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Version 2.0

SYBR Green荧光定量PCR试剂盒 Super Hot-Start SYBR Green qPCR Master Mix

Cat.No. MRT4460V **Size :** 1mL (100 rxns) ☐ 5mL (500 rxns) ☐ 25mL (2500 rxns) ☐

Technical literature is available at: www.mesgenbio.com. E-mail MesGen Technical Services if you have questions on use of this system: tech@mesgenbio.com

产品描述

The Super Hot-Start SYBR Green qPCR Master Mix is a ready-to-use qPCR cocktail. It contains a novel Taq DNA Polymerase, unique hot start reagents, optimized buffer, SYBR Green I, dNTPs, PCR Enhancer and PCR stabilizer. qPCR Master Mix is provided at 2× concentration and can be used at 1× concentration by adding template, primer, passive reference dye (optional) and ddH_2O. qPCR Master Mix can be directly used for robust and low-template quantitative PCR with high sensitivity, specificity and reliability. Two separate tubes of ROX Reference Dye are included for use with instruments that require a high or low level of reference dye for rectify the error of fluorescence signals between different wells.

实验方法

1. Prepare PCR reaction mixture

To obtain reliable quantitative PCR reaction results, it is recommended to run three replicates for each sample. The suggested template amount is 10 ng to 100 ng for genomic DNA or 1 ng to 10 ng for cDNA template. **Please prepare the PCR reaction solution according to the list below (All reagents should be placed on ice)**

Reagent	10 μL	20 μL	50 μL	Final con.
SYBR Master mix (2×)	5 μL	10 μL	25 μL	1×
PCR Forward Primer (10 μM)	0.2 μL	0.4 μL	1 μL	0.2 μM
PCR Reverse Primer (10 μM)	0.2 μL	0.4 μL	1 μL	0.2 μM
Reference Dye (optional)	0.2 μL	0.4 μL	1 μL	1×
DNA	1 μL	2 μL	4 μL	
ddH_2O	3.4 μL	6.8 μL	18 μL	
Total	10 μL	20 μL	50 μL	

Notes:

a. 200 nM of primer final concentration is applicable for most cases. The concentration can be adjusted within 0.1~1.0 μM when amplification efficiency is not satisfactory.

b. Too much or too little template used may lead to inaccuracy of quantitative result. A range of 1-100 ng is recommended to result in a good Ct value (15<Ct<35). If template is stocked at high concentrations, dilute it prior to loading to prevent possible loading errors.

c. It is recommended that the amplicon length should be within the range of 100-500 bp, with 100-200 bp preferred.

d. For consistency within an experimental set, prepare a sufficient volume of reaction mix without template DNA for the DNA standard reactions and experimental sample reactions.

2. Perform quantitative PCR

Perform quantitative PCR using optimized cycling conditions. Provided below is a standard two-step program and three-step program.

Two-step PCR Program

Step	1	2		3		
	Hot-Start DNA Polymerase Activation	PCR		Melt Curve		
	HOLD	40 cycles		1 cycle		
		Denature	Anneal/Extend			
Temp.	95.0°C	95.0°C	60.0°C	95.0°C	60.0°C	95.0°C
Time	5-10 mins	15 secs	30-60 secs	15 secs	60 secs	15 secs
Volume	10 μL-50 μL			10 μL-50 μL		

Three-step PCR Program

Step	1	2			3		
	Hot-Start DNA Polymerase Activation	PCR			Melt Curve		
	HOLD	40 cycles			1 cycle		
		Denature	Anneal	Extend			
Temp.	95.0°C	95.0°C	50.0°C-60.0°C	72.0°C	95.0°C	60.0°C	95.0°C
Time	5-10 mins	15 secs	30 secs	30 secs	15 secs	60 secs	15 secs
Volume	10 μL-50 μL				10 μL-50 μL		

Notes:

a. Please note that the hot-start polymerase in this system needs to be activated at 95°C for 5 minutes prior to amplification.

b. If the amplicon sequence is GC-rich, the time for pre-denaturation/enzyme activation can be prolonged to 10 minutes.

c. Extension time may be adjusted according to the qPCR instruments used. For example, the extension time should be set to no less than 30 seconds when using ABI 7700 and 7900HT, 31 seconds when using ABI 7000 and 7300, 34 seconds when using ABI 7500.

3. Attention Points in Operation (Please Read Carefully)

a. Avoid repetitive freeze-thaw cycles to prevent polymerase activity from decreasing. Aliquot the mix into small batches for frequent usage.

b. Gently invert the tube upside down several times before use. DO NOT vortex. Brief centrifugation prior to use is recommended.

c. Keep the mix from bright light during storage and usage due to the fact that the fluorescent SYBR Green I dye may fade under light over time, resulting in a decrease in performance sensitivity.

d. Due to the high sensitivity nature of the qPCR reaction, contamination of air or aerosols may lead to reaction failure or result inaccuracy. Please set up the qPCR reaction in a clean environment using filtered tips, and sterilized tubes and pipette sets.

产品组成

Components	1 mL (100 rxns)	5 mL (500 rxns)	25 mL (2500 rxns)
SYBR Green qPCR Master Mix (2×)	1 mL	1 mL × 5	1 mL × 25
ROX Reference Dye I (High)	40 µL	200 µL	1000 µL
ROX Reference Dye II (Low)	40 µL	200 µL	1000 µL
Contains hot-start DNA Polymerase, dNTPs, Mg2+, and SYBR Green I dye			

应用&特点

1. Dye-based quantitative PCR detection
2. Nucleic acid amplification and expression profiling
3. Quantitative genotyping studies
4. Genomics-related applications

储存条件

Stored at -20°C protecting from light, and is stable for up to 18 months. Avoid repetitive freeze-thaw cycles while using. For immediate use, components may be stored at 2-8°C

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