PanGreen™ One-Step RT-qPCR Kit w Low ROX

03 JUL 2023

Catalog Number	Size	Concentration
QSR01-L100	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™ One-Step RT-qPCR Kit w Low ROX delivers high sensitivity of the target RNA level due to its RScript reverse transcriptase, a reduced RNase H+ activity MMLV enzyme in addition to a powerful RNase inhibitors mix which aim to diminish RNA degradation and mispriming during reaction setup and reverse transcription to guarantee optimal RT efficiency.

The Universal SYBR® Green Master Mix 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 hot-start Taq DNA polymerase, dNTPs, $MgCl_2$, SYBR® Green I dye, enhancers, stabilizers and essentials for a success PCR reaction.

Kit Content(s)

2X Universal SYBR® Green Master Mix	1 ml x 1 vial
RScript Enzyme Mix	20 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

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

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Reaction Setup

1. Thaw RScript Enzyme mix, 2X 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.
2. Prepare (on ice or at room temperature) enough assay Master Mix for all reactions by adding all necessary components, except the RNA template, according to the recommendations in Table 1 (below).

Table 1. Reaction Setup

Component	Volume per 20 µl Reaction	Volume per 10 µl Reaction	Final Concentration
2X Universal SYBR® Green Master Mix	10 µl	5 µl	1x
RScript Enzyme mix (RScript reverse transcriptase & RNase inhibitor)	0.2 µl	0.1 µl	1x
Forward and reverse primers	Variable	Variable	300 nM* each
RNA **(add at step 4)**	Variable	Variable	Total RNA: 1 ng – 5 µg
Low ROX Reference Dye	0.4 µl	0.2 µl	50 nM
Nuclease-free H_2O	Variable	Variable	---
Total reaction setup volume	*20 µl*	*10 µl*	---

* Optimization may be needed for better performance.

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.
4. Add RNA template (and DNase-free H_2O 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).
5. Spin the tubes or plate to remove any air bubbles and collect the reaction mixture in the vessel bottom.
6. Setup the thermal cycling protocol on a real-time PCR instrument according to Table 2. **Note:** optimization may be needed for better performance.
7. Load the PCR tubes or plate into the real-time PCR instrument and commence the run.
8. Perform data analysis according to the instrument-specific instructions.

- Process in the thermal cycler for 35~45 cycles as follows:

Table 2. Thermal Cycling Protocol

Steps	Temperature	Time	Cycle(s)
cDNA Synthesis	42°C	15 minutes	1
Pre-Denaturation	95°C	5 minutes	1
Denaturation	95°C	10 seconds	35~45
Annealing	60°C	60 seconds	
Instrument Cooling	40°C	10 seconds	1

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 RNA is needed for a success RT-qPCR reaction. The final concentration of RNA 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 the table 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	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 template 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	1. Use a thermal gradient to identify the optimal thermal cycling conditions for a specific primer set.