SYBR Green qPCR Mix(2X)

Catalogue No.: K093

Size: 1mL/1ml*5

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 (2X) is a high-quality premixed solution for real-time Quantitative PCR, namely qPCR(Quantitative PCR) or real-time PCR, which is mainly used for specific ultra-sensitive quantitative detection of cDNA and genomic DNA.

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 does not contain ROX and is suitable for fluorescent quantitative PCR instruments that do not require ROX as a correction dye.

For conventional 96-well plate qPCR assay (recommended reaction system is 20 μl), 100 tests can be performed per ml ; If used for a conventional 384-well plate qPCR assay (10 μl is recommended), this product can perform 200 tests.

Assay Procedure:

一. PCR reaction system was set up:

1. Melt and mix the solutions required for PCR reaction. Place SYBR Green qPCR Mix(2X) over an ice bath or in an ice box.

2. Refer to the table below to set up the PCR reaction system under ice bath conditions.(96-well plate as an example)

reagents	volume(μ L)
RNase-free water	6
Template DNA	2
Primer mixture（3 μ M each）	2
SYBR Green qPCR Mix(2X)	10
Total volume	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.

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

4. 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.

三. Result detection

The results were analyzed using the software provided by the fluorescence quantitative PCR instrument.

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.