SupRealQ Ultra Hunter SYBR qPCR Master Mix (U+)

NB-54-0417-02
NB-54-0417-03
NB-54-0417-04

Contents

For Research Use Only. Not for use in diagnostic procedures.

SupRealQ Ultra Hunter SYBR qPCR Master Mix (U+)

#Cat: NB-54-0417-02 Size: 500rxns
#Cat: NB-54-0417-03 Size: 2500rxns
#Cat: NB-54-0417-04 Size: 500rxns

01/Product Description

This product is a specialized premix for qPCR reactions using the SYBR Green I fluorescence method, with a purple color to facilitate sample loading. The core enzyme is a Taq polymerase selected through BioSmart platform-directed screening, featuring strong 3' end mismatch recognition and high specificity. It is combined with high-closure dual-species antibodies to form a hot-start Taq enzyme, which maintains strict closure at 55°C. Paired with an optimally formulated buffer for qPCR, it enables precise detection and efficient amplification of target genes, even with low template amounts or low-expression genes. The reagent includes a dUTP/UDG contamination prevention system that works at room temperature, preventing aerosol contamination and ensuring the accuracy of qPCR results. Additionally, this product contains a special ROX Passive Reference Dye, making it compatible with a wide range of qPCR instruments. No need to adjust the ROX concentration for different instruments-simply add primers and templates during reaction setup to begin amplification.

02/Components

Components	NB-54-0417-02 500rxns(20µl/rxn)	NB-54-0417-03 2,500 rxns (20 µl/rxn)	NB-54-0417-04 (50 rxns) 500 rxns (20 µl/rxn)
2 × SupRealQ Ultra Hunter SYBR qPCR Master Mix (U+)	4 × 1.25 ml	20 × 1.25 ml	5 ml

▲ It contains dNTP/dUTP Mix, Mg2+, SYBR Green I, Specific ROX Reference Dye, Heat-labile UDG, etc.

03/Storage

Store at -30 ~ -15°C and protect from light. Ship at ≤0°C.

04/Applications

It is applicable for DNA quantification from various type of templates such as genomic DNA, cDNA, plasmid DNA and λDNA.

05/Notes

1. Avoid repeated freeze-thaw cycles to prevent a decrease in enzyme activity. If small amounts are used each time, it is recommended to aliquot the product for convenience.
2. Before use, gently invert the Master Mix to mix. Do not vortex to avoid bubble formation, which may affect quantification results. After mixing, briefly centrifuge the Master Mix, and it will be ready for use. During sample loading, pipette gently. If bubbles accidentally form in the Master Mix, centrifuge again briefly before proceeding.
3. As this kit contains a fluorescent dye SYBR Green I, it should be stored protect from light. Avoid strong light when preparing the reaction solution.
4. The purple dye in the kit does not interfere with the fluorescence signal collection of SYBR Green I.

06/Experiment Process

1.Prepare the following reaction mixture in a qPCR tube:

Components	Volume
2 × SupRealQ Ultra Hunter SYBR qPCR Master Mix (U+)	10.0 µl
Primer 1 (10 µM)	0.4 µl
Primer 2 (10 µM)	0.4 µl
Template DNA/cDNA	x µl
ddH_2O	To 20.0 µl

The volume of each component in the reaction system can be adjusted according to the following principles:

▲ Generally, a final primer concentration of 0.2 µM provides optimal amplification. If the reaction performance is suboptimal, the primer concentration can be adjusted within the range of 0.1 - 1.0 µM.

▲ For undiluted cDNA template stock, the volume used should not exceed 1/10 of the total qPCR reaction volume.

2. Perform the qPCR reaction according to the following conditions

Stage 1	Initial Denaturation[a]	Rep: 1	95°C	30 sec
Stage 2	Cycles	Reps: 40	95°C 60°C*	3 - 10 sec[b] 10 - 30 sec[c]
Stage 3	Melting Curve[d]	Default Instrument Settings		

▲To achieve better contamination removal, a 37°C digestion step for 2 min (1 cycle) can be added before the Initial Denaturation step.

a.The standard Initial Denaturation condition is suitable for most amplification reactions. For complex template structures, the denaturation time can be extended to 5 min to improve its effectiveness.

b.For standard programs, select 10 sec; for fast programs, the minimum time can be set to 3 sec.

c.For the extension step, standard programs typically use 30 sec. In fast programs, the extension time can be set to as short as 10 sec for amplicons smaller than 200 bp. For amplicons exceeding 200 bp, an extension time of 30 sec is recommended.

d.Melting curve acquisition programs vary with instrument type. The instrument's default melting curve acquisition program is recommended.

