Universal SYBR Green qPCR Master Mix

NB-64-97262-1mL
NB-64-97262-5mL

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#Cat: NB-64-97262-1mL Size: 1mL
#Cat: NB-64-97262-5mL Size: 5mL

Description:

Universal SYBR Green qPCR Master Mix is a ready-to-use 2× concentrated premix formulated for real-time quantitative PCR (qPCR). It contains a hot-start DNA polymerase, SYBR Green I fluorescent dye, dNTPs, Mg^{2+}, and an optimized ROX reference dye, making it compatible with all qPCR instruments. To perform a reaction, simply add the DNA template, primers, and nuclease-free water—streamlining the workflow and minimizing the risk of contamination. This Master Mix incorporates a temperature-responsive polymerase activator that precisely regulates enzyme activity in real time. The formulation also includes proprietary components that effectively suppress non-specific amplification and enhance overall reaction efficiency. As a result, it enables accurate and reproducible quantification of target genes across a broad dynamic range, ensuring high sensitivity, specificity, and reliability in qPCR applications.

Application:

1. Detection by quantitative real-time PCR (qPCR).
2. Nucleic acid amplification and gene expression analysis.
3. Automated and high-throughput quantitative genotyping.
4. Genomics-related research.

Storage:

1. Store at -20 °C protected from light. Stable for 2 years.
2. Avoid repeated freeze-thaw cycles. Prepare aliquots for future use.
3. Contains the fluorescent dye SYBR Green I. Avoid exposure to strong light.

Prepare Reaction Mix

Example: Volume of 50 µL and 20 µL

Component	Volume(µL)	Volume(µL)	Final Concentration
qPCRSYBRGreenMasterMix	25	10	1×
ForwardPrimer(10µM)	1	0.4	0.2µM
ReversePrimer(10µM)	1	0.4	0.2µM
DNA	X	X	
ddH2O	To 50	To 20	
Volume	50	20	

*It is recommended to prepare on ice.

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Note: Mix thoroughly before use. Avoid vigorous shaking to prevent excessive bubble formation.

a) Primer concentration: 0.2 µM of primer final concentration is applicable for most cases. The concentration can be adjusted within 0.1~1.0 µM when amplification efficiency is not satisfactory.
b) Template concentration: If using undiluted cDNA as the template, the volume should not exceed 1/10 of the total qPCR reaction volume.

c) Template dilution: A 5–10 fold dilution of cDNA is recommended. The optimal amount of template should yield a Ct value between 20 and 30 cycles.

d) Reaction volume: A reaction volume of 20 µL or 50 µL is recommended to ensure effective and reproducible amplification of the target gene.

e) Reaction setup: Prepare the reaction mix in a biosafety cabinet using nuclease-free pipette tips and tubes. Filter tips are strongly recommended. Take care to avoid cross-contamination and aerosol generation.

Perform Quantitative PCR

Two-step PCR Program

Step	1	2		3
	Hot-Start DNA Polymerase Activation	PCR		Melt Curve
	Hold	40 cycles		1 cycle
		Denature	Anneal/Extend	
Temp	95°C	95°C	60°C	/
Time	5 min	10 sec	30 sec	
Volume	20 µL-50 µL			

Three-step PCR Program

Step	1	2			3
	Hot-Start DNA Polymerase Activation	PCR			Melt Curve
	Hold	40 cycles			1 cycle
		Denature	Anneal	Extend	
Temp	95°C	95°C	55-60°C	72°C	/
Time	5 min	20 sec	20 sec	20 sec	
Volume	20 µL-50 µL				

Note: For higher specificity, a two-step protocol is recommended. For higher amplification efficiency, consider using a three-step protocol.
a) Initial Denaturation Time: Depending on the template and primers, the time can be shortened to 2 minutes if appropriate.
b) Annealing Temperature and Time: Adjust according to the primer sequences and the length of the target gene.
c) Fluorescence Signal Acquisition (*): Set the cycling program according to the instrument manufacturer' s instructions.
Recommended acquisition times for common instruments are as follows:
≥30 sec: Applied Biosystems: StepOne, StepOne Plus, 7500 Fast
Roche Applied Science: LightCycler 480
Bio-Rad: CFX96
≥31 sec: Applied Biosystems: 7300
≥34 sec: Applied Biosystems: 7500

Data Analysis

1. Quantitative experiments require at least three biological replicates. After the reaction is completed, it is necessary to check the amplification curve and the melting curve.
2.Amplification curve:
The typical Ct value range is between 15 and 35, with the most accurate quantification usually falling between 20 and 28.

- If the Ct value is too low, the template should be diluted.
- If the Ct value is too high, increase the template concentration, increase primer concentration, or optimize the qPCR program accordingly.

3.Melting curve:
Generally, only a single peak in the melting curve indicates a valid quantification result.

- If multiple peaks appear in the melting curve, experimental conditions need to be optimized.
- Common solutions include redesigning the primers, among others.

Attention

1.Storage at -80°C may cause the formation of white or pale yellow precipitates. To redissolve, gently warming the tube in your hand. Leave the mix at room temperature protected from light for a short period. Gently invert the tube until the precipitate is completely dissolved. The performance of the product will not be affected.
2.Before use, gently invert the tube several times to mix and avoid bubbles. Brief centrifugation prior to use is recommended.
3.Avoid repeated freeze-thaw cycles to maintain polymerase activity. Aliquot the mix for future frequent use.
4.Keep the mix from bright light during storage and usage due to the fact that the SYBR Green I may fade under light over time,resulting in a decrease in performance sensitivity.
5.Due to the high sensitivity nature of the qPCR reaction, contamination of air or aerosols may lead to reaction failure or result inaccuracy. Please set up the qPCR reaction in a clean environment using filtered tips, and sterilized tubes and pipette sets.
6.For your safety and health, please wear a lab coat and disposable gloves during operation. When preparing

the reaction mix or aliquoting the product, always use new, uncontaminated pipette tips and microtubes to avoid cross-contamination.

7. This product is intended for use by qualified professionals for scientific research only. It is not for clinical diagnosis or treatment, not for use in food or pharmaceuticals, and must not be stored in residential areas.