MagicSYBR Mixture

Catalog Number:

M665651 (1 mL)

M665651 (5 mL)

M665651 (40 mL)

Storage conditions: -20 ℃ to avoid light, such as the need for frequent use, can be stored in 2-8 ℃, try to avoid repeated freezing and thawing.

Products content

Component	1 mL	5 mL	40 mL
2× MagicSYBR Mixture	1 mL	5 x 1 mL	40 x 1 mL
ROX Reference Dye I	40 μL	200 μL	1 mL
ddH2O	1 mL	5 x 1 mL	40 x 1 mL

Products Introduction

MagicSYBR Mixture is a pre-mixed system for dye-based (SYBR Green I) real-time fluorescence quantitative PCR. The concentration is 2×, which contains 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.

It contains the fluorescent dye SYBR Green I that binds to all double-stranded DNAs, allowing 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 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 30 s, which greatly reduces the PCR reaction time. 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 with stronger fluorescence signal and higher sensitivity.

caveat

1. Before use, 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 solutions.

3. Avoid repeated freezing and thawing of the product, which may degrade its

performance. This product can be stored at -20℃ for a long period of time, protected from light. If frequent use is required within a short period of time, store at 2-8° C.

4. This product cannot be used in the probe method of fluorescence quantitative PCR.

5. For more information about ROX Reference Dye I, please contact your local sales representative or call CombiSense customer service.

4006-222-360.

Usage

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

1. PCR reaction system

reagents	50 μL system	25 μL system	20 μL system	final concentration
2×MagicSYBR Mixture	25 μL	12.5 μL	10 μL	1×
Forward Primer, 10 μM	1 μL	0.5 μL	0.4 μL	0.2 μM 1)
Reverse Primer, 10 μM	1 μL	0.5 μL	0.4 μL	0.2 μM 1)
Template DNA2)	X μL	X μL	X μL	
Template DNA2)	-	-	-	
ddH2O	up to 50 μL	up to 25 μL	up to 20 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 copy numbers of target genes contained in the templates of different species are different, the templates can be subjected to gradient dilution to determine the optimal amount of template to be used.

(3) ROX dye is used to correct the fluorescence signal error generated between the wells of the quantitative PCR instrument, and it is generally used in Real

Time PCR amplifiers of ABI, Stratagene, etc. The excitation optics of different instruments are different, so the amount of ROX dye used must be matched with the corresponding fluorescence quantitative PCR instrument. The excitation optics vary from instrument to instrument, so the amount of ROX dye used must be matched to the corresponding quantitative fluorescence PCR instrument. The optimal amount of ROX Reference Dye I for several common instruments is shown in the table below:

Instrument Type	ROX usage
Roche instruments, Bio-rad instruments,	No ROX dye calibration
ABI Prism7500/7500 Fast, QuantStudio® Series, Stratagene Mx3000/Mx3005P, Corbett Rotor Gene 3000, etc.	0.5 μL /50 μL system, 0.25 μL/25 μL system, the 0.2 μL/20 μL system
ABI Prism 7000/7300/7700/7900. ABI Step One/Step One Plus, etc.	5 μL /50 μL system, 2.5 μL/25 μL system, the 2 μL/20 μL system

2.PCR reaction conditions

move	temp	timing	
premutability	95° C	30 s	
denaturation	95° C	5 s	40-45 cycles
Annealing/Extension	60° C	30 s	40-45 cycles
Melting curve analysis	95° C	15 s	
	60° C	1 min	
	95° C	15 s	
	50° C	30 s	

Attention:

(1) The enzyme used in this product must be activated under the condition of pre-denaturation at 95℃ for 30 s. Most of the templates can be deconvolved well under this condition. Under this condition, most of the templates can be well unchained. For the templates with high GC content and complex secondary structure, the appropriate pre-denaturation time can be extended to 1-10min, so that the starting template can be fully unchained, and the optimal pre-denaturation time can be determined according to the template condition.

(2) It is recommended to use two-step PCR program, the annealing temperature should be 60-64℃ as the 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 values, 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 using the Roche 480 fluorescence quantitative PCR instrument as a reference.

4) Most of the templates can get good amplification curves in 40 cycles, which can be increased to 45 cycles for low-copy templates. Can be customized according to

The optimal number of cycles was determined experimentally to get a better amplification profile.