



# PanGreen™ Universal MasterMix w High ROX

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Catalog Number	Size	Concentration
QSD01-H100	100 reactions (20 µl vol)	2X

## Storage Conditions

Stable for up to 3 months at 4°C.

Stable for up to 24 months at -20°C.

## Description

PanGreen™ Universal MasterMix w High ROX is a 2x concentrated, ready for use Master Mix reaction enhanced for dye-based quantitative PCR (qPCR) and compatible with the majority of commercially available real-time PCR systems (ROX-independent and ROX-dependent). It contains NanoTaq hot-start DNA polymerase, dNTPs, MgCl<sub>2</sub>, SYBR® Green I dye, enhancers, stabilizers and essentials for a success PCR reaction. PanGreen™ Universal SYBR® Green Master Mix is a new generation qPCR Master Mix which can greatly reduce the time of qPCR reaction to 30-40 minutes. Note: The reaction time may vary due to a bigger PCR product sequence or the efficiency of the primers.

## Kit Content(s)

2X Universal SYBR® Green Master Mix	1ml x 1 vial
High 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

## Reaction Setup

1. Thaw PanGreen™ Universal SYBR® Green Master Mix 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.





- 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 1 (below).

Components	Volume per 20 $\mu$ l Reaction	Volume per 10 $\mu$ l Reaction	Final Concentration
PanGreen™ Universal SYBR® Green Master Mix (2x)	10 $\mu$ l	5 $\mu$ l	1x
Forward and reverse primers	Variable	Variable	300–500 nM each primer
DNA template ( <b>add at step 4</b> )	Variable	Variable	cDNA: 1pg–10ng Genomic DNA: 50ng–250ng
High ROX Reference Dye	0.4 $\mu$ l	0.2 $\mu$ l	500 nM
Nuclease-free H <sub>2</sub> O	Variable	Variable	—
<i>Total reaction mix volume</i>	<i>20 <math>\mu</math>l</i>	<i>10 <math>\mu</math>l</i>	—

- 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.
- Add DNA samples (and DNase-free H<sub>2</sub>O if needed) to the PCR tubes or wells containing assay Master Mix (Table 1), seal the tubes or wells with flat caps or optically transparent film. **Note:** to ensure thorough mixing of reaction components, vortex for approximately 30 seconds (or more).
- Spin the tubes or plate to remove any air bubbles and collect the reaction mixture in the vessel bottom.
- Setup the thermal cycling protocol on a real-time PCR instrument according to Table 2. **Note:** optimization may be needed for better performance.
- Load the PCR tubes or plate into the real-time PCR instrument and commence the run.
- Perform data analysis according to the instrument-specific instructions.
  - Process in the thermal cycler for 35~45 cycles as follows:

Initial Denaturation	3-5 minutes at 95°C (5 mins for GC rich or complex templates)
Denaturation	15 seconds at 95°C
Annealing & Extension	60 seconds at 60°C and Plate Read
Melting curve	Refer to specific guidelines for instrument used

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.



## Template

Purified high quality DNA is needed for a success PCR reaction. The final concentration of cDNA 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.
3. The ROX reference dye keeps away from light.

## Troubleshooting

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

Trouble	Cause	Solution
Poor Signal or No Signal	Inhibitor Present	<ol style="list-style-type: none"> <li>1. Perform a dilution series of the PCR template to determine whether the effect of the inhibitory agent can be reduced.</li> <li>2. Take extra care with the nucleic acid extraction steps to minimize carryover of PCR inhibitors.</li> </ol>
	Degraded Template Material	<ol style="list-style-type: none"> <li>1. Do not store diluted template in water or at low concentrations.</li> <li>2. Check the integrity of template material by automated or manual gel electrophoresis.</li> </ol>
	Inadequate Thermal Cycling Conditions	<ol style="list-style-type: none"> <li>1. Try using a minimum extension time of 30 sec for genomic DNA and 15 sec for cDNA.</li> </ol>
Signal in Negative Control	Contamination of Reaction Components with Target Sequence	<ol style="list-style-type: none"> <li>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.</li> <li>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.</li> </ol>
Poor Reproducibility Across Replicate Samples	Inhibitor Present	<ol style="list-style-type: none"> <li>1. Perform a dilution series of the PCR template to determine whether the effect of the inhibitory agent can be reduced.</li> <li>2. Take extra care with the nucleic acid extraction steps to minimize carryover of PCR inhibitors.</li> </ol>
	Primer Design	<ol style="list-style-type: none"> <li>1. Verify primers design at different annealing temperatures.</li> </ol>





Low or High Reaction Efficiency	Primer- Dimer	<ol style="list-style-type: none"> <li>1. Reduce primer concentration.</li> <li>2. Evaluate primer sequences for complementarity and secondary structure. Redesign primers if necessary.</li> <li>3. Perform melt-curve analysis to determine if primer- dimers are present.</li> </ol>
	Insufficient Optimization	<ol style="list-style-type: none"> <li>1. Use a thermal gradient to identify the optimal thermal cycling conditions for a specific primer set.</li> </ol>