2 X SYBR Green qPCR Mixture

Item No.: HPR012 5x1ml
HPR012M 10x5ml

Storage conditions: 12 months at -20°C, 2 weeks at 4°C. Avoid repeated freezing and thawing

Product introduction:

This system is a premixed system for real-time fluorescence quantification using dye method (SYBR Green I). The product contains optimized concentrations of HotStart Taq DNA Polymerase, dNTPs, Mg2+, reaction buffer and stabilizers. It is mainly used for the detection of genomic DNA target sequence and cDNA target sequence after RNA reverse transcription, such as gene expression analysis, copy number analysis, SNP genotype analysis, etc. It is suitable for different types of fluorescent quantitative PCR. This system is a 2× pre-mixed real-time fluorescent quantitative PCR reaction system. It only needs to add template, primers and water to make the working concentration 1×. It has the advantages of quickness and simplicity, high sensitivity, strong specificity, and good stability.

Standard operating procedure

1**. Prepare the reaction system according to the following table**

Component	Volume
DNA template	1 µl
Upstream primer (10 µM)	0.5 µl
Downstream primer (10	0.5 µl
2 x Green qPCR Mixture	10 µl
DEPC-ddH_2O	Make up

2. Reaction settings

Two-step process	temperature	Time	
Predenaturation	94°C	2 min	
transsexual	94°C	1	40-50 cycles
Annealing-extension	60°C	3	
Melting curve	Machine default setting		

¬ When the amplification efficiency of the two-step method is not good, it is recommended to choose the three-step method for qPCR reaction.

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Three-step process	Temperature	Time	
Predenaturation	94°C	2 min	
Transsexual	94°C	1	40-50 Cycle
Annealing	60°C	1	
Extend	72°C	3	

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Melting curve	Machine default

The meaning of reference fluorescence, how to add;

Reference Fluorescence (ROX) is to solve the edge effect caused by the different optical paths of the different reaction wells of the 96-well plate to the detector. By analyzing the reference fluorescence intensity, the fluorescence signal intensity between the wells and the wells is calibrated to achieve more accurate detection. .

According to different concentrations, it is divided into ROXI (ROX High; high concentration) and ROXII (ROX Low; low concentration). When using, select the corresponding ROX Dye according to the machine used (see the list below); before use, add the dye in the amount of 1/50 of the final volume (add 40ul in 1ml 2x qPCRmix) and mix it for use.

It should be noted that most machines do not require the addition of ROX. Even the recommended machines (such as ABI series machines) can use reagents without ROX. You need to check the "ROX" option in the Dye option to "None" during the setup phase. "That's all.

It is recommended to use ROXII (low density; light color) machines

- Applied Biosystems 7500 and 7500 FAST Real-Time PCR System
- Bio-Rad CFX96, DNA EHRPine Opticon® and Opticon® 2 Real-Time PCR Detection Systems
- Bio-Rad/MJ Research Chromo4™ Real-Time Detector
- Cepheid SmartCycler® System
- Corbett Rotor-Gene™ 3000 and 6000 Real-Time Rotary Analyzer
- Eppendorf Mastercycler® ep realplex Real-Time PCR System
- Roche LightCycler® 480 Real-Time PCR System
- Stratagene Mx3000P® and Mx3005P® Real-Time PCR Systems
- Stratagene Mx4000® Multiplex Quantitative PCR System

It is recommended to use ROX (high density and deep color) models

- ABI PRISM® 7000 and 7700 Sequence Detection System
- Applied Biosystems 7300 and 7900HT Real-Time PCR System
- Applied Biosystems GeneAmp® 5700 Thermal Cycler
- Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems

Troubleshooting:

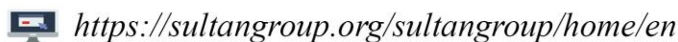
1. What is the significance of adding a template control group (NTC) in a fluorescence quantitative experiment?

In the fluorescence quantitative experiment of dye method, the combination of SYBR Green dyes with DNA is non-specific, so once non-specific amplification (error amplification and dimer) is generated, non-specific fluorescence signals will be generated, which will make the detection results Produce a large deviation. The addition of NTC control group combined with melting curve analysis can effectively evaluate the influence of heterogeneous amplification on experimental data:

Under normal circumstances, NTC Ct value greater than 35 is considered acceptable, if the sample Ct-NTC Ct>=6, the data is considered acceptable. If the sample Ct>=32, you need to focus on NTC, and redesign the primers if necessary.

2. What is the difference between 2-step and 3-step reactions?

In the reaction using antibody method qPCRmix, since the detection fragment of qPCR is small (usually less than 250bp) and the target genome is generally selected for amplification, we can usually omit the 72°C extension step in conventional PCR in the experiment. Incorporate into the second annealing step at 60°C. Achieve the effect of further reducing the reaction time.
It should be noted that although the 2-step method is suitable for most genetic testing, the 3-step reaction procedure can effectively increase the amplification efficiency to obtain better experimental data when encountering high GC or poorly amplified templates
3. What effect does the length of the pre-denaturation time have on the qPCR reaction;
The qPCRmix of the antibody method requires 30sec-2min at 94℃ to completely release the taq enzyme activity.
The HRP antibody method qPCRmix can also tolerate the activation at 94°C for 10 minutes without loss of activity, which is suitable for the amplification of high GC templates.
4. What is the significance of the miscellaneous peaks in the melting curve, and how to deal with it;
4. What is the significance of the miscellaneous peaks in the melting curve, and how to deal with it;
The significance of the melting curve is that as the temperature rises, DNA melts and melts, and different DNA fragments have different melting temperatures. Then analyze the specificity of the amplified product (polymorphism analysis based on saturated dye-HRM analysis is derived from this). Once the melting curve appears the miscellaneous peak, the set peak, etc., it indicates that there are many kinds of DNA double-strands in the amplification system, and the amplification specificity is not good. Need to consider the authenticity of the data.
Double peaks in the amplification can be optimized by increasing the annealing temperature, reducing the amount of primers, reducing the amount of template, and using a 3-step procedure. But if the effect cannot be satisfied, the primers need to be redesigned.
The melting temperature represents the length of the chain. Generally, if it is lower than 75°C, it is necessary to consider whether it is a dimer;
The melting temperature obtained by using different reagents to amplify the same gene is different and will not be exactly the same.
5. How to analyze the abnormal response curve;
The curve is not smooth: the template is not pure enough, use the purified DNA as the template; optimize the primers
The curve is low, not a typical S curve: the amplification efficiency is poor, use the 3-step method or optimize the primers
The platform is too low: the system has fewer PCR products and the template dilution factor is too high.
6. How to choose a suitable internal reference gene: The abundance of the internal reference gene should be similar to that of the target gene, and the difference in Ct value between the two at the same template concentration should not be more than 6 cycles.
7. How to choose the reaction volume of qPCR;
From the perspective of PCR reaction, the optimal reaction volume is 50ul; in conventional 100ul and 200ul PCR tubes, it is recommended not less than 20ul per reaction; it is not recommended to



use a reaction system less than 10ul (even 384-well plates). Conducive to the progress of the reaction. At the same time, due to the small volume, it is easy to cause deviation of fluorescence reading.
However, the volume greater than 50ul is too large, and the reaction is not easy to be uniform.