SYBR Green qPCR Mix with Tracking Dyes

Catalogue No.: K092

Size: 100T/500T

Kit component:

Item	100T	500T
SYBR Green qPCR Mix (2X, Blue)	1 mL	1 mL*5
Template Dilution Buffer (40X, Yellow)	0.2 mL	1 mL
Low ROX (50X)	40 μL	0.2 mL
High ROX (50X)	40 μL	0.2 mL

Storage:

The kit should be stored at -20℃ away from light for one year; Store at 4℃ away from light for one month. Try to avoid repeated freezing and thawing.

Introduction:

SYBR Green qPCR Mix with Tracking is a high quality premix with a dual-dye tracking system for real-time Quantitative PCR, qPCR(Quantitative PCR) or real-time PCR. The color-changing effect produced by the mixing of two tracer dyes can be used to trace the pipetting process, so that users can distinguish between blank holes and holes added to qPCR Mix, and confirm whether DNA samples with small volume have been added to qPCR Mix, which can be quickly and easily used for specific ultra-sensitive quantification of cDNA or genomic DNA.

When preparing the solution of the qPCR reaction system, it is difficult to tell whether the sample has been added by the naked eye because the volume of the DNA sample is usually only about 1-2 microliters. A tracer inert Blue dye is added to SYBR Green qPCR Mix (2X, Blue), and a Template Dilution Buffer (40X, Yellow) with an inert yellow dye is provided. When preparing the qPCR reaction system, the color of the solution will change significantly with the mixing of the two components, that is, when the yellow solution is added to the blue solution, it will become a green solution, so that the DNA template and qPCR mix can be accurately determined according to the color change of the liquid, which will play a tracer role in the sample addition process, so improve the accuracy of qPCR system setting and avoid missing or wrong adding templates.

SYBR Green qPCR Mix (2X) uses SYBR Green I as a dye. SYBR Green I is a green fluorescent dye that binds to the double-helix sulci region of double-stranded DNA.The fluorescence of SYBR Green I is weak in the free state, and its fluorescence is greatly enhanced once it is bound to double-stranded DNA. In this way, the amount of double-stranded DNA produced by PCR amplification can be quantitatively detected by detecting the intensity of fluorescence.

The Taq DNA Polymerase used in the polymerase is a high quality thermostarter enzyme that binds to antibodies, enabling a convenient and efficient thermostarter. The Taq enzyme binds to anti-Taq enzyme

monoclonal antibodies, thereby inhibiting the DNA polymerase activity of Taq enzyme, which effectively avoids non-specific amplification caused by non-specific annealing of primer and template DNA or primer dimers at low temperatures.In the pre-denaturation step of PCR reaction, the antibody will be heated to inactivate, which can ensure that the Taq enzyme activity will be released only after the pre-denaturation, and no DNA polymerization will occur before the pre-denaturation, thus greatly improving the specificity, sensitivity and accuracy of quantitative detection of PCR reaction.

This product contains all common components such as Taq DNA Polymerase, PCR Buffer, dNTPs, SYBR Green I fluorescent dye, stabilizer and magnesium ion, making it easier to operate and use. Users only need to add their own primers, sample DNA and deionized water.

This product includes Low ROX and High ROX and is widely compatible with fluorescent quantitative PCR instruments that do not require ROX and that require Low ROX or High ROX as a correction dye. The role of ROX is to correct for fluorescence fluctuations independent of PCR, thereby minimizing pore differences. This difference can be caused by a variety of factors, such as pipetting errors and sample evaporation. Different fluorescent quantitative PCR instruments have different requirements for ROX. Please choose High ROX, Low ROX or no ROX when preparing the reaction system according to the actual instrument used.

Assay Procedure:

一. PCR reaction system was set up:

1. Melt and mix the solutions required for PCR reaction. Place all reagents over an ice bath or in an ice box.

2. Template Dilution (Optional) : If you do not need to trace the Template, do not use the Template Dilution Buffer to dilute the template.

