miRNA Universal SYBR qPCR Master Mix

MQ101

Version 22.1

Product Description

miRNA Universal SYBR qPCR Master Mix is a special premix for qPCR assays based on SYBR Green I chimeric fluorescence method. The quantification of miRNA requires high specificity because the miRNA sequences are short and miRNA sequences of the same family are generally highly similar. This product contains hot-start AceTaq DNA Polymerase based on the chemical modification. With the optimized Buffer, it can greatly reduce nonspecific amplification; meanwhile, the special ROX Reference Dye makes the master mix suitable for most qPCR instruments. It only needs to add primers and templates to perform amplification reaction, without adjusting the ROX concentration on different instruments. It is recommended to use with Vazyme's miRNA 1st Strand cDNA Synthesis Kit (by stem-loop) (Vazyme #MR101).

Components

Components	MQ101-01 125 rxns (20 μl/rxn)	MQ101-02 500 rxns (20 μl/rxn)
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. It contains dNTP, Mg^{2+}, AceTaq DNA Polymerase, SYBR Green I, Specific ROX Reference Dye, etc.
b. The sequence is AGTGCAGGGTCCGAGGTATT.

Storage

Store at -30 ~ -15℃ and protect from light. Transport at ≤0℃.

Applications

It is applicable for dye-based fluorescence quantitative PCR of miRNAs such as animals and plants.

Applicable Instruments

Applied Biosystems 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne, StepOnePlus, 7500, 7500 Fast, ViiA7.
Bio-Rad CFX96, CFX384, iCycler iQ, iQ5, 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 uses a special ROX Reference Dye that is suitable for most qPCR instruments (no ROX calibration instruments, low concentration ROX calibration instruments, high concentration ROX calibration instruments). The concentration of ROX does not need to be adjusted on different instruments.

Notes

For research use only. Not for use in diagnostic procedures.

Experiment Process

1. Prepare the following mixture in a qPCR tube

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 primers designed by Vazyme's miRNA Design software. The stem-loop sequence used is GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGAC; when using different stem-loop sequences, please design and synthesize qPCR reverse primers by yourself.

The volume of each component in the reaction system can be adjusted according to the following principles:

▲ Generally, a good result can be obtained when the final concentration of primer in the reaction system is 0.2 μM. If the result is not as expected, the primer concentration can be adjusted between 0.1 - 1.0 μM.

▲ Due to the high sensitivity of qPCR, the accuracy of template volume has a significant impact on qPCR results. In order to effectively improve the repeatability of the experiment, it is recommended to add the template to the reaction system after dilution.

▲ The volume of undiluted cDNA template should be ≤1/10 of total volume.

2. Run the qPCR program as follows:

Stage 1	Initial Denaturation	Rep: 1	95℃	5 min
Stage 2	Cycling Reaction	Reps: 40	95℃	10 sec
			60℃	30 sec
Stage 3	Melting Curve*	Rep: 1	95℃	15 sec
			60℃	60 sec
			95℃	15 sec

* The melting curve acquisition programs of different qPCR instrument types are not the same. Please select the default melting curve acquisition program of the instrument.

FAQ & Troubleshooting

◇Abnormal shape of amplification plot

①Rough amplification plot: The signal is too weak and generated after system correction. Increase template concentration and retry.

②Broken or downward amplification plot: The template concentration is too high and the baseline endpoint is greater than C_T value. Reduce the baseline endpoint (C_T value - 4) and repeat data analysis.

③Amplification plot goes downward suddenly: There are bubbles remaining in the reaction tube. Pay attention to centrifugation when processing samples and carefully check the reaction tube for any remaining bubbles before performing reaction.

◇No amplification plot

①Insufficient number of reaction cycles: In general, the number of cycles is set to 40, but it should be noted that too many cycles will increase too many background signals and reduce the reliability of the data.

②Confirm whether the signal acquisition step is set up in the program: The two-step amplification program generally sets the signal acquisition at the annealing and extension stage, while the three-step amplification program should set the signal acquisition at the 72℃ extension stage.

③Confirm whether the primers are degraded: Primers that have not been used for a long time should be tested for integrity using PAGE electrophoresis before use in order to rule out the possibility of degradation.

④Low template concentration: Reduce the dilution factor and repeat the test. In general, samples with unknown concentration should be started at the highest concentration.

⑤Template degradation: Prepare new template and retry.

◇C_T value appears too late

①Low amplification efficiency: Optimize the reaction system, try the three-step amplification program or redesign the synthetic primers.

②Low template concentration: Reduce the dilution factor and repeat the test. In general, samples with unknown concentration should be started at the highest concentration.

③Template degradation: Prepare new template and retry.

④PCR inhibitors in the system: They are usually introduced along with the template. Increase the dilution factor or prepare new template and retry.

◇Amplification observed in negative control

①Contaminated of reaction system: Replace with new mix, ddH_2O and primers to repeat the experiment. The reaction system should be prepared in clean bench to reduce aerosol contamination.

②Primer dimer: Carry out analysis in association with the melting curve.

◇Standard curve linearity is poor for absolute quantification

①Sample loading error: Increase the template dilution factor and the loading volume of sample.

②Standard product degradation: Prepare the standard again and repeat the test.

③Template concentration is too high: Increase the template dilution factor.

◇Multiple peaks in melting curve

①Inappropriate primer design: Design and synthesize new primers according to the primer design principles.

②High primer concentration: Decrease the primer concentration.

③cDNA template with contamination of genomic DNA: Prepare new cDNA templates.

◇Poor experiment repeatability

①Inaccurate pipetting volume: Use higher performance pipette; increase the template dilution factor, and increase the sample loading volume.

②Differences in temperature control between wells in qPCR instrument: Calibrate the instrument regularly.

③Low template concentration: The lower the template concentration, the worse the repeatability. Reduce the template dilution factor or increase the volume of sample addition.