Version Number：1.0

Real Time PCR Easy™ – SYBR Green I

For real time PCR using cDNA, purified DNA

Kit composition(20μl system)	QP-01011	QP-01012	QP-01013	QP-01014
	200T	500T	1000T	2000T
2× Real PCR Easy™ Mix-SYBR	1 ml × 2	1.7 ml × 3	1.7 ml × 6	1.7 ml × 12
50× ROX Reference Dye	400 μl	1 ml	1 ml × 2	1 ml × 4
DNase-Free ddH_2O	1.7 ml	1.7 ml × 2	10 ml	20 ml
Instruction	1	1	1	1

Product introduction

The 2× Real PCR Easy™ Mix-SYBR provided by the kit is a new premix system that uses SYBR Green I for Real Time PCR amplification reactions, which can greatly improve product specificity and reaction sensitivity. At the same time, ROX is provided as an internal control dye. The fluorescence intensity of this kit is 3-5 times that of similar products, and can more sensitively and intuitively reflect the concentration of target template DNA.

2× Real PCR Easy™ Mix-SYBR contains our company's unique hot-start Foregene Taq DNA Polymerase. Compared with ordinary Taq enzymes, this enzyme has the advantages of high amplification efficiency, strong specific amplification ability, and low mismatch rate. Used for fluorescent quantitative PCR reaction can reduce non-specific amplification and improve the accuracy of PCR.

Transport and storage conditions

1. Transportation conditions: The whole process is transported in a low-temperature ice box to ensure that the kit is in a state of <4°C.
2. Storage conditions: Store at -20°C in the dark; if used frequently, it can also be stored at 4°C for short-term storage (use up within 10 days).

Kit component information

- 2× Real PCR Easy™ Mix-SYBR： Contains Foregene special modified hot-start Taq DNA Polymerase, MgCl2, optimized ratio of dNTPs and SYBR Green I, reaction buffer, PCR reaction enhancer, optimizer and stabilizer, etc. During the PCR reaction, just add the appropriate lysis mixture, primers, and DNase-ddH2O to the 2× Real PCR EasyTM Mix-SYBR to be used in the PCR reaction.
- ROX Reference Dye： It is generally used on Real Time PCR amplifiers of companies such as ABI and Stratagene to adjust the difference between PCR tubes and tubes caused by PCR sample loading errors. The concentration of ROX Reference Dye required by different instruments is different, and the user can add it according to the recommended concentration of the instrument.

Precautions:

- 2× Real PCR Easy™ Mix-SYBR is stored at -20°C. Repeated freezing and thawing should be avoided, otherwise the PCR efficiency will be affected.
- When using, please turn the2× Real PCR Easy™ Mix-SYBR upside down and mix gently to avoid foaming, and centrifuge briefly before use. If the reagents are not evenly mixed, the reaction performance will decrease. Do not use a shaker to mix well.
- 2× Real PCR Easy™ Mix-SYBR contains SYBR Green I. Avoid strong light when storing or preparing PCR reaction solution.
- Please use new (non-contaminated) pipette tips, PCR tubes, etc. for the preparation and aliquoting of the reaction solution to avoid contamination as much as possible.

Operation guide

A：Real Time PCR system preparation

1. Take out 2× Real PCR Easy™ Mix-SYBR, 50× ROX Reference Dye, primers, etc. and place them in an ice box to let them melt naturally. After thawing, mix the reagents upside down, and use a centrifuge to collect the liquid scattered on the tube wall and lid.

   Note: 2× Real PCR Easy™ Mix-SYBR will become turbid when placed at room temperature or held in your hand for a long time. You can put it on ice for 2-5 minutes. When the solution is clear, mix upside down and mix 3-5 times before using.

2. Add an appropriate amount of DNA template, primers or 50× ROX Reference Dye to 2× Real PCR Easy™ Mix-SYBR, and dilute to 1× with DNase-Free ddH2O (see Table 1 for PCR system preparation).

   Note: This operation should be performed on an ice bath. Long-term storage at room temperature will reduce product performance.

Table 1: PCR reaction system preparation

PCR system additions	Consumption	
2× Real PCR Easy™ Mix-SYBR	10 μl	1×
Forward Primer (10 μM)	0.8 μl	0.4 μM 1*
Reverse Primer (10 μM)	0.8 μl	0.4 μM 1*
Template(cDNA/DNA)	X μl	2*
50× ROX Reference Dye	-	3*
DNase-FreeddH$_2$O	(8.4-X) μl	
Total Volume	20 μl	

Note: For qPCR with a 50 μl system, please refer to the 20 μl system to adjust the amount of reagents proportionally.

1*: Generally, a final primer concentration of 0.4 μM can give better results. When the reaction performance is poor, the primer concentration can be adjusted within the range of 0.2 ~ 1.0 μM.

2*: The amount of DNA template added is usually below 100 ng. Because different types of DNA templates contain different copy numbers of target genes, gradient dilution can be performed if necessary, to determine the optimal DNA template addition amount.

3*: Choose the appropriate final concentration of ROX Reference Dye according to different quantitative PCR instruments. The optimal concentration of ROX Reference Dye for common quantitative PCR machines is shown in the following table:

Fluorescence quantitative PCR instrument	ROX Reference Dye final concentration
ABI PRISM7000/7300/7700/ 7900HT/Step One, etc.	5× (For 20 μl system, add 2 μl 50×ROX Reference Dye)
ABI 7500/7500 Fast and Stratagene Mx3000P/Mx3005P/Mx4000, etc.	1× (For 20 μl system，add 0.4 μl 50×ROX Reference Dye)

B: Real Time PCR reaction

Prepare the PCR system according to step A, mix well, and perform PCR reaction according to the optimized PCR conditions (annealing temperature, etc.) (see the following table 2-1 for the two-step reaction conditions, and the following table 2-2 for the three-step reaction conditions).

Table 2-1: Two-step method

Steps	Temperature	Time	Number of cycles	Content
1	94-95℃	3 min	1	Predenaturation
2(Two steps)	94-95℃	5-10 sec	40	Template denaturation
	60-65℃	20-30 sec		Annealing/Extension

Table 2-2: Three-step method

Steps	Temperature	Time	Number of cycles	Content
1	94-95℃	3 min	1	Predenaturation
2(Three-step)	94-95℃	5-10 sec	40	Template denaturation
	55-65℃	10 sec		Annealing
	72℃	20 sec		Extension

Note: In order to get the best PCR effect, gradient PCR can be used to optimize the reaction conditions for different templates and different primers. The PCR reaction conditions vary depending on the quantitative PCR instrument, template, primers, etc. In specific operations, it is necessary to design the best reaction conditions, including annealing temperature, reaction time, etc., according to specific conditions such as the quantitative PCR instrument, template type, target fragment size, base sequence of the amplified fragment, and GC content and length of primers.