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Ver.08/2019

Magic SYBR Mixture

Catalog Number: CW3008S (1 ml)
CW3008M (5 ml)
CW3008H (40 ml)

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

Kit Components

Component	CW3008S (1 ml)	CW3008M (5 ml)	CW3008H (40 ml)
2×MagicSYBR Mixture	1 ml	5×1 ml	40×1 ml
ROX Reference Dye	40 μl	200 μl	1 ml
ddH_2O	1 ml	5×1 ml	40 ml

Product Introduction

The MagicSYBR 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.

Note

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.
6. If you need more ROX Reference Dye I, please call us 617-800-3785

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	50 μl	25 μl	20 μl	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 DNA[2)]	X μl	X μl	X μl	
50×ROX Reference Dye[3)]	—	—	—	
ddH_2O	up to 50 μl	up to 25 μl	up to 20 μl	

Note:

1) Usually 0.2 μM of primer concentration gives better results, and the final concentration of primers should be between 0.1 and 1.0 μM. 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 10-100 ng for genomic DNA or 1-10 ng 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.

Type of instrument	Final ROX Concentration(Volume)
Roche,Bio-rad	No need for ROX dye normalization
Series of ABI Prism7500/7500 Fast，QuantStudio®, ratagene Mx3000/Mx3005P，Corbett Rotor Gene 3000 etc.	0.5 μl /50 μl，0.25 μl/25 μl，0.2 μl/20 μl
ABI Prism7000/7300/7700/7900，ABI Step One/Step One Plus etc.	5 μl /50 μl，2.5 μl/25 μl，2 μl/20 μl

1. PCR reaction program:

Component	Temperature	Time	
Pre-denaturation	95°C	30 s	
Denaturation	95°C	5 s	40-45 Cycles
Annealing/Extension	60°C	30 s	
Melting curve analysis	95°C	15 s	
	60°C	1 min	
	95°C	15 s	
	50°C	30 s	

Note:

1) The enzymes used in this kit must be activated under the conditions of pre-denaturation at 95°C and 30 seconds. Under this conditions, most of DNA templates are well unwound. For high GC content and complex secondary structure templates, the pre-denaturation time can be extended to 1 minute to fully unwind process. However, the enzyme activity will be affected with high temperature treatment for a long time. The optimum pre-denaturation time can be determined according to the template condition.

2) It is suggested that a two-step PCR procedure should be adopted. The annealing temperature should be set at 60°C~64°C as a reference range . If a non-specific reaction occurs, the annealing temperature can be increased. If the experimental results are not good, caused by lower Tm value primers, three-step PCR amplification might be considered. The annealing temperature should be set as 56°C ~64°C as a reference range.

3) The melting curve analysis should be set according to the procedure recommended by the qPCR instrument. This procedure is based on Roche 480 fluorescence quantitative PCR.

4) Most of the templates can get a good amplification curve in 40 cycles, for the low copy template, it can be increased to 45 cycles.