Fluorescence Signal Acquisition.

Optimization Plan for the Reaction System

An ideal reaction system should exhibit the following characteristics: a single peak in the melting curve (amplification specificity), amplification efficiency close to 100% (amplification efficiency), and reasonable C_T values (amplification sensitivity). If the default reaction conditions do not perform well, optimization can be carried out based on the following guidelines

1. Relationship Between Primer Concentration and Reaction Performance:

When the final primer concentration ranges from 0.1 to 1.0 µM, higher primer concentrations generally lead to higher amplification efficiency, but may reduce amplification specificity.

2. Relationship Between Amplification Program and Reaction Performance:

To improve amplification specificity, the annealing temperature can be increased:

Two-step Protocol 95°C/10 sec 60°C/30 sec	Increase the annealing temperature (increase by 3°C each time): 95°C/10 sec 63°C/30 sec

To improve amplification efficiency, extend the extension time in the two-step protocol or use the three-step protocol:

Two-step Protocol 95°C/10 sec 60°C/30 sec	Extend the extension time: 95°C/10 sec 60°C/60 sec	Three-step Protocol 95°C/10 sec 56°C/30 sec 72°C/30 sec	Extend the extension time: 95°C/10 sec 56°C/30 sec 72°C/60 sec

07/FAQ & Troubleshooting

◇ **Abnormal Amplification Curve**

① **Amplification curve is not smooth:** This may occur due to weak signals, which are corrected by the system. Increase the template concentration and repeat the experiment.

② **Amplification curve breaks or declines:** This happens when the template concentration is too high, causing the baseline endpoint to exceed the C_T value. Reduce the baseline endpoint (CT value - 4) and reanalyze the data.

③ **Sudden drop in individual amplification curves:** This is caused by air bubbles in the reaction tubes. Ensure proper centrifugation of samples and carefully check for any air bubbles before starting the amplification reaction.

◇ **No Amplification Curve After Reaction Completion**

① **Insufficient number of cycles:** Typically, 40 cycles are set, but be cautious as too many cycles can increase background signal and reduce data reliability.

② **Check if signal collection is set in the program**: In the two-step protocol, signal collection is generally set during the annealing-extension phase; in the three-step protocol, signal collection should be set during the 72°C extension phase.

③ **Check if primers have degraded:** Primers that have not been used for a long time should be tested for integrity using PAGE electrophoresis to rule out degradation.

④ **Template concentration is too low:** Reduce dilution and repeat the experiment, starting with the highest concentration for unknown samples.

⑤ **Template degradation:** Reprepare the template and repeat the experiment.

◇ **C_T Value Appears Too Late**

① **Extremely low amplification efficiency:** Optimize reaction conditions, try the three step amplification protocol, or redesign and synthesize new primers.

② **Template concentration is too low:** Reduce dilution and repeat the experiment, generally starting with the highest concentration for unknown samples.

③ **Template degradation:** Reprepare the template and repeat the experiment.

④ **PCR product is too long:** It is recommended that PCR products be between 80 and 150 bp in length.

⑤ **PCR inhibitors present in the system:** These are usually introduced by the template. Increase template dilution or reprepare the template and repeat the experiment.

◇ **Poor Linear Relationship of Standard Curve in Absolute Quantification**

① **Pipetting error:** Increase the template dilution and improve the pipetting volume.

② **Standard material degradation:** Reprepare the standard material and repeat the experiment.

③ **Template concentration too high:** Increase the template dilution.

◇ **Melt Curve Shows Multiple Peaks**

① **Poor primer design**: Redesign and synthesize new primers following design principles. This may involve optimizing primer length, GC content, or ensuring specificity to avoid nonspecific binding or secondary structures.

② **High primer concentration:** Reduce the primer concentration to prevent primer-dimer formation or non-specific binding, which can lead to additional peaks.

③**Genomic contamination in cDNA template:** Reprepare the cDNA template, ensuring thorough removal of genomic DNA. Using a gDNAremoval step during RNApreparation or a specific DNase treatment during cDNA synthesis can help eliminate this issue.

◇ **Poor Reproducibility of Experiment**

① **Pipetting volume inaccuracies**: Use a high-quality pipette and dilute the template to a higher concentration for more accurate volume addition to the reaction mixture.

② **Inconsistent temperature control at different positions in the qPCR instrument:** Regularly calibrate the instrument to ensure uniform temperature distribution.

③ **Low template concentration:** The lower the template concentration, the worse the reproducibility. Reduce template dilution or increase the volume added to the reaction.