2× Color SYBR Green qPCR Master Mix (ROX1)

Catalog No.: A0016-R1

Description

This kit uses a hot start DNA polymerase with strong amplification and anti-interference capabilities, combined with its highly optimized buffer and dye systems, to enhance amplification efficiency, anti-interference ability, sensitivity, and specificity. Under the same conditions, it has the characteristics of earlier peak initiation, stronger fluorescence signal, smaller Ct value, and higher melting curve specificity.

The 2× Color SYBR Green qPCR Master Mix (ROX1) of this kit is pre mixed with ROX1 dye (high ROX), so that qPCR reaction can be performed by adding template cDNA, primers, and ddH_2O. Meanwhile, the 2× Color SYBR Green qPCR Master Mix (ROX1) contains a highly stable red dye that can be combined with ***EZ*Bioscience**® Color Reverse Transcription Kit (Cat. No.: A0010CGQ), the latter containing blue dye. When performing a qPCR reaction, the cDNA (blue) generated by the the Color Reverse Transcription Kit and the 2× Color SYBR Green qPCR Master Mix (red) of this kit will turn the solution into purple. This provides excellent visual assistance during sample addition, greatly reducing the risk of sample errors. Therefore, it is recommended to use these two types of kits together to achieve the best results.

Components

Components	A0016-R1 (500 Rxns, 20 μl/Rxn)	A0016-R1-L (2500 Rxns, 20 μl/Rxn)
2× Color SYBR Green qPCR Master Mix (ROX1) *	5 ml	25 ml

*: Contains Hot Start DNA Polymerase, dNTPs, Mg^{2+}, ROX1, SYBR Green I dye and red dye.

Storage

The kit should be stored at -20°C and protected from light. The quality of this product can be guaranteed for 24 months under recommended storage conditions.

Applicable instruments

Appropriate real-time PCR instruments for this kit are as follows (If the instrument is not listed in the table below, please use A0016-R2):

ABI 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast; StepOne™, StepOne Plus™.
Bio-Rad CFX96™, CFX384™, iCycler iQ™, iQ™5, MyiQ™, MiniOpticon™, Opticon®, Opticon 2, Chromo4™; **Roche** LightCycler™ 96, Roche LightCycler™ 480; **Eppendorf** Mastercycler® ep realplex, realplex 2s; **Illumina** Eco qPCR; **Qiagen/Corbett** Rotor-Gene® Q, Rotor-Gene® 3000, Rotor-Gene® 6000; **Thermo** Scientific PikoReal Cycler; **Analytikjena** qTOWER 3G; **Cepheid** SmartCycler®; **Bioer** Linegene 9600; **HONGSHI** SLAN®-96P.

Note

1. Before starting the experiment, first verify whether the primers are suitable, refer to the last standard in this manual, and mainly observe the amplification curve and melting curve.

2. After primer validation, several portions should be divided and stored at -20°C to prevent contamination or degradation.

3. The quality of RNA and cDNA has a significant impact on the results of qPCR, so it is recommended to ensure that RNA does not degrade as much as possible. It is usually recommended to perform reverse transcription as soon as possible after RNA extraction and avoid repeated freezing and thawing. If the expected usage is large, multiple cDNA tubes can be reversed at once. If cDNA is not used immediately, it is recommended to store it in a -80°C freezer.

Protocol

1. Before use, take the 2× Color SYBR Green qPCR Master Mix (ROX1) reagent from the -20°C refrigerator and let it sit at room temperature for 5 ~ 10 minutes or grip the reagent tube tightly by hand to fully melt it. Invert the tube 10 times and mix thoroughly (very important), then briefly centrifuge it to the bottom of the tube using a centrifuge and place it on ice for later use.

2. It is recommended to dilute and thoroughly mix the cDNA obtained from reverse transcription reaction before using it as a template to improve the reproducibility of the experiment. It is usually recommended to dilute 5 to 10 times before use (the specific dilution factor depends on the abundance of gene expression). In a 20 μl qPCR reaction system, if the template cDNA is diluted 5-fold, it is recommended to use 5 μl of cDNA (2 ~ 9.2 μl).

Assuming that the template cDNA has been diluted 5-fold with Nuclease-free ddH_2O before use (20 μl cDNA mixed with 80 μl ddH_2O diluted to 100 μl), prepare the qPCR reaction system according to the following table (in order to minimize sample addition errors, it is generally recommended to prepare a premix of cDNA and ddH_2O, and the remaining components in the table are prepared into a premix, mixed separately, and then added to each reaction well in sequence; or add samples according to personal proficiency in sample addition methods):

Components	Volume/10 μl	Volume/20 μl
2× Color SYBR Green qPCR Master Mix (ROX1)	5 μl	10 μl
Forward Primer (10 μM)	0.2 μl	0.4 μl
Reverse Primer (10 μM)	0.2 μl	0.4 μl
cDNA	2.5 μl (1 ~ 4.6 μl)	5 μl (2 ~ 9.2 μl)
Nuclease-free ddH_2O	Add to 10 μl	Add to 20 μl

3. After adding the sample, cover it with a sealing film and seal it tightly. Centrifuge at 1000 rpm for 1 minute and centrifuge the liquid to the bottom of the qPCR well plate.

4. Follow the qPCR reaction procedure below to set the amplification curve program, and the melting curve program is usually set according to the instrument's default program without modification:

Step	1	2		3
	Hot Start DNA Polymerase Activation*1	**PCR**		**Melting curve**
		Cycle (40 cycles)		Instrument's default program
		Denature	Annealing & Extension (**Collect fluorescence signals**) *2	
Temp.	95°C	95°C	60°C	
Time	5 min	10 sec	30 sec	
Volume	10 μl/20 μl			

Important: *1: The Hot Start DNA Polymerase requires activation at 95°C for 5 minutes. *2: Collect fluorescence signals during the Annealing & Extension step.

The standard for qPCR reaction

1 The amplification curve shows a typical S-shaped curve, with complete visible fluorescence background signal stage, fluorescence signal index amplification stage, and plateau stage. The melting curve is unimodal, and the internal reference Ct value is within a reasonable range (usually between 10 and 22, with typical internal reference Ct values between 15 and 20), indicating that the reaction is normal.

2 The Ct values of duplicate wells of the same template and primer differ by less than 0.5. Data that meets both of the above conditions can be considered usable.