Version No.: FAL1715

GenFQ SYBR qPCR Master Mix

GenFine Code: A104-01, A104-02

Storage condition: Store at -30 ~ -15℃ and protect from light, Transportation at ≤0℃, Valid period 2 years.

Description:

GenFQ SYBR Green qPCR Master Mix is prepared at 2× reaction concentration, which is specially designed for SYBR Green I based real-time PCR. The reaction can be started by adding only primers and template, which simplifies the operation. Antibody-modified hot-start Taq DNA Polymerase combined with the optimized buffer system, it can significantly improve the specificity and sensitivity of low copy genes. It also can get excellent amplification curve in a wide range. The product can accurately quantify and detect the target genes.

Components:

Components	A104-01	A104-02
2×SYBR Green qPCR Mix*	1.25 ml	1.25 ml×4
ROX Reference Dye 1**	50 µl	200 µl
ROX Reference Dye 2 **	50 µl	200 µl
ddH_2O	1 ml	1 ml×4

*.Including dNTPs, Mg^{2+}, HS-Taq DNA Polymers, SYBR Green I etc.

**. ROX used to rectify the error of fluorescence signals between different wells. Select the appropriate ROX reference dye according to the Real-time PCR instrument used:

1) Do not use ROX Reference Dye:

SLAN-96P/48P, Bio-Rad CFX96™, CFX384™, iCycler iQ™, iQ™5, MyiQ™, Mini Opticon™, Opticon® , Opticon 2, Chromo4™, Cepheid SmartCycler® , Eppendorf Master cycler ® ep realplex, realplex 2 s, Illumina Eco qPCR, Qiagen/Corbett Rotor-Gene ® Q, Rotor-Gene ® 3000, Rotor-Gene ® 6000, Roche Applied Science Light Cycler TM 480, Thermo Scientific PikoReal Cycle.

2) Use ROX Reference Dye 1:

Applied Biosystems 7500, 7500 Fast, Quant studio 5, ViiA™7, Stratagene MX4000™, MX3005P™, MX3000P™.

3) Use ROX Reference Dye 2:

Applied Biosystems 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, Step One™, Step OnePlus™.

Product features:

1. Suitable for Real Time PCR reaction, it can quickly and accurately detect and quantify the target genes.
2. When the PCR reaction solution is prepared, only templates, primers and sterilized water need to be added for RT-PCR reaction. The operation is simple and convenient.
3. Taq DNA polymerase modified with anti Taq antibody can be used for hot start PCR reaction, with optimized buffer system can achieve high amplification efficiency and high amplification sensitivity.

Applications:

Widely used for amplification and quantification of DNA samples. The sample types can be genomic DNA, cDNA, plasmid DNA, λ DNA, etc.

Quality control:

Different genes were amplified using Hela cell cDNA. Only single peak was observed from melting curve. The amplification curve was similar between different batches.

Protocol: (using ABI 7500)

1. Prepare the reaction solution as follows:

Components	Volume (20 μl)
Template DNA/cDNA	X μl
2 × SYBR Green qPCR Master Mix	10 μl
Forward Primer (10 μM)	0.4 μl
Reverse Primer (10 μM)	0.4 μl
50×ROX Reference Dye 1	0.4 μl
ddH_2O	Up to 20 μl

For each component, the volume of can be adjusted according to the following principle:

1） The final concentration of primer is usually 0.2 μM, it can be adjusted between 0.1μM and 1μM.

2） qPCR is highly sensitive, the accuracy of template volume impacts significantly on the final quantitative results. It is recommended that the template should be diluted and added to the reaction system, which can effectively improve the repeatability of the experiment.

3） For undiluted cDNA, the volume of template should be ≤ 1/10 of total volume.

2. Thermal cycling conditions:

Stage 1	Reps: 1	95 °C	3 min
Stage 2	Reps: 40	95 °C	10 sec
		60 °C*	30 sec
Stage 3**	Reps: 1	95 °C	15 sec
		60 °C	
		95°C **	15sec

*. The fluorescence signal was collected when at 60℃.

**. Use the instrument default dissolution curve acquisition program, the fluorescence signal was collected when at 95℃ for dissolution curve acquisition program.

