PanGreen™ Universal SYBR Green Mastermix UNG

29 JAN 2026

Catalog Number	Size	Concentration
QSD02-0100	100 reactions (20 µl vol)/40 reactions (50 µl vol)	2X

Storage Conditions

Stable for up to 24 months at -20°C.

Description

This product is a new contamination-proof, dye-based real-time PCR master mix developed, based on PanGreen™ Universal MasterMix (Cat. QSD01-0100), by adding an optimized ratio of dUTP and UNG enzymes. It contains optimized concentrations of the Hotstart DNA polymerase, SYBR Green I, dNTPs, dUTP, UNG enzyme (Uracil DNA glycosylase), Mg^{2+}, reaction buffer, and stabilizer. In the PCR reaction, dUTP is used instead of dTTP, and the T in the amplification product fragment is replaced by the U to form a PCR amplification product containing dU bases, and the highly active UNG enzyme can quickly degrade the U-containing DNA fragment in the reaction system, effectively eliminating the residual contamination of PCR products in the environment and greatly reducing the false positive caused by the amplification product contamination, thus ensuring the specificity and accuracy of the amplification. This product is a 2×contamination-proof, chemically modified real-time PCR premix reaction system, only requiring to add the template, primers, and water to make its working concentration 1× for carrying out the reaction (ROX-independent and ROX-dependent). It has the advantages of rapidness and simplicity, high sensitivity, strong specificity, and good stability, which can minimize the human error, save PCR experimental operation time, and reduce the probability of contamination.

Kit Content(s)

2X Universal SYBR® Green Master Mix (UNG)	1 ml x 1 vial
High ROX Reference Dye	40 µl x 1 vial
Low ROX Reference Dye	40 µl x 1 vial

Required materials but not provided

- A compatible real-time PCR instrument
- Vortex or equivalent
- Microcentrifuge
- Plates and seals for your instruments

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Instrument Compatibility

Instrument	ROX
ABI Prism7000/7300/7700/7900HT, ABI Step One, ABI Step One Plus	High ROX reference dye
ABI Prism 7500/7500 Fast, MJ Research Chromo4, Option (II), Corbett Rotor Gene 3000	Low ROX reference dye
Thermal Cycler Dice Real Time System, LightCycler, Smart Cycler System, Corbett Rotor-gene 6000, Agilent Technologies Mx3000P	Without ROX reference dye needed

Reaction Setup

1 Thaw PanGreen™ Universal SYBR® Green Master Mix (UNG) and the rest of the frozen reaction components to a temperature of 4°C. To entirely collect solutions, combine thoroughly and centrifuge briefly, then store at 4°C and avoid light.

2 Prepare (on ice or at room temperature) enough assay Master Mix for all reactions by adding all necessary components, except the DNA template, according to the recommendations in the table below.

Table 1. Reaction Setup without ROX Reference Dye

Components	Volume per 20 µl Reaction	Volume per 50 µl Reaction
2X Universal SYBR® Green Master Mix (UNG)	10 µl	25 µl
Forward and reverse primers [a]	1 µl	2.5 µl
DNA template [b]	1 µl	1 µl
Nuclease-free H_2O	Variable	Variable
Total reaction mix volume	*20 µl*	*50 µl*

Table 2. Reaction Setup with ROX Reference Dye

Components	Volume per 20 µl Reaction	Volume per 50 µl Reaction
2X Universal SYBR® Green Master Mix (UNG)	10 µl	25 µl
Forward and reverse primers [a]	1 µl	2.5 µl
DNA template [b]	1 µl	1 µl
High ROX Reference Dye or Low ROX Reference Dye [c]	0.4 µl	1 µl
Nuclease-free H_2O	Add to 20 µl	Add to 50 µl
Total reaction mix volume	*20 µl*	*50 µl*

[a] Primer concentration: Typically, optimal results are achieved with a primer concentration of 0.2 µM, with a suggested range of 0.1-1.0 µM. Adjustments can be made to the primer concentration to enhance amplification efficiency or mitigate nonspecific reactions. To achieve ideal qPCR outcomes, aim for amplification fragments ranging from 80 to 200 bp in length.

[b] Template: Use 0-100 ng of genomic DNA or 1-10 ng of cDNA as a reference. Due to variations in the copy numbers of target genes among different species, gradient dilutions of the template can be performed to determine the optimal amount for usage. Additionally, when using cDNA from Two-step RT-PCR reactions as a template, ensure that the amount added does not exceed 10% of the total volume of the PCR reaction mixture.

[c] ROX Reference Dye requirement varies among different instruments, and its addition may be necessary or unnecessary. Please consult the instrument manual for specific instructions.

