FastSYBR Mixture

Item No. F665884 (5 mL)
Storage condition: -20℃, if need to use frequently, can be stored in 2-8℃, try to avoid repeated freezing and thawing.

Product content

individual parts making up a compound	F665884 5 ml
2×FastSYBR Mixture	5 x 1 ml
50 x Low ROX	/
50 x High ROX	/
ddH20	5 x 1 ml

Product Introduction

FastSYBR Mixture is a premixed system for dye-based (SYBR Green I) real-time fluorescence quantitative PCR. It contains Fast Taq DNA Polymerase, PCR Buffer, dNTPs, SYBR Green I fluorescent dye, and Mg2+ at a concentration of 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 can be combined with all double-stranded DNAs, allowing 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 needs to be incubated 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 is suitable for a wide range of applications and is suitable for both general and rapid quantitative PCR programs. ROX dye is used to correct the fluorescence signal error generated between the wells of quantitative PCR instruments and is generally used in Real Time PCR amplifiers from ABI, Stratagene, etc. The excitation optical

system varies from instrument to instrument. The excitation optics vary from instrument to instrument, so the concentration of ROX dye must be matched to the corresponding quantitative fluorescence PCR instrument.
Instruments that do not require ROX correction: Roche LightCycler 480, Roche LightCyler 96, Bio-rad iCyler iQ, iQ5, CFX96, etc.
Instruments requiring Low ROX correction: 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, etc. System, Stratagene Mx3000/Mx3005P, Corbett Rotor Gene 3000, and others.
Instruments requiring High ROX calibration: ABI Prism7000/7300/7700/7900, Eppendorf, ABI Step One/Step One Plus, etc.

Caveat

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

Usage

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

1. PCR reaction system

reagents	50 μL reaction system	final concentration
2×FastSYBR Mixture	25 μL	1 ×
Forward Primer, 10 μM	1 μL	0.2 μM [1)]
Reverse Primer, 10 μM	1 μL	0.2 μM [1)]
Template DNA	2 μl [2)]	/

50×Low ROX or High ROX (optional)[3)]	1 μL	1 ×
ddH2O	up to 50 μl	/

Attention:

(1) Usually, a primer concentration of 0.2 μM can give better results, 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 primer can be increased; if non-specific reaction occurs, the concentration of primer can be decreased, thus optimizing the reaction system.

(2) Usually, the amount of DNA template is 10-100 ng of genomic DNA or 1-10 ng of cDNA as a reference. Since the templates of different species contain different copy numbers of target genes, the templates can be diluted in a gradient to determine the optimal amount of template to be used.

(3) The excitation optical systems of different instruments are different, choose to add 50×Low ROX or 50×High ROX according to the instrument using fluorescence quantification.

2. **PCR reaction conditions**

move	temp	timing	/
premutability	95° C	20 s [1)]	/
denaturation	95° C	3 s	35-40 cycles
Annealing/Extension[2)]	60° C	30 s	35-40 cycles
Melting curve analysis[3)]	/	/	/
/	95° C	15 s	/
/	60° C	1 min	/
/	95° C	15 s	/
/	60° C	15 s	/

Attention:

(1) The enzyme used in this product should be activated under the condition of pre-denaturation at 95℃ for 20s. 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 allow the starting template to fully unravel the chain. If the high temperature treatment time is too long, the enzyme activity will be affected. 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 set at 60-64℃ as a reference range, and the annealing temperature can be increased when non-specific reaction occurs. If you can not 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 by the fluorescence quantitative PCR instrument you are using, and this program is set up with the ABI 7500 fluorescence quantitative PCR instrument as a reference.