Genious 2X SYBR Green Fast qPCR Mix (High ROX Premixed)

Cat. No. : RK21207

Product components

Components	Component number	Size-1	Size-2	Size-3
Genious 2X SYBR Green Fast qPCR Mix (High ROX Premixed) *	RM21206	1 mL	5 × 1 mL	25 × 1 mL

*** Including ABclonal Genious Hot Start Taq DNA polymerase, Mg^{2+}, dNTPs, SYBR® Green I, etc.**

Product Description

Real-time Quantitative PCR (qPCR) is a powerful technique in detecting initial DNA input in a PCR reaction by fluorescence signal accumulation. The DNA double strand bonded dye, SYBR® Green I, is the most commonly used dye in qPCR. ABclonal Genious 2X SYBR Green Fast qPCR Mix (High ROX Premixed) is an optimized SYBR Green qPCR reaction mix which can used in machines with High ROX mode, It contains all required components in qPCR except primers and template. It is convenient for experiment and suitable for multiple species. The above features make it as an ideal experiment tool for gene quantitative research.

Instruments

ROX Reference Dye	qPCR Instruments
High ROX	ABI 7000 / 7300 / 7700/7900, StepOne / StepOnePlus, etc.

Storage

This product should be stored at -20℃ for long-term storage and should be protected from light.

Materials Required

1. PCR tubes and other related materials.
2. qPCR specific primers and DNA templates.
3. qPCR 96-well plate and sealing membrane (adhesive film).

Notes

1. Genious 2X SYBR Green Fast qPCR Mix (High ROX Premixed) must be completely thawed before use. Protect from direct light exposure and store in darkness.
2. Genious 2X SYBR Green Fast qPCR Mix (High ROX Premixed) contains glycerol. Gently mix before use to avoid bubble formation; vortex and centrifuge before use. Immediately return to -20℃ storage after use.
3. This product contains DNA polymerase. Maintain on ice throughout use. For short-term repeated usage within a day, temporary storage at 4℃ is permitted. Minimize freeze-thaw cycles.
4. This product is an ROX premixed product, and no additional reference dye needs to be added during use.

Operation Description

Experimental Preparation

1. It is recommended to choose the amplification product length within the range of 70-200 bp.
2. It is recommended to take a reaction volume of 20 μL, add 1 pg-50 ng of DNA as a template, and set NTC (No Template Control).
3. To ensure experimental accuracy, perform triplicate technical replicates for all samples and controls.

E-mail:info@abclonal.com

Experimental Procedure

1. It is recommended to prepare a reaction system mix on ice and quickly transfer the mix to a qPCR instrument.

Components	Volume
Genious 2X SYBR Green Fast qPCR Mix (High ROX Premixed)	10 μL
gDNA or cDNA *	2 μL
Forward Primer (10 μM) **	0.4 μL
Reverse Primer (10 μM) **	0.4 μL
ddH_2O	To 20 μL

*** Using 10 pg-10 ng genomic DNA or 10 pg-100 ng cDNA as the template reference quantity, gradient dilution can be performed on the template to determine the optimal template usage due to the different copy numbers of the target genes contained in the templates of different species. In addition, when using cDNA (RT reaction solution) from the two-step RT qPCR reaction as a template, the addition amount should not exceed 10% of the qPCR reaction system.**

**** Typically, the final concentration of the primer is 0.2 μM, and good results can be obtained , and the final concentration of 0.1-1.0 μM can be used as a reference for setting the range. In the case of low amplification efficiency, the concentration of primers can be increased; When non-specific reactions occur, the concentration of primers can be reduced and the reaction system can be optimized.**

2. Program qPCR reaction as follows:

Step	Temp	Time	Cycles
Pre-denaturation	95 ℃	3 min	1
Cylcling	95 ℃	5 s	40-45
	60 ℃	30-34 s *	
Melting Curve	Instrument default		

*** Confirm there is a signal collection step after each extending step. The extending time is varied according to different machines: 30 s for StepOne Plus, 31 s for 7300 and 34 s for 7500. If not otherwise specified, default to 30 s.**

