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2×SYBR Green PCR Mix

Composition

2×SYBR Green PCR Mix	1 ml	5 ml
Cat. No.	7106100	7106500
2×SYBR Green PCR Mix[1]	1 ml	1 ml×5
50×ROX Reference Dye[2]	40 μl	200 μl
ddH_2O	1 ml	1 ml×5
Instructions	1	1

1. Contains hot-start Taq DNA Polymerase, dNTP Mix, Mg2+, SYBR Green I., and other PCR enhancers.
2. For correct the fluorescence signal error generated between the wells. Apply Applied Biosystems 5700,7000,7300,7700,7900,7900 HT, 7900HT Fast, StepOne™, StepOnePlus™ and other fluorescent quantitative PCR instruments need to add a high concentration of ROX Reference Dye, the addition of 50× ROX Reference Dye is 1/50 of the PCR reaction system; Applied Biosystems 750, 7500 Fast, ViiA™7, Stratagene MX4000™, MX3005P™, MX3000P™ and other fluorescent quantitative PCR instruments with low concentration ROX Reference Dye need to be added, the addition of 50×ROX Reference Dye was 1/250 of the PCR reaction system. The following fluorescent quantitative PCR instruments do not need to add ROX Reference Dye: Bio-Rad CFX96™, CFX384™, iCycler iQ™, iQ™5, MyiQ™, MiniOpticon®™, Opticon, Opticon 2,Chromo4™, Cepheid SmartCycler®, Eppendorf Mastercycler®ep realplex, realplex 2, Illumina Eco qPCR, Qiagen/Corbett Rotor-Gene®Q, Rotor- Gene®3000, Rotor-Gene®6000, Roche Applied Science LightCyclerTM480, Thermo Scientific PikoReal Cycler, et al.

Storage

Store at -20°C in the dark, valid for 2 years.

Introduction

This product is a special reagent for Real Time PCR using the SYBR Green I chimeric fluorescence method. It is mainly used for the detection of genomic DNA target sequences or cDNA target sequences after RNA reverse transcription. 2×SYBR Green PCR Mix is a 2× concentration master mix designed for use with dye-based (SYBR Green I) real-time PCR. For experiments, the preparation of PCR solution is very convenient and simple, just take 0.5 times the volume of the PCR system and add 2× SYBR Green PCR Mix, add primers and template, and make up the volume with ddH_2O.

This product is suitable for fast Real Time PCR amplification reactions, and can obtain a good standard curve in a wide quantitative region, and quickly and accurately detect and quantify target genesStrong specificity, good reproducibility, and high confidence. The included fluorescent dye SYBR Green I binds to all double-stranded DNA, allowing the product to be used for the detection of different target sequences without the need to synthesize specific probes. Hot-start Taq DNA Polymerase is a highly efficient hot-start enzyme with no polymerase activity at room temperature, effectively avoiding non-specific amplification by non-specific binding of primers and templates or primer dimers at room temperature. The unique combination of PCR buffer system and hot-start enzymes, as well as the PCR enhancer and protein stabilizer contained in the product, can effectively inhibit non-specific PCR amplification, greatly improving the specificity of PCR, and allowing for high-precision real-time PCR amplification reactions. The ROX dye contained corrects the fluorescence signal error generated between the wells of the quantitative thermal cycler and is suitable for fluorescence quantitative thermal cyclers that use ROX as the correction dye.

Equipment and Reagents to Be Supplied by User

1. PCR primers
2. DNA or cDNA template.
3. Single-tube, 8-tube strip, or 96-well PCR tube (plate) suitable for real-time PCR.
4. Micropipettes and clean tips.
5. Real-time PCR instrument (authorized instrument).

Precautions

1. Gently mix upside down before use, avoid foaming and spin down.
   (1) Do not vortex to mix well.
   (2) 2× SYBR Green PCR Mix may produce white or yellowish precipitates when stored at -20°C, so it can be dissolved slowly by holding it in your hand, placed at room temperature for a short time in the dark, and gently inverted upside down to mix until the precipitate disappears..
   (3) Precipitation can lead to uneven solution composition, so be sure to mix the reagent well before use.
2. This product contains SYBR GreenI. fluorescent dye and ROX dye, and should be protected from strong light when storing this product or preparing PCR reactions.
3. Avoid repeated freeze-thaw cycles of this product, as repeated freeze-thaw may degrade the performance of the product.
4. This product cannot be used for probe-based PCR.
5. When preparing reactions, use clean tips (filtered tips are recommended) and centrifuge tubes to minimize contamination.
6. When preparing the reaction, place the reagent on ice.