The Template Dilution Buffer (40X, Yellow) provided by this product is 40X, which must be 1X in the final PCR reaction system.

For dilution of Original Template DNA to 100μl (Final volume), the dilution method of the original template can be referred to the following table, a suitable volume of the diluted template was then added to the 20μl qPCR reaction system. For 20μl qPCR reaction system, usually the amount of diluted template added is 2μl or 4μl, and cannot exceed 8μl.

Volume for Diluted Template DNA in 20μl qPCR Reaction System (μl)	1	2	3	4	5	6	7	8
Template Dilution Buffer (40X, Yellow) (μl)	50	25	16.7	12.5	10	8.4	7.2	6.3
Original Template DNA (μl)	x	x	x	x	x	x	x	x
Ultrapure Water (μl)	50-x	25-x	16.7-x	12.5-x	10-x	8.4-x	7.2-x	6.3-x
Final volume (μl)	100	100	100	100	100	100	100	100

3. Refer to the table below to set up the PCR reaction system at room temperature or on an ice bath.(96-well plate as an example)

Reagents	Volume(μ L)
SYBR Green qPCR Mix(2X, Blue)	10
Template DNA	X
Primer mixture（2.5 μ M each）	2
Without or Low/High ROX (50X)	0.4
Ultrapure Water	To 20

Note: Good detection results can be obtained when the final concentration of primer is 0.2-0.5 μ M, and the final concentration of the primer can be adjusted in the range of 0.1-1.0 μ M according to the situation. Usually the amount of DNA template is 1-10ng cDNA or 10-100ng genomic DNA as a reference amount. Because the template of different species contains different number of copies of the target gene, if necessary, the template can be gradiently diluted to determine the optimal template usage. When the cDNA obtained by RT-PCR reaction is directly used as a template, the amount added should not exceed 10% of the total volume of the PCR reaction.

4. Gently blow and mix with a pipette, centrifuge at room temperature for a few seconds.

5. Put the PCR reaction liquid on the PCR instrument to start the PCR reaction.

二. The setting of PCR reaction parameters:

Predenaturation of the template is performed before the Real-time PCR reaction, usually set at 95 ° C for 2 minutes, with complex or high GC templates appropriately extended to 5 minutes. The Taq DNA Polymerase in this product can complete the amplification of at least 300bp in 15 seconds, which can meet most qPCR experiments. For amplicons with more than 350bp or high GC content, it is recommended to increase the extension time to 60 seconds or use the three-step method to improve amplification efficiency.

STEP1(initial denaturation): 95℃ 2min

STEP2(denaturation): 95℃ 15sec

STEP3(annealing/extension): 60℃ 15-30sec

STEP4(cycle): Go To STEP2 for 40 cycles

Note: The three-step method only requires a step of 72 ℃ 30sec after annealing/extension.

Notes:

1. Pay attention to the annealing temperature of the primer. When the annealing temperature is <60℃, three-step PCR amplification is recommended.

2. This product contains SYBR Green I fluorescent dye. When storing this product or setting up PCR reaction, avoid strong light irradiation to avoid fluorescence quenching as much as possible.

3. For amplified fragments with more than 350bp or high GC content, it is recommended to increase the extension time to 60 seconds or adopt the three-step method to improve the amplification efficiency.

4.qPCR detection is an ultra-sensitive detection, and the PCR reaction setting area should try to avoid all possible contamination of products to be amplified. The PCR products should be sealed and discarded to avoid contaminating the experimental environment with ultra-high concentration of PCR products.

5. Although this product still has almost the same PCR amplification effect after 10 times of repeated freeze-thaw, it is still appropriate to avoid repeated freeze-thaw this product, repeated freeze-thaw may degrade product performance.

6. This product is only for the use of scientific researchers, can not be used for clinical diagnosis or treatment, food or medicine, shall not be stored in ordinary homes.

7. For your safety and health, please wear good clothes and disposable gloves.