



# PanGreen™ Universal One-Step RT-qPCR Kit

09 DEC 2020

Catalog Number	Size	Concentration
QSR01-0100	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 One-Step RT-qPCR Kit 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<sub>2</sub>, SYBR® Green I dye, enhancers, stabilizers and essentials for a success PCR reaction.

## Kit Content(s)

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

This Master Mix is compatible with the majority of commercially available real-time PCR systems.

## Reaction Setup

1. Thaw RScript Enzyme mix, 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 RNA template, according to the recommendations in Table 1 (below).

Component	Volume per 20 $\mu$ l Reaction	Volume per 10 $\mu$ l Reaction	Final Concentration
PanGreen™ universal SYBR® Green reaction mix (2x)	10 $\mu$ l	5 $\mu$ l	1x
RScript Enzyme mix (RScript reverse transcriptase & RNase inhibitor)	0.2 $\mu$ l	0.1 $\mu$ l	1x
Forward and reverse primers	Variable	Variable	300 nM* each
RNA (add at step 4)	Variable	Variable	Total RNA: 1 ng – 5 $\mu$ g
Nuclease-free H <sub>2</sub> O	Variable	Variable	—
<i>Total reaction setup volume</i>	<i>20 <math>\mu</math>l</i>	<i>10 <math>\mu</math>l</i>	—

\* Optimization may be needed for better performance.

- 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 RNA template (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:

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 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>