute the template before use to improve the repeatability of experiment.

▲ If the template is an undiluted cDNA stock solution, the volume of template used should not exceed 1/10 of the total volume of the qPCR reaction.

2. Perform qPCR reaction at the following cycling conditions:

Stage 1	Pre-denaturation	Reps: 1	95℃	5 min
Stage 2	Cycling reaction	Reps: 40	95℃	10 sec
			60℃	30 sec
			95℃	15 sec
Stage 3	Melting curve	Reps: 1	60℃	60 sec
			95℃	15 sec

* For different instrument types, the melting curve acquisition program is not the same, use the instrument default melting curve acquisition program.

Quality Control

Purity Test: All of the components are tested to be free of exonuclease, exonuclease or RNase.

Function Test: Take RT product dilution of total RNA from HELA as template to amplify 6 genes, result in a single peak following melt curve analysis. With good specificity and the amplification curve between batches of the product is stable.

Reaction system optimization

An excellent reaction system should have the following characteristics: a single peak of the melting curve (amplification specificity), an amplification efficiency close to 100% (amplification efficiency), and a small CT value (amplification sensitivity).If the performance of the default reaction conditions is not good, it can be optimized according to the following scheme.

1. Relationship between primer concentration and reaction performance: When the final primer concentration is varied from 0.1 μM to 1.0 μM, the higher the primer concentration, the worse the amplification specificity, but the higher the amplification efficiency.
2. Relationship between amplification procedures and reaction performance:
   To increase the amplification specificity, increase the annealing temperature.

Two-step standard procedure	→	Increase the annealing temperature (3℃ per increase)
95℃/10 sec		95℃/10 sec
60℃/30 sec		63℃/30 sec

   To increase the amplification efficiency, extend the extension time of the two-step procedure or use the three-step procedure

Two-step standard procedure	→	Extended extension time	→	Three-step procedure	→	Extended extension time
95℃/10 sec		95℃/10 sec		95℃/10 sec		95℃/10 sec
60℃/30 sec		60℃/60 sec		56℃/30 sec		56℃/30 sec
				72℃/30 sec		72℃/60 sec

FAQs and Troubleshooting

◇ Abnormal shape of amplification plot

① Rough amplification plot: It is caused by system rectification due to weak signal. Elevate the template concentration and repeat in the reaction.

② Broken or downward amplification plot: The concentration of templates is too high. The end value of the baseline is bigger than Ct value. Decrease the end of the baseline (Ct value - 4), and re-analyze the data.

③ Amplification plot goes downward suddenly: There are bubbles left in the reaction tube, which break up when the temperature rises,thus the instrument detects the sudden decrease of the fluorescence value. Spin briefly and check closely if there are bubbles left before reaction.

◇ No amplification plot

① Cycling number is insufficient: Generally the cycling number is set to be 40. But notice that too many cycles will result in excessive background,thereby reducing the reliability of the data.

② Check if there is signal collection procedure during cycling: in two-step program, signal collection is usually positioned at annealing and extension stage; for three-step program, signal collection should be positioned at 72℃ extension stage.

③ Check if the primers are degraded: Test the integrity of primers after long-term storage through PAGE electrophoresis to confirm the presence of primers in solution.

④ The concentration of templates is too low: Reduce the dilution fold and retry. For target gene with unknown expression level, begin without template dilution.

⑤ Degradation of templates: Prepare new templates and retry.

◇ Ct value appears too late (high)

① Low amplification efficiency: Optimize the reaction. Try three-step program or re-design primers.

② The concentration of templates is too low: Reduce the dilution fold and retry. For target gene with unknown expression level, begin without template dilution.

③ Degradation of templates: Prepare new templates and retry.

④ There are PCR inhibitors in the reaction: They are usually brought in when adding templates. Increase the dilution folds or prepare new templates and retry.

◇ Apparent amplification can be observed in negative control

① The reagents or water used is contaminated: Change new reagents or water and retry. The reaction should be set up in a clean bench to minimize contamination from the air.

② Appearance of primer dimer: it is normal that amplification occurs in negative control after 35 cycles. Analysis can be performed combining with the melt curve.

◇ The linear relation of the standard curve is not satisfactory when performing absolute qualification

① Pipetting error: Dilute the templates to increase the pipetting volume.

② Degradation of standards: Prepare new standards and retry.

③ Too high template concentration: Increase the dilution fold.

◇ Multimodal dissociation curve

① The primers are not optimal: Design new primers according the design principles.

② Too high concentration of primers: Appropriately decrease the concentration of primers.

③ cDNA template is contaminated by genomic DNA: Prepare new cDNA templates.

◇ Experiment has low reproducibility

① Inaccurate pipetting volume: Use a more accurate pipettor, elevate the reaction volume, and dilute the templates to increase the pipetting volume.

② Difference in temperature control in different wells of qPCR instrument: Regularly maintain the instruments.

③ Too low template concentration: The lower the template concentration, the worse the repeatability. Decrease the dilution fold or increase the volume of template used.

ISO 9001: 2015