PanProbes™ Universal qPCR MasterMix UNG
PanProbes™ Universal qPCR MasterMix UNG w High ROX
PanProbes™ Universal qPCR MasterMix UNG w Low ROX

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Catalog Number	Size	Concentration
QPD02-0100	100 reactions (20 µl vol)/40 reactions (50 µl vol)	2X
QPD02-H100	100 reactions (20 µl vol)/40 reactions (50 µl vol)	2X
QPD02-L100	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 the PanProbes™ Universal qPCR MasterMix (Cat. QPD01-0100), by adding an optimized ratio of dUTP and UNG enzymes. It contains optimized concentrations of the Hotstart DNA polymerase, 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, probe-based real-time PCR premix reaction system, only requiring to add the template, primers, probe, ROX Reference Dye (used according to different real-time PCR instruments) and water to make its working concentration 1× for carrying out the reaction. 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)

QPD02-0100	2X Universal qPCR Master Mix (UNG)	1 ml x 1 vial
QPD02-H100	2X Universal qPCR Master Mix (UNG)	1 ml x 1 vial
	High ROX Reference Dye	40 µl x 1 vial
QPD02-L100	2X Universal qPCR Master Mix (UNG)	1 ml 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

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 PanProbes™ Universal qPCR Master Mix (UNG) and the rest of frozen reaction components to a temperature of 4°C. In order to entirely collect solutions, combine thoroughly and centrifuge briefly, then store at 4°C and avoid from 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 table below.

Components	Volume per 20 µl Reaction			Volume per 50 µl Reaction		
	QPD02-0100	QPD02-H100	QPD02-L100	QPD02-0100	QPD02-H100	QPD02-L100
2X PanProbes™ Universal qPCR Master Mix (UNG)	10 µl			25 µl		
Forward primer [a]	0.5 µl			1 µl		
Reverse primer [a]	0.5 µl			1 µl		
Probe [b]	0.5 µl			1 µl		
DNA template [c]	0.4 µl			1 µl		
High ROX Reference Dye[d]	-	0.4 µl	-	-	1µl	-
Low ROX Reference Dye[d]	-	-	0.4 µl	-	-	1 µl
Nuclease-free H_2O	Add to 20 µl			Add to 50 µl		
Total Volume	20 µl			50 µl		

[a] Primers: Typically, a primer concentration of 0.2 µM yields satisfactory results, with a recommended range of 0.1-1.0 µM as a guideline.

[b] Probe concentration is contingent upon the specific fluorescent quantitative PCR instrument, probe type, and fluorescent labeling substance employed. Consult the instrument manual or specific requirements for each fluorescent probe when adjusting the concentration.

[c] DNA Template: Utilize 10-100 ng of genomic DNA or 1-10 ng of cDNA. Since the copy numbers of target genes vary among different species, performing gradient dilutions of the template can determine the optimal amount for usage. Adjusting the primer concentration can enhance amplification efficiency or minimize nonspecific reactions. To achieve optimal qPCR performance, aim for target amplification fragments with lengths ranging from 80 to 200 bp.

[d] Different instruments necessitate varied ROX Reference Dyes or may require the addition or omission of this dye. Please consult the instrument manual for appropriate handling procedures.

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 Table 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 mins at 50°C	
Initial Denaturation	10 mins at 95°C	
Denaturation	15 secs at 95°C	35-45 cycles
Annealing- Extension	30 secs at 60°C	

Note: Optimal conditions for amplification will vary depending on the primers and thermal cycler used. It may be necessary to optimize the system for individual primers, template, and thermal cycler.

5.2 Three-step reaction setting

Procedure	Time and Temperature	
UNG enzyme treatment	5 mins at 50°C	
Initial Denaturation	10 mins at 95°C	
Denaturation	15 secs at 95°C	35-45 cycles
Annealing	15-30 secs at 55-65°C	
Extension	30 secs at 72°C	

Note: Optimal conditions for amplification will vary depending on the primers and thermal cycler used. It may be necessary to optimize the system for individual primers, template, and thermal cycler.

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 DNA template please refer to Table 1.

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.

Troubleshooting

Refer to Table 1 below to troubleshoot problems that you may encounter when quantifying of nucleic acid targets with the kit.

Table 1. 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. 3. Perform melt-curve analysis to determine if primer- dimers are present.
	Insufficient Optimization	Use a thermal gradient to identify the optimal thermal cycling conditions for a specific primer set.

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