BioArk® 2×SYBR Green qPCR Master Mix (None ROX)

Cat. #：BSY3320NR

Product Information

Product Name	Cat. No.	Spec.
2×SYBR Green qPCR Master Mix (None ROX)	BSY3320NR-01	1 mL
	BSY3320NR-05	5×1 mL
	BSY3320NR-15	15×1 mL

Product Description/Introduction

2×SYBR Green qPCR Master Mix (None ROX) is a special 2× premix for qPCR reaction using SYBR Green I chimeric fluorescence method, which contains all qPCR components except primers and DNA templates, which can reduce the operation steps, shorten the time of adding samples, and reduce the chance of contamination. The core component is genetically engineered hot-start Taq DNA Polymerase, which effectively seals off DNA polymerase activity and prevents non-specific amplification at low temperatures by efficiently combining monoclonal antibody and Taq DNA Polymerase, with many advantages such as high specificity and high sensitivity, and is coupled with a reaction buffer optimized for qPCR. It is very suitable for high specificity and high sensitivity qPCR reaction. This product is a 2× premixed reagent containing the optimal concentration of SYBR Green I for qPCR reaction, which can obtain a good standard curve in a wide quantification area, accurate quantification of target genes, good reproducibility and high confidence.

Storage and Shipping Conditions

Ship with wet ice. Store at -20°C without light, valid for 12 months. Avoid freeze-thaw cycles. After thawing, it can be stably stored at 4℃ for one month without light.

Product Contents

Component	BSY3320NR-01	BSY3320NR-05	BSY3320NR-15
2×SYBR Green qPCR Master Mix (None ROX)	1 mL	5×1 mL	15×1 mL
Manual	One copy		

Assay Protocol / Procedures

Before starting

1. Real Time PCR amplification apparatus;
2. Special reaction tube or reaction plate for experiment;
3. PCR primers (reference primer design principles);
4. Micropipette and autoclaving-tips;

Procedures

1. Recommend the qPCR reaction system:

Component	20 μL rxn	50 μL rxn	Final Concentration
2×SYBR Green qPCR Master Mix (None ROX)	10 μL	25 μL	1×
Forward Primer (10 μM)[a]	0.4 μL	1 μL	0.2 μM
Reverse Primer (10 μM)[a]	0.4 μL	1 μL	0.2 μM
Template[b]	Variable	Variable	as required
Nuclease-Free Water	Add to 20 μL	Add to 50 μL	

a. Usually, a good amplification effect can be obtained with the final concentration of 0.2 μM. When the reaction

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performance is poor, the primer concentration can be adjusted in the range of 0.2-1.0 μM.

b. The amount of template added varies depending on the number of copies of the target gene, and the appropriate amount of template addition is studied by gradient dilution. The best addition amount of template DNA in the 20 μL reaction system was less than 100 ng. When the cDNA (RT reaction solution) of RT-PCR reaction was used as template, the addition amount should not exceed 10% of the final qPCR volume.

2. PCR reaction program (can be adjusted appropriately according to the instrument)

A. Two-step process *					B. Three-step process*				
Stage	Step	Cycles	Temperature	Time	Stage	Step	Cycles	Temperature	Time
Stage 1	Predegeneration	1	95℃	30 sec	Stage 1	Predegeneration	1	95℃	30 sec
Stage 2	Degeneration	40	95℃	15 sec	Stage 2	Degeneration	40	95℃	15 sec
	Annealing-Extension		60℃	30 sec[a]		Annealing		55-65℃	10 sec
						Extension		72℃	30 sec[a]
Stage 3	Melting curve	1	Instrument default Settings		Stage 3	Melting curve	1	Instrument default Settings	

***: If amplification specificity needs to be improved, two-step procedure or annealing temperature can be used; To improve the amplification efficiency, a three-step procedure or extension time can be used.**

a: For fluorescence signal collection, please set the experimental procedure according to the instruction manual of the instrument.

Note

1. Mixed gently upside down before use. Do not swirl and shake to avoid bubbles. Reagents are mixed well before use.
2. Reagents should be placed on ice when preparing reaction solution.
3. The product contains fluorescent dye SYBR Green, so strong light should be avoided when preparing PCR reaction solution.
4. Please using new disposable tips for the preparation and packaging of the reaction solution to avoid contamination between samples.
5. Avoid repeated freeze-thawing of Master Mix and try to use it within one month after thawing.

