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SYBR Green qPCR Master Mix

1 Components

Components	HY-K0501-100 rxns	HY-K0501-500 rxns
SYBR Green qPCR Master Mix (2×)	1 mL	1 mL × 5
ROX Reference Dye (50×)	40 μL	200 μL
ROX Reference Dye II (50×)	40 μL	200 μL

2 Introduction

The Super Hot-Start SYBR Green qPCR Master Mix utilizes a special performance-enhanced Taq DNA polymerase protected via a hot-start activation technique, and optimized qPCR buffer system to perform SYBR Green I based quantitative PCR (qPCR). MCE qPCR Master Mix is provided as a simple-to-use, stabilized 2× formulation that includes all components for qPCR except sample DNA, primers and water. Hot Start Polymerase, $MgCl_2$, dNTPs and a proprietary reaction buffer, produce optimal results in qPCR experiments.

MCE 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 rectification the error of fluorescence signals between different wells.

Select appropriate ROX according to the Real-Time PCR instrument used

DO NOT USE ROX Reference Dye	Bio-Rad: CFX384, CFX96, MiniOpticon, iCycler IQ, MyiQ and iQ5; Eppendorf: MasterCycler RealPlex and RealPlex2; Qiagen/Corbett Rotor-Gene: 6000; Roche: LightCycler 480; Cepheid: SmartCycler; Illumina: Eco qPCR
ROX Reference Dye	ABI GeneAmp 5700; ABI PRISM 7000, 7700; ABI 7300, 7900HT (Fast); ABI StepOne (Plus)
ROX Reference Dye II	ABI: 7500 (Fast), ViiA 7, QuantStudio 6 and 7 Flex Systems; Stratagene: Mx3000P, Mx3005P and Mx4000; Qiagen/Corbett Rotor-Gene: 3000; Bio-Rad/MJ: Chromo4, Opticon 2 and Opticon

3 Protocol

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	

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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	50.0-60.0°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.

4 Storage Conditions

-20°C 18 months

Protecting from light. Avoid repetitive freeze-thaw cycles while using. For immediate use, components may be stored at 2-8°C.

5 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.

6 Data Analysis

a. Relative Quantitation

Target gene expression is measured against an internal standard.

Set CtA1 as the Ct value of the target gene of sample 1, and CtB1 as the Ct value of the internal control gene of sample 1; set CtA2 as the Ct value of the target gene of sample 2, and CtB2 as the Ct value of the internal control gene of sample 2. The expression difference (in folder) of the target gene in sample 1 and in sample 2 can be calculated this way

(2-ΔΔCt approach) : ΔΔCt=(CtA2-CtB2)-(CtA1-CtB1)

The expression level of the target gene in sample 2 is $2^{-\Delta\Delta Ct}$ times that of sample 1.

Notes: This calculation method is based on the assumption that the amplification efficiency is 100% (the amount of products after each cycle is doubled). If the amplification efficiency is not 100%, the calculation formula needs to be amended according to actual reaction efficiency.

b. Absolute Quantitation:

Compare the Ct of an unknown sample against a standard curve with known copy numbers.

Absolute quantitation is applicable only if isolation procedure and sample contents do not effect PCR amplification. The quantitation of genomic DNA may lend itself for absolute quantitation against a standard curve.

c. Passive Reference ROX

Passive reference ROX is a dye molecule included in the SYBR Green qPCR Master Mix that does not participate in the PCR amplification. On applied Biosystems real-time PCR system, the passive reference provides an internal reference to which the SYBR Green/dsDNA complex signal can be normalized during data analysis. Normalization is necessary to correct for well-to-well fluorescent fluctuation.