3 Combine the assay Master Mix thoroughly to ensure consistency and equally dispense the solution into each qPCR tube or into the wells of a qPCR plate. Employ good pipetting practice to ensure assay precision and accuracy.

Note: to ensure thorough mixing of reaction components, vortex for approximately 30 seconds (or more).

4 Spin the tubes or plate to remove any air bubbles and collect the reaction mixture in the vessel bottom.

5 Setup the thermal cycling protocol on a real-time PCR instrument according to Step 5.1 and 5.2.

Note: optimization may be needed for better performance.

Thermal Cycling Protocol

Starting with a two-step PCR reaction protocol is recommended, and if it doesn't yield satisfactory results, PCR conditions can be optimized. In situations where the amplification performance of the two-step PCR reaction is subpar, possibly due to factors like the use of primers with lower Tm values or longer amplification fragments, transitioning to a three-step PCR amplification reaction can be considered.

5.1 Two-step reaction setting

Procedure	Time and Temperature	
UNG enzyme treatment	5 minutes at 50°C	
Initial Denaturation	10 minutes at 95°C	
Denaturation	15 seconds at 95°C	40 cycles
Annealing- Extension	1 minutes at 60°C	
Melting curve		

5.2 Three-step reaction setting

Procedure	Time and Temperature	
UNG enzyme treatment	5 minutes at 50°C	
Initial Denaturation	10 minutes at 95°C	
Denaturation	15 seconds at 95°C	40 cycles
Annealing	30 seconds at 60°C	
Extension	30 seconds at 72°C	
Melting curve		

Note: The Hotstart DNA Polymerase utilized in this product is chemically modified to better suppress background activity, remaining inactive at temperatures below 75°C. This effectively prevents nonspecific amplification resulting from primer-template nonspecific binding or primer dimer formation at room temperature. Activation of the enzyme requires incubation at 95°C for 10 minutes. Reaction mixtures can be prepared at room temperature without the need for ice, offering convenience in operation. Please note that the examples provided are for standard qPCR reaction systems and are intended for reference purposes only. Actual reaction conditions may vary depending on the structure of the template, primers, and target fragments, necessitating optimization based on their characteristics. Adjustments to the reaction system should be made accordingly.

6 Load the PCR tubes or plate into the real-time PCR instrument and commence the run.

7 Perform data analysis according to the instrument-specific instructions.

Template

Purified high-quality DNA is needed for a successful PCR reaction. For the final concentration of cDNA template please refer to the table in Reaction Setup.

Important notes

1. Shake gently before use to avoid foaming and low-speed centrifugation.
2. During operation, always wear a lab coat, disposable gloves, and protective equipment.
3. The ROX reference dye keeps away from light.

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Troubleshooting

Refer to Table 3 below to troubleshoot problems that you may encounter when quantifying of nucleic acid targets with the kit.

Table 3 Troubleshooting

Trouble	Cause	Solution
Poor Signal or No Signal	Inhibitor Present	1. Perform a dilution series of the PCR template to determine whether the effect of the inhibitory agent can be reduced. 2. Take extra care with the nucleic acid extraction steps to minimize carryover of PCR inhibitors.
	Degraded Template Material	1. Do not store diluted templates in water or at low concentrations. 2. Check the integrity of template material by automated or manual gel electrophoresis.
	Inadequate Thermal Cycling Conditions	1. Try using a minimum extension time of 30 sec for genomic DNA and 15 sec for cDNA.
Signal in Negative Control	Contamination of Reaction Components with Target Sequence	1. To minimize the possibility of contamination of PCR components by PCR product or other template, designate a work area exclusively for PCR assay setup. 2. Use a solution of 10% bleach instead of ethanol to prepare the workstation area for PCR assay setup. Ethanol will only induce precipitation of DNA in your work area, while the 10% bleach solution will hydrolyze, as well as dissolve, any residual DNA.
Poor Reproducibility Across Replicate Samples	Inhibitor Present	1. Perform a dilution series of the PCR template to determine whether the effect of the inhibitory agent can be reduced. 2. Take extra care with the nucleic acid extraction steps to minimize carryover of PCR inhibitors.
	Primer Design	1. Verify primers design at different annealing temperatures.
Low or High Reaction Efficiency	Primer- Dimer	1. Reduce primer concentration. 2. Evaluate primer sequences for complementarity and secondary structure. Redesign primers if necessary. 2. Perform melt-curve analysis to determine if primer-dimers are present.
	Insufficient Optimization	3. Use a thermal gradient to identify the optimal thermal cycling conditions for a specific primer set.