2×Realtime SYBR qPCR Mix（NO ROX）

Code No.：
NG012S 5×1 ml
NG012M 50×1 ml

Storage：Store at -20 ℃ for up to two years and avoid repeated freezing and thawing. For regular use, please store at 2-8 ℃ within a month.

Introduction：

This product is a 2× premix for real-time quantitative PCR using the SYBR Green I. It contains optimized concentrations antibody-modified hot-start Taq DNA Polymerase, SYBR Green I, dNTPs, Mg^{2+} reaction buffer, and stabilizers. It is mainly used for the detection of genomic DNA target sequences and cDNA target sequences after RNA reverse transcription. PCR reaction system can be carried out only by adding template, primer， water and mix to the reaction buffer. which simplifies the experimental operation process and reduces the pollution in the operation process. The advantages of 2×Realtime SYBR qPCR Mixture（NO-ROX）include high convenience, sensitivity, specificity and stability.

Content:

content	NG012S	NG012M
2×Realtime SYBR qPCR Mix（N0 ROX）	5×1 ml	50×1 ml

Note:

Dye ROX：For certain fixed models of instruments, ROX must be added to accurately determine the Ct value. The concentration of ROX used can be referenced.

NG012 2×Realtime SYBR qPCR Mixture（NO-ROX）: quantitative PCR instrument that no need to add ROX Dye including Thermal Cycler Dice Real Time System/ LightCycler/Smart Cycler System, Corbett Rotor-gene 6000, Agilent Technologies Mx3000P etc.

NG022 2×Realtime SYBR qPCR Mixture(LOW-ROX): quantitative PCR instrument that requiring low ROX Reference Dye including ABI Prism7500 /7500 Fast, MJ Research Chromo4, Opticon (II), Corbett Rotor Gene 3000 etc.

NG032 2×Realtime SYBR qPCR Mixture（High-ROX）: quantitative PCR instrument that requiring high ROX Reference Dye including ABI Prism7000/7300/7700/7900HT/ABI Step One /ABI Step One Plus etc.

Attention:

1. Please invert gently to mix before use, avoiding foaming, and use after a brief centrifugation.
2. Minimize exposure time 2×Realtime SYBR qPCR Mix to light; prolonged exposure may lead to a decrease in fluorescence signal.
3. Please use uncontaminated pipette tips, microtubes, etc., for preparing and dispensing the reaction mixture to avoid cross-contamination.
4. This product is not suitable for hybridization probe methods.

Operation Procedure:

Reagents to be provided by the user: cDNA or DNA template, primers.

Please perform the experiment according to the operating instructions of the different real-time PCR instrument.

Operation Example: Using 20 μl and 50 μl PCR reaction systems as examples:

1. Preparation of PCR reaction system

content	20 μl	50 μl
template	1 μl	1 μl
Forward Primer (10 μM)	0.5 μl	1 μl
Reverse Primer (10 μM)	0.5 μl	1 μl
2×Realtime SYBR qPCR Mix（N0 ROX）	10 μl	25 μl
High/Low ROX Reference Dye	0.4 μl	1 μl
Sterile Water	Up to 20 μl	Up to 50 μl

2. PCR reaction conditions:

2-step PCR amplification standard procedure:

Step	Temperature	Time	
Initial denaturation	95 ℃	2 min	
denaturation	95 ℃	15 sec	40 Cycles
Annealing - Extension	60 ℃	15-30 sec	
Dissociation stage			

3-step PCR amplification standard procedure:

Step	Temperature	Time	
Initial denaturation	95 ℃	2 min	
denaturation	95 ℃	15 sec	
Annealing	60 ℃	15-30 sec	40 Cycles
Extension	72 ℃	30 sec	
Dissociation stage			

The examples above represent a standard qPCR reaction system for reference only. Actual reaction conditions vary depending on the structure of the template, primers, mal reaction conditions and target fragment.

Template amount: 10-100 ng genomic DNA or 1-10 ng cDNA is recommended. Since the copy number of the target gene varies among different species, perform a serial dilution of the template to determine the optimal amount. Additionally, when using cDNA (RT reaction mixture) from Two Step RT-PCR reaction as the template, the volume added should not exceed 10% of the total PCR reaction volume.

Primers: A primer concentration of 0.2 μM typically yields good results, with a range of 0.1-1.0 μM as a reference for setting. In cases of low amplification efficiency, the primer concentration can be increased; in cases of non-specific reactions, the primer concentration can be decreased to optimize the reaction system. To achieve ideal qPCR results, the length of the amplified fragment is recommended to be 80-200 bp.

The Taq DNA Polymerase used in this product is a HotStart DNA polymerase blocked by anti-Taq antibodies. If pre-denaturation of the template formed prior to the PCR reaction, it is typically set at 95 ℃ for 2 min; for complex or high GC templates, the time should be extend the time appropriately to 5 min. This DNA polymerase can complete the amplification of at least 300 bp within 15 s, which is sufficient for the vast majority of qPCR experiments. For amplicons exceeding 350 bp or with high GC content, it is recommended to increase the extension time to 60 s or use a 3-step method to improve amplification efficiency..

3. Analyze experimental data.