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Master SYBR Mixture

Product Number: PCM08

Shipping and Storage

-20°C; if used frequently, store at 2-8°C to avoid repeated freezing and thawing.

Components

Component	PCM08 1ml	PCM08 1ml	PCM08 1ml
2× Master SYBR Mixture	1 ml	5×1 ml	40×1 ml
ROX Reference Dye I	40μl	200μl	1 ml
ddH_2O	1 ml	5×1 ml	40×1 ml

Description

The Master SYBR Mixture is a premixed system for real-time fluorescence quantitative PCR (SYBR Green I), and the concentration is 2×. It contains Fast Taq DNA Polymerase, PCR Buffer, dNTPs, SYBR Green I Fluorescent Dye, and Mg^{2+}. The operation is simple and convenient. This product is mainly used for the detection of genomic DNA target sequences and cDNA target sequences after RNA reverse transcription.

This product contains the fluorescent dye SYBR Green I which can bind with all double-strand DNA, so that the product can be used for the detection of different target sequences without the need for the synthesis of specific labeled probes. The Fast Taq DNA Polymerase in the mixture that does not have polymerase activity at room temperature which prevents non-specific amplification efficiently, and it is activated by incubation at 95°C only for 30 seconds which greatly reduces the reaction time. The combination of a unique PCR buffer system and a hot-start enzyme effectively inhibits non-specific PCR amplification and significantly increases the amplification efficiency of PCR.

Notes

1. Mix gently before use, avoid foaming, and use after brief centrifugation.
2. This product contains SYBR Green I fluorescent dye. Avoid strong light irradiation when storing this product or preparing PCR reaction solution.
3. Avoid frequently freezing and thawing of this product. It may comprise product performance.
4. This product cannot be used for probe based qPCR .
5. When preparing the reaction solution, use new or non-contaminated tips and centrifuge tubes to prevent contamination.

Protocol

The following protocol is an example of conventional PCR reaction system and condition. The actual protocol should be improved and optimized based on the template, primer structure and the size of the target.

1. PCR reaction system

Reagent	50μL	25μL	20μL	Final Conc.
2×Master SYBR Mixture	25μl	12.5μl	10μl	1×
Forward Primer, 10 μM	1μl	0.5μl	0.4μl	0.2μM [1)]
Reverse Primer, 10 μM	1μl	0.5μl	0.4μl	0.2μM [1)]
Template DNA [2)]	Xμl	Xμl	Xμl	
ROX Reference Dye I [3)]	—	—	—	
ddH_2O	up to 50μl	up to 25μl	up to 20μl	

Note:1) Usually 0.2μM of primer concentration gives better results, and the final concentration of primers should be between 0.1 and 1.0μM. When the amplification efficiency is low, increases the concentration of primers ; when non-specific reaction occurs, the concentration of primers can be reduced, and optimize reaction system.

2) Usually the amount of DNA template is 10-100ng for genomic DNA or 1-10ng for cDNA. Template can be gradient diluted to optimize.

3) ROX dye is used for well-to-well fluorescence normalization. It is generally used in Real Time PCR amplifier of ABI, Stratagene and other companies. Different instruments have different excitation optical systems, so the concentration of ROX dye must be matched with the corresponding fluorescence qPCR machine. Optimum ROX Reference Dye concentrations for several common instruments are shown in the table below.

Instrument Type	ROX used
Roche，Bio-rad etc.	Correction without ROX dye
ABI Prism7500/7500 Fast，QuantStudio® series，Stratagene Mx3000/Mx3005P，Corbett Rotor Gene 3000 etc.	0.5μl /50μl system 0.25μl/25μl system 0.2μl/20μl system
ABI Prism7000/7300/7700/7900，ABI Step One/Step One Plus etc.	5μl /50μl system 2.5μl/25μl system 2μl/20μl system

2. PCR reaction condition

Step	Temperature	Time	
Pre denaturation	95℃	30 s	
Denaturation	95℃	5 s	40-45cycles
Annealing/Extension	60℃	30 s	
Fusion curve analysis			
	95℃	15 s	
	60℃	1 min	
	95℃	15 s	
	50℃	30 s	

Note:1)The enzyme used in this product must be activated under pre denaturation conditions of 95℃ in 30 seconds.Under this condition, most templates can perform well in de chaining. For templates with high GC content and complex secondary structures, the appropriate pre denaturation time can be extended to 1-10 minutes to fully unwind the initial template. The optimal pre denaturation time can be determined based on the template situation.

2)It is recommended to use a two-step PCR reaction program, and the annealing temperature should be set at 60-64 ℃ as a reference range. When non-specific reactions occur, the annealing temperature can be increased. If good experimental results cannot be obtained due to the use of primers with lower Tm values, a three-step PCR amplification method can be attempted. The annealing temperature should be set within the range of 56℃-64℃ as a reference.

3)Please set the fusion curve analysis using the recommended program for the fluorescence quantitative PCR instrument used. This program is based on the Roche 480 fluorescence quantitative PCR instrument as a reference setting.

4)Most templates can achieve a good amplification curve in 40 cycles, while for low copy templates, it can be increased to within 45 cycles. The optimal number of cycles can be determined based on experiments to obtain a better amplification curve.