Protocol

1. Prepare the PCR reaction solution according to the following components (please prepare the reaction solution on ice)

Reagent	Usage	Usage	Final concentration
2×SYBR Green PCR Mix	10.0 μl	25.0 μl	1×
PCR Forward Primer(10 μM)	0.4 μl	1.0 μl	0.2 μM*1
PCR Reverse Primer(10 μM)	0.4 μl	1.0 μl	0.2 μM*1
50× ROX Reference Dye*2	0.4 μl	1.0 μl	1×
DNA template	2.0 μl*3	5.0 μl*3	
ddH_2O	7.2 μl	18.0 μl	
Total	20.0 μl*4	50.0 μl*4	

*1 In general, a final primer concentration of 0.2 μM is a good result. When the reaction performance is poor, the primer concentration can be adjusted in the range of 0.1~1.0 μM.

*2 50× ROX Reference Dye is 1/50 of the PCR system when using a PCR instrument that requires a high concentration of ROX Reference Dye, the addition of 50 × ROX Reference Dye is 1/250 of the PCR reaction system when using a low-concentration ROX requirement PCR instrument. 50× ROX Reference Dye is not required when using a PCR instrument that does not require the addition of ROX Reference Dye. (Please consult the instrument manufacturer for the use of the instrument).

*3 In a 20 μl reaction, the amount of DNA template added is typically less than 100 ng. Because different types of DNA templates contain different copy numbers of target genes, serial dilutions can be performed if necessary to determine the optimal amount of DNA template to add. If you want to use this product for the second step PCR amplification reaction of the two-step RT-PCR reaction, do not add more than 10% of the total volume of the PCR reaction when the RT reaction is used as a DNA template.

*4 The reaction solution is prepared according to the recommended system of each instrument.

2. Perform a Real-Time PCR reaction

It is recommended to use the two-step PCR procedure in the table below, and if the procedure does not yield good experimental results, then optimize the PCR conditions. If the amplification performance of the two-step PCR is poor due to the use of primers with low Tm values, a three-step PCR amplification can be tried. The three-step operation steps are detailed in "Optimization of Reaction Conditions".

Steps	Number of cycles	Temperature (℃)	Reaction time (min:sec)
1: Initial denaturation	1	95	00:30
2: PCR reaction	40	95	00:10
		60	00:30 (fluorescence acquisition).
3: Melting curve	1	95	00:15
		60	01:00
		95	00:15 (fluorescence acquisition)
		60	00:15

Notes:1. The annealing temperature should be 60-64℃ as a reference for the set range, and the annealing temperature can be increased in the event of a non-specific reaction.

2. This program is set with the ABI 7500 fluorochronocycler as a reference, and the solution curve analysis should be set according to the recommended program of the fluorochronocycler used.

3. Analysis of experimental results

After the reaction, the amplification curve and melting curve of Real Time PCR are confirmed, and a standard curve is created for PCR quantification. The analytical method is referred to the operator's manual of the instrument.

Optimization of reaction conditions

When optimizing the reaction conditions for fluorescence quantification, primer concentration, annealing temperature, and extension time should be considered to improve the reaction specificity and amplification efficiency.

1. The experimental system with high reaction specificity and amplification efficiency should have the following conditions:

(1) High reaction specificity: The negative control does not produce non-specific amplification such as primer-dimers and does not produce amplification other than the target fragment.

(2) High amplification efficiency: the amplification product peaks earlier (Ct value is small). PCR amplification efficiency is high (close to the theoretical value of 100%).

2. Reaction condition optimization method:

(1) Primer concentration: Usually 0.2 μM primer concentration can give good results, and the final concentration of 0.1-1.0 μM can be used as a reference for the set range. To increase the specificity of the reaction, you can decrease the primer concentration, and to increase the amplification efficiency, you can increase the primer concentration.

(2) Annealing temperature: To increase the specificity of the reaction, the annealing temperature can be increased to 60-64℃ as a reference for the set range. If you do not get good results due to the low Tm value of the primers used, you can try to perform a three-step PCR amplification, and the annealing temperature of the three-step method should be set in the range of 55℃-64℃.

(3) Extension time: To increase amplification efficiency, try increasing the extension time to 1 minute, or try three-step PCR.

(4) Initial denaturation: Initial denaturation conditions are usually set at 95℃ at 30 sec, and this condition is generally good for denaturing of refractory circular plasmid DNA and genomic DNA templates. If you want to change the denaturation conditions for the refractory template, you can extend it to 1~2 minutes, but if the time is too long, the enzyme is easy to inactivate, so it is not recommended to use the denaturation conditions for more than 2 minutes.