FastSYBR Mixture

Project number: F665747

Storage condition: -20℃, if need to use frequently, can be stored in 2-8℃, try to avoid repeated freezing and thawing.

Product content

Component	F665747-5ml
2×FastSYBR Mixture	5 x 1ml
50 x High ROX	200 μl
ddH2O	5 x 1ml

Product Introduction

FastSYBR Mixture is a premixed system for dye-based (SYBR Green I) real-time fluorescent quantitative PCR, with a concentration of 2×, containing Fast Taq DNA Polymerase, PCR Buffer, dNTPs, SYBR Green I fluorescent dye and Mg^{2+}, which is easy to operate. It is mainly used for the detection of genomic DNA target sequence and cDNA target sequence after RNA reverse transcription. The fluorescent dye SYBR Green I contained in this product can bind to all double-stranded DNAs, enabling the product to be used for the detection of different target sequences without the need to synthesize specific labeling probes. The Fast Taq DNA Polymerase can effectively reduce the non-specific amplification caused by the non-specific binding of primers and templates or primer dimerization at room temperature, and the activation of the enzyme only requires incubation at 95℃ for 20 s. The whole PCR reaction can save about 40 minutes compared with the normal reaction, which greatly shortens the reaction time of PCR. The unique combination of PCR buffer system and hot-start enzyme effectively inhibits the generation of non-specific products and significantly improves the amplification efficiency of PCR. The product has a wide range of applications and is suitable for both normal and rapid quantitative PCR programs.

ROX dye is used to correct the fluorescence signal error generated between wells of a quantitative PCR instrument, and is generally used in Real Time PCR amplifiers from ABI, Stratagene, and other companies. The excitation optics vary from instrument to instrument, so the concentration of ROX dye must be matched to the corresponding fluorescence quantitative PCR instrument.

Instruments that do not require ROX calibration:

Roche LightCycler 480, Roche LightCyler 96, Bio-rad iCyler iQ, iQ5, CFX96 and others.

Instruments requiring Low ROX calibration:

ABI Prism7500/7500 Fast, QuantStudio®3 System, QuantStudio®5 System, QuantStudio®6 Flex System, QuantStudio®7 Flex System, ViiA 7 system. Stratagene Mx3000/Mx3005P, Corbett Rotor Gene 3000, and more.

Instruments requiring High ROX calibration:

ABI Prism 7000/7300/7700/7900, Eppendorf, ABI Step One/Step One Plus, and others.

matters needing attention

1. Before use, please mix gently by turning up and down, avoid foaming as much as possible, and use after brief centrifugation.
2. This product contains the fluorescent dye SYBR Green I. Avoid strong light when storing this product or preparing PCR reaction solution.

3. Avoid repeated freezing and thawing of this product, repeated freezing and thawing may degrade the product performance. This product can be stored for long term at -20℃, protected from light. If frequent use is required within a short period of time, it can be stored at 2-8℃.
4. This product cannot be used for fluorescent quantitative PCR by the probe method.

Usage

The following examples are conventional PCR reaction systems and reaction conditions, which should be improved and optimized according to the template, primer structure and target fragment size in actual operation.

1.PCR reaction system

reagents	50μl reaction system	final concentration
2×FastSYBR Mixture	25 μl	1×
Forward Primer, 10μM	1μl	0.2μM1)
Reverse Primer, 10μM	1μl	0.2μM1)
Template DNA	2μl2)	
50 x Low ROX or High ROX (optional)3)	1μl	1×
ddH2O	up to 50μl	

Note: 1) Usually, better results can be obtained with a primer concentration of 0.2 μM, and 0.1-1.0 μM can be used as a reference for setting the range. If the amplification efficiency is not high, the concentration of the primer can be increased; when a non-specific reaction occurs, the concentration of the primer can be decreased, thus optimizing the reaction system.
2) Usually the amount of DNA template is 10-100 ng genomic DNA or 1-10 ng cDNA as a reference. Since the number of copies of the target gene contained in the template varies from species to species, the template can be diluted in a gradient to determine the optimal amount of template to be used.
3) The excitation optical system varies from instrument to instrument, so choose whether to add 50×Low ROX or 50×High ROX depending on the instrument used for fluorescence quantification.

2.PCR reaction conditions

Steps	Temperature	Time	
Pre denaturation	95° C	20s1)	
denaturation	95° C	3s	35-40 cycles
Annealing/Extension2)	60° C	30s	
Melting curve analysis3)			
	95° C	15s	
	60° C	1min	
	95° C	15s	
	60° C	15s	

Note: 1) The enzyme used in this product must be pre-denatured at 95° C, 20s to achieve enzyme activation. Under this condition, most of the templates can be well unchained. For templates with high GC content and complex secondary structure, the pre-denaturation time can be extended to 1 minute in order to make the starting template fully unchained. If the high temperature treatment time is too long, it will affect the enzyme activity. The optimal pre-denaturation time can be determined according to the template.
(2) It is recommended to use two-step PCR reaction program, the annealing temperature should be 60-64 ℃ as the reference of the setting range, and the

annealing temperature can be increased when a non-specific reaction occurs. If you can't get good results due to the use of primers with low Tm value, you can try three-step PCR amplification, and the annealing temperature should be set in the range of 56℃-64℃ as a reference.

(3) For melting curve analysis, please set up the program recommended for the fluorescence quantitative PCR instrument used, and this program is set up using the ABI 7500 fluorescence quantitative PCR instrument as a reference.