ChamQ Blue Universal SYBR qPCR Master Mix

NB-54-0416-02
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Contents

For Research Use Only. Not for use in diagnostic procedures

ChamQ Blue Universal SYBR qPCR Master Mix

#Cat: NB-54-0416-02 Size: 500rxns
#Cat: NB-54-0416-03 Size: 2,500rxns

1/Product Description

This product is a specialized premix for qPCR reactions using the SYBR Green I fluorescence method, with a blue color to facilitate sample loading. Its core component, Champagne Taq DNA Polymerase, is an innovative antibody-based hot-start DNA polymerase that offers excellent specificity and high detection sensitivity. Combined with an optimized qPCR-specific buffer and a specificity-enhancing factor, it is ideally suited for high-specificity, high-sensitivity qPCR reactions. The product includes a unique ROX Passive Reference Dye that is compatible with a wide range of qPCR instruments, eliminating the need to adjust ROX concentration for different platforms. Simply add primers and templates to the reaction system for amplification.

2/Components

Components	500 rxns (20μl/rxn)	2,500 rxns (20μl/rxn)
2 × ChamQ Blue Universal SYBR qPCR Master Mix*	4 × 1.25 ml	20 × 1.25 ml

3/Storage

Store at -30 ~ -15°C and protect from light. Ship at ≤0°C. Once thawed, the Master Mix can be stably stored at 2 ~ 8°C under light-protected conditions for up to 6 months.

4/Applications

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

5/Notes

1. If precipitation is observed after thawing the Master Mix, allow it to sit at room temperature briefly. Gently invert the tube until the precipitate is fully dissolved before use.
2. Repeated freeze-thaw cycles should be avoided to prevent loss of enzyme activity. For smaller usage volumes, aliquoting is recommended.
3. Prior to use, gently invert the Master Mix to ensure thorough mixing. Do not vortex to prevent bubble formation, which may interfere with accurate quantification. After mixing, briefly centrifuge the Master Mix before use. During pipetting, avoid vigorous actions; if bubbles form, centrifuge again before proceeding.
4. This product contains the fluorescent dye SYBR Green I and should be stored protected from light. Minimize exposure to strong light during reaction setup to preserve dye integrity
5. The product's high sensitivity makes it susceptible to contamination from aerosols. It is strongly recommended to prepare the reaction system in a clean bench using sterilized pipette tips and reaction

tubes. Whenever possible, use dedicated pipettes and filter tips to further reduce the risk of contamination.

6. The blue dye included in this product has been validated to not interfere with the fluorescence signal detection of SYBR Green I.

6/Experiment Process

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

Components	Volume
2 × ChamQ Blue Universal SYBR qPCR Master Mix	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	Rep: 1	95°C	30 sec[a]
Stage 2	Cycles	Reps: 40	95°C	3 - 10 sec[b]
			60°C*	10 - 30 sec[c]
Stage 3	Melting Curve	Default Instrument Settings		

a. The initial denaturation condition is suitable for most amplification reactions. For complex template structures, the initial denaturation time can be extended to 5 min to enhance the effect.

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

c. For standard protocols, set 30 sec; for fast protocols: for amplicons within 200 bp, the minimum extension time can be set to 10 sec; for amplicons exceeding 200 bp, an extension time of 30 sec is recommended.

* Fluorescence Signal Acquisition.

7/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	Increase the annealing temperature (increase by 3°C each time)
95°C/10 sec	95°C/10 sec
60°C/30 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	Extend the extension time	Three-step Protocol	Extend the extension time
95°C/10 sec	95°C/10 sec	95°C/10 sec	95°C/10 sec
60°C/30 sec	60°C/30 sec	56°C/30 sec	56°C/30 sec
		72°C/30 sec	72°C/60 sec

8/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 CT 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 gDNA removal step during RNA preparation 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.