BioArk® 2×SYBR Green qPCR Master Mix (High ROX)

Cat. #：BSY3320HR

Product Information

Product Name	Cat. No.	Spec.
2×SYBR Green qPCR Master Mix (High ROX)	BSY3320HR-01	1 mL
	BSY3320HR-05	5×1 mL
	BSY3320HR-15	15×1 mL

Product Description/Introduction

2×SYBR Green qPCR Master Mix (High ROX) is a ready-to-use solution optimized for quantitative real-time PCR and two-step real-time RT-PCR. The master mix includes Hot Start Taq DNA polymerase and dNTPs in an optimized PCR buffer to ensures PCR specificity and sensitivity. The SYBR Green I intercalating dye allows for DNA detection and analysis without using sequence-specific probes. Only template and primers need to be added. The use of 2×SYBR Green qPCR Master Mix (High ROX) in real time PCR ensures reproducible, sensitive and specific quantification of genomic, plasmid, viral and cDNA templates.

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	BSY3320HR-01	BSY3320HR-05	BSY3320HR-15
2×SYBR Green qPCR Master Mix (Low ROX)	1 mL	5×1 mL	15×1 mL
Manual	One copy		

Assay Protocol / Procedures

Before starting

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

Procedures

1. Recommended PCR reaction systems

Component	20 μL rxn	50 μL rxn	Final Concentration
2×SYBR Green qPCR Master Mix (Low 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 μ M. When the reaction 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 μ L reaction system was less than 100 ng. When the cDNA (RT reaction solution) of RT-PCR reaction was used as template, the

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addition amount should not exceed 10% of the final qPCR volume.

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

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 seca		Annealing		55-65℃	10 sec
						extension		72℃	30 seca
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. Mix gently upside down before use. Do not swirl and shake to avoid bubbles. Mix the reagents well before using.
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	PikoReal™ 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		

Primer design principles

1. The length of amplification product is recommended to be between 80-300 bp;

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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

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Problem description	Possible reasons	Solutions
At the end of the reaction, no amplification curve appeared or CT value appeared too late	The template concentration is too low	Repeat the experiment to reduce the template dilution multiple, and start from the highest concentration when the sample concentration is unknown
	Template degradation	The template was prepared again and the experiment was repeated
	There are PCR inhibitors in the system	Generally, the template is carried in, the dilution ratio of the template is increased or the template with high purity is reprepared and repeated
	Primers may degrade	Primers that have not been used for a long time should first be tested for integrity by PAGE electrophoresis to rule out the possibility of degradation
	Low amplification efficiency	Increase the primer concentration, try a three-step amplification procedure, or redesign the primer
	The amplification product is too long	The amplification product length was controlled in the range of 80-300 bp
The blank control shows the signal	Reaction system pollution	Firstly, the blank control water should be replaced. If the same situation still occurs, the primers, aspirators and PCR tubes should be replaced or a new Master Mix should be started. The reaction system is prepared in a super clean table to reduce aerosol pollution

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	Non-specific amplification such as primer dimers appears	Generally, it is normal for the amplification products to appear in blank control after 35 cycles, which should be analyzed with the melting curve. Redesign primer, adjust primer concentration or optimize PCR reaction procedure
The melting curve has multiple peaks	Primer design is poor	The new primer was re-designed according to the primer design principles
	Primer concentration is too high	Reduce primer concentration appropriately
	There is 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	Repeat the experiment to reduce the dilution times of the template
	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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