Compatible instruments

Brand of PCR machine	BSY3320NR (None ROX)	BSY3320LR (Low ROX)	BSY3320HR (High ROX)
ABI Thermo life	PikoRealTM Cycler	7500/7500 Fast, ViiA 7™ QuantStudio™ series	5700/7000/7300/7700/7900/ 7900HT/7900 HT Fast, StepOne™, StepOne Plus™
Stratagene		Mx3000P®/3005P™ /4000™	
Bio-Rad	All series		
Eppendorf	Realplex 2s, Mastercycler®ep realplex		
Illumina	Eco QPCR		
Cepheid	SmartCycler®		
Qiagen Corbett	Rotor-Gene® series		
Roche	LightCycler™ series		
Takara	Thermal Cycler Dice series		
Analytikjena	qTOWER series		
qTOWER	LineGene series		

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Primer design principles

1. The length of amplification product is recommended to be between 80-300 bp;
2. Primer length: 18-25 bp;
3. The content of base G+C in primers should be between 40%-60%;
4. The Tm value difference between forward primers and reverse primers is less than 2℃, and the Tm value between 58-62℃ is the best;
5. Randomness of base distribution;
6. Primers had better not contain self-complementary sequences, otherwise they will form a secondary hairpin structure;
7. There should be no more than 4 complementary or homologous bases between two primers, otherwise primer dimer will be formed, especially complementary overlap at the 3' end;
8. The 3' terminal base of the primer is suggested to be G or C;
9. No other non-specific products were found in NCBI comparison results.

Trouble-shooting

Problems	Possible cases	Solutions
At the end of the reaction, no amplification curve appeared or CT value appeared too late	Template concentration too low	Reduce the number of template dilutions to repeat the experiment, starting with the highest concentration if the sample concentration is unknown.
	Degradation of primers	Reprepare the template and repeat the experiment.
	PCR inhibitors present in the reaction mixture.	Generally template carryover, increase template dilution or re-prepare template with high purity to repeat the experiment.
	Possible degradation of primers	Primers that have not been used for a long period of time should first be tested for integrity by PAGE electrophoresis to rule out their degradation.
	Low amplification efficiency	Increase the primer concentration, try a three-step amplification program, or redesign the primers.
	Amplification products too long	Amplification product length was controlled at 80-300 bp.
Amplification signal in non-template control	Reaction system contamination	First change the water of the blank control, if the same thing still happens, continue to change the primers, tips, PCR tubes or enable a new Master Mix; The reaction system is prepared in an ultra-clean bench to minimize aerosol contamination.

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	Non-specific amplification such as primer dimers	Generally, it is normal for amplification products to appear in the blank control after 35 cycles, which should be analyzed together with the melting curve; redesign the primers, adjust the primer concentration or optimize the PCR reaction procedure.
The melting curve has multiple peaks	Primer design is suboptimal	New primers were redesigned according to primer design principles.
	Primer concentration is too high	Reduce primer concentration appropriately
	Genomic contamination in cDNA template	The extracted RNA solution is digested using DNA enzymes, such as dsDNase, to remove genomic contamination, or to design transintron primers
Poor reproducibility of experiments	The error of adding sample is large	The use of accurate pipette, with high quality suction head accurate pipette; High dilution template, adding large volume template to reduce sampling error; The reaction volume of qPCR was enlarged
	The template concentration is too low	Reduce the template dilution times to repeat the experiment.
	Temperature deviation at different locations of the qPCR instrument	Calibrate the qPCR instrument regularly
The amplification curve is not smooth	Fluorescence signal is too weak, produced after system correction	Ensure that the dyes premixed in the Master Mix are not degraded; Replace fluorescent signal to collect better qPCR consumables
Amplification curve breaks or slips	The template concentration was higher and the baseline endpoint value was greater than the CT value	The baseline endpoint (Ct value -3) was reduced and the data were reanalyzed
Amplification curves of individual Wells suddenly dropped sharply	There are bubbles in the reaction tube	Ensure that MIX is completely dissolved, and do not swirl and oscillate evenly; After the sample is added, the bubbles are removed by centrifugation with light elastic. The pre-denaturation time was extended to 10 min to remove the bubbles

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