Trouble shooting:

1. Abnormal shape of amplification curve

a. Amplification curve is not smooth: Signal is too weak to be generated after systematic correction，increase the concentration of template and repeat the experiment.

b. Amplification curve was broken or slipped: the template concentration was too high, and the endpoint value of baseline was greater than CT value. Reduce the baseline endpoint (CT value - 4) and reanalyze the data.

c. Individual amplification curve suddenly dropped: there were bubbles in the reaction tube. Centrifugation should be paid attention to when processing samples, and whether there are bubbles in the reaction tube should be carefully checked before amplification reaction.

2. No amplification curve

a. The number of reaction cycles is not enough: generally, the number of cycles is 40, but it should be noted that

too many cycles will increase too many background signals and reduce the credibility of the data.
b. Confirm whether the signal acquisition step is correct: the two-step expansion program generally sets the signal acquisition in the annealing extension stage; Three step amplification program should set the signal acquisition at 72 ° C extension stage.
c. To confirm whether the primer is degraded or not: the integrity of the primer should be detected by page electrophoresis to exclude the possibility of degradation if it has not been used for a long time
d. Template concentration is too low: reduce the dilution and repeat the experiment. Generally, the sample with unknown concentration starts from the highest concentration.
e. Template degradation: the template was prepared again and the experiment was repeated.

3. CT value appears too late

a. The amplification efficiency is too low: optimize the reaction conditions, try three-step amplification procedure, or redesign the synthetic primers.
b. Template concentration is too low: reduce the dilution and repeat the experiment. Generally, the sample with unknown concentration starts from the highest concentration.
c. Template degradation: the template was prepared again and the experiment was repeated.
d. The PCR product is too long: the recommended length is 80 bp-150 bp
e. There are PCR inhibitors in the system: generally, the template is brought in, the dilution ratio of the template is increased or the template is prepared again to repeat the experiment.

4. Amplification curve was observed in negative control

a. Reaction system pollution: replace mix, water and primer, repeat the experiment. The reaction system is prepared in the ultra clean worktable to reduce the aerosol gel pollution.
b. The appearance of primer dimer was analyzed with melting curve.

5. The linear relationship of standard curve is not good in absolute quantification

a. Sample addition error: increase the dilution ratio of the template, increase the sample volume
b. Degradation of standard: prepare the standard again and repeat the experiment.
c. Concentration of the template is too high, increase the dilution ratio of the template.

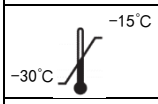
6. More than one peaks in the melting curve

a. Poor primer design: new primers were designed and synthesized according to the design principle
b. Too high primer concentration: reduce the primer concentration appropriately.
c. cDNA template with genomic contamination: re preparation of cDNA template.
d. Poor primer design: new primers were designed and synthesized according to the design principle

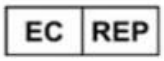
7.Poorly repeatability

b. Sample volume inaccuracy: the pipette with better performance was used; The template was highly diluted and added to the reaction system in large volume.
c. The temperature of different positions is inconsistent: calibrate the instrument regularly.
d. Too low template concentration: the lower the template concentration is, the worse the repeatability is. Reduce the template dilution or increase the sample volume.

[Symbols]

Symbols	Meanings
	Manufacturer
	Authorized representative in the European Community
	*In vitro* diagnostic medical device
	This product fulfills the requirements of the European Directive 98/79 EC for in vitro diagnostic medical devices.
	Catalogue number
	Batch code
	Date of manufacture
	Use-by date
	Temperature limit
	Consult instructions for use
	Keep dry
	Keep away from sunlight
	Do not re-use
	Do not use if package is damaged

GENFINE BIOTECH (CHANGZHOU) CO., LTD.
4th Floor, Building E4, No.9, Changyang Road, West Taihu Technology Industrial Park, Changzhou City, Jiangsu Province
PEOPLE'S REPUBLIC OF CHINA
Tel: +86 051983761557
E-mail: marketing@genfine.com
Web: en.genfine.com



Lotus NL B.V.
Koningin Julianaplein 10, 1e Verd, 2595AA, The Hague, Netherlands.
E-mail：peter@lotusnl.com
Tel：+31644168999