Product Information

2× SYBR Green qPCR Master Mix

Catalog Number: S2014

Product Size: 100T, 500T

Contents:

Components	100T	500T
A. 2×SYBR Green Master Mix	1 mL	5×1 mL
B. 10 × ROX reference dye	0.5 mL	1 mL

The product contains two components. Component A contains SYBR Green dye, dNTP, PCR buffer (including Tris and $MgCl_2$) and hot-start Taq DNA polymerase. Component B is 10× ROX reference dye, which may be required on certain ABI instruments (See protocol below).

Storage

Upon arrival, the SYBR Green qPCR Master Mix should be stored at -25°C to -15°C and protected from light. After each experiment, the leftover mix (completely thawed and thoroughly homogenized) can be stored at 4°C if it is to be used within the next 3 months. Avoid repeated freeze-thaw cycles to retain maximum performance. The SYBR Green qPCR Master Mix is stable for 2 years from the date of shipping when stored and handled properly.

Description

The 2×SYBR Green qPCR Master Mix is a ready-to-use cocktail containing all components (except primers and template) for the amplification and detection of DNA in qPCR. The regent is supplied as a 2× master mix with Taq DNA polymerase, dNTPs, $MgCl_2$, fluorescent dye (detection), and proprietary buffer components. Furthermore, US Everbrigtht's proprietary chemical modification of the DNA polymerase included in this Master Mix allows for Hot Start PCR, conferring a significant reduction in non-specific PCR amplification that is otherwise of a common occurrence with regular Taq polymerases.

Protocols

1. Thaw the 2×SYBR Green qPCR Master Mix without ice. Then the master Mix was mixed with Primer, template, RNase-free water, vortexed gently and mix well.

2. Prepare the reaction mixture as follows:

Components	20 μL Reaction	Final Concentration
2×SYBR Green Master Mix	10 μL	1×
F, R primers	2 μL	0.1-0.5 μM
Template DNA	Variable	See Note ①
10× ROX	Variable	See Table 1
H_2O	Add to 20 μL	

Note:

① Template concentration: The amount of DNA template added is usually below 100 ng. Because the copy number of the target gene contained in different kinds of DNA templates is different, gradient dilution may be performed if necessary to determine the appropriate amount of DNA template addition. To ensure optimal amplification efficiency, the cDNA sample to be added should not exceed 10% of the total volume of the PCR reaction.

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3. Mix the reaction mixture gently by vortexing and transfer the fixed volume to the PCR tube.

4. You may choose one of the following three protocols, depending on the nature of your amplification and the function of the instrument.

A. Two-step fast amplification

This procedure is suitable for amplification of most primers with a Tm of 60 °C. The melting curve is obtained in accordance with the standard process provided by the instrument you are using.

Cycling Step	Temperature	Holding Time	Number of Cycles
Enzyme Activation	95 ℃	2 min	1
Denaturation	95 ℃	5 s	45
Annealing & Extension	60 ℃	30 s	

B. Three-step fast amplification

This procedure is suitable for experiments where the amplification temperature is higher than the annealing temperature. For example, if the amplified fragment has a relatively long primer, then it is easy to produce non-specific amplification. Carrying out the extension step at a higher temperature can reduce nonspecific amplification. The melting curve is obtained in accordance with the standard process provided by the instrument you are using.

Cycling Step	Temperature	Holding Time	Number of Cycles
Enzyme Activation	95 ℃	2 min	1
Denaturation	95 ℃	5 s	45
Annealing	50-60 ℃	5 s	
Extension	72 ℃	25 s	

C. Universal procedure

This procedure is suitable for almost all qPCR instruments, as well as for amplifications that do not achieve good results under fast cycling conditions.

Cycling Step	Temperature	Holding Time	Number of Cycles
Enzyme Activation	95 ℃	5 min	1
Denaturation	95 ℃	15 s	45
Annealing & Extension	60 ℃	60 s	

5. Perform qPCR reactions.

6. Analyze experimental data.

Notes

1. ROX reference dye： For certain instruments, ROX is necessary for obtaining accurate Ct value. The concentration of ROX can be referred to the following table 1. ROX will cause some background interference to the melting curve analysis. Therefore, in order to avoid ROX peak interference, do not select the ROX fluorescence value option in the “Passive Reference Dye” of the application software, and then collect and analyze the data.

2. Annealing temperature: The annealing temperature should be set at your primer’s Tm value, and it is usually 50-60 ℃ for optimal result. However, primer’s Tm value (and thus extension temperature) should be designed as closer as possible to 60 ℃ (but still within 50-60℃ range) to reduce the gap between annealing and denaturation temperatures. This requires less time for temperature increase and thus higher amplification efficiency.

3. For Real Time RT-PCR reactions, the reagents used for the reverse transcription reaction are recommended: UEIris II RT-PCR System for First-Strand cDNA Synthesis (no DNase I) (Catalog No. R2027), UEIris II RT-PCR System for First-Strand cDNA Synthesis (with DNase I) (Catalog No. R2028).

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Table 1. Recommended ROX Concentration for PCR Instruments

PCR Instrument	Recommended ROX concentration	Amount of 10× ROX per 20 μL Reaction
BioRad: iCycler, MyiQ, MiQ 2, iQ 5, CFX-96, CFX-384, MJ Opticon, Option2, Chromo4, MiniOpticon Qiagen: Roto-Gene Q, Roto-Gene3000, Roto-Gene 6000 Eppendorf: Mastercycler realplex Illumina: Eco RealTime PCR System Cepheid: SmartCyler Roche: LIghtCycler 480, LightCycler 2.0	No ROX	None
ABI: 7500, 7500 Fast, ViiA 7 Stratagene: MX4000P, MX3000P, MX3005P	Low ROX (0.05-0.1× final)	Dilute 10× ROX at 1:10 with ddH_2O 1× ROX: 1~2 uL
ABI: 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne, StepOne plus	High ROX (1× final)	2 uL

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