Product Information

Universal SYBR Green qPCR Supermix

Catalog Number: S2024

Product Size: 100T, 500T

Contents:

Component	100T	500T
2× Universal SYBR Green qPCR Supermix	1 mL	5×1 mL

The supermix contains SYBR Green dye, dNTP, PCR buffer, hot-start Taq polymerase and Rox reference.

Storage

Store at -20°C and protect from light. When stored as directed, product is stable for at least 12 months. It can be stored at 4 ℃ for 6 months after opening.

Description

SYBR Green is a dye with a green excitation wavelength that binds to all dsDNA double helix minor groove regions. After SYBR Green binds to dsDNA, the fluorescence signal would be enhanced 800-1000 times. It is a commonly used qPCR fluorescent dye. With high sensitivity and high signal-to-noise ratio, it can be applied to gene expression difference analysis, gene chip and so on.

S2024 Universal SYBR Green qPCR Supermix is a star product for qPCR gene expression analysis. This product has greatly improved the stability, specificity, the amplification ability of high GC content sequence and anti-inhibition ability of the product. The key is to optimize the use steps of the product.

This supermix contains a special ROX reference dye, which is suitable for all qPCR instruments. There is no need to adjust the ROX concentration on different instruments, and only primers and templates are added to the reaction system for amplification.

Protocol

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

2. Prepare the reaction mixture as follows:

Components	20 μL Reaction system	Final Concentration
2× Universal SYBR Green qPCR Supermix	10 μL	1×
F, R primers	Suitable	0.4 μM
Template DNA	Suitable	See Note 1
H_2O	Add to 20 μL	

Note：

①Template 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.

②The final concentration of PCR primers is usually 0.4 μM, which gives better results. If the reactivity of the system is poor the primer concentration can be adjusted within 0.2 ~ 1 μM.

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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 two 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 ℃	5 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 ℃	5 min	1
Denaturation	95 ℃	5-10 s	40
Annealing	55-65 ℃	15 s	
Extension	72 ℃	25-30 s	

Note: The extension time should be adjusted according to the minimum time limit of data collection required by the real-time quantitative PCR instrument.

Notes

1. 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 and speed up PCR amplification.

2. Annealing time: The annealing time should be set for 5~20 seconds. Longer annealing time results in increased efficiency, and a shorter time decreases non-specifiv amplification.

3. To ensure the effect of the product, repeated freezing-thawing should be avoided as much as possible.

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