Data Analysis

1. Plot the standard curve based on Ct values and sample input quantity. The correlation coefficient (R^2) of the standard curve should be >0.98, with a slope between -3 and -3.5. The PCR amplification efficiency (E) generally falls between 90-120%.
2. The standard deviation (STD) of Ct values between replicate wells should be <0.2. The STD of Ct values for the same experiment across different batches should be <0.5 (when comparing the same experiment across batches, ensure the threshold setting is essentially consistent).
3. The melting curve of the amplification product shows no significant non-specific amplification products (non-specific peaks) or primer-dimer peaks (confirm by agarose gel electrophoresis if necessary). Furthermore, the Tm value of the melting curve is typically between 80-95°C.
4. Validation of Valid Ct: A valid amplification Ct value must be less than the Ct value of the no-template control curve, while its melting curve must show no non-specific peaks.

Troubleshooting

Melt Curve Show Multiple Peaks

a. Primer Design: Design the primer following basic primer design protocols.

b. Primer Concentration Too High: lower down the concentration of primers.

Unusual Amplification Curves

a. Amplification Curve Not Smooth: Too low amplification signal, increase the template input and make sure the qPCR Mix is stored properly.

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b. Inconsistent Amplification Curve: Bubbles causes abnormal qPCR results, centrifuge the plate prior to running it.

c. Abnormal Amplification Curves: the default baseline value of machine is set to be from 3 to 15, the baseline setting can be changed according actual amplification conditions. Besides, the degradation of template may affect the curve.

No Amplification Curves after Reaction

a. Not Enough PCR Cycles: the PCR cycle number is usually set to be 40. It should be noted a higher cycle number may increase the background signal.

b. Primer Degradation: Use electrophoresis to confirm the integrity of primers.

c. Confirm the Signal Collection Step: the signal collection step are usually set to be after the annealing-extending step for two-step qPCR and after extending step for Three-step qCPR.

d. Template Input Too Low: Increase template concentration or add extra repetition.

e. Template Degradation: Use freshly prepared template (Use electrophoresis to confirm integrity of template).

f. Not Enough Initial Denaturation Time: Genious 2X SYBR Green Fast qPCR Mix (High ROX Premixed) uses Hot-Start Taq polymerase, the pre-denaturation time should be at least 3 min.

Ct Value Too Late

a. Low Amplification Efficiency: Optimize reaction condition or change primer.

b. Template Input Too Low: Increase template concentration or add extra repeat.

c. Template Degradation: Use freshly prepared template (Use electrophoresis to confirm Integrity of template).

d. Too Long PCR Products: The length of amplification products is usually in the range of 70 bp-200 bp.

e. PCR Inhabitation Reagent: use new template or dilute the template.

f. Too Short Pre-denaturation Time: Genious 2X SYBR Green Fast qPCR Mix (High ROX Premixed) contains Hot-Start Taq polymerase, the pre-denaturation time should be at least 3 min.

NTC Shows Amplification

a. Contamination: Use sterile water to conduct experiment and the all operation is suggested to be done in clean room to avoid aerosol contamination.

b. Non-Specific PCR Products: analyze with melt curve.

Inconsistent Results

a. Inconsistent Sample Added: Use proper pipetting techniques

b. Inconsistent Temperature in qPCR Machine: ensure periodic machine calibration.

c. Template Concentration Too Low: the lower template input, the poorer qPCR result is. Increase the template concentration.

d. Inconsistent Threshold Set: when comparing the qPCR results in different plates, make sure the threshold value of each experiments is same.

Related Products

Name	Catolog	Size
ABScript Neo RT Master Mix for qPCR	RK20432	20 RXN / 100 RXN
ABScript Neo RT Master Mix for qPCR with gDNA Remover	RK20433	20 RXN / 100 RXN
ABScript II cDNA First-Strand Synthesis Kit	RK20400	20 RXN / 100 RXN

E-mail:info@abclonal.com