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PanProbes™ One-Step RT-qPCR Kit UNG
PanProbes™ One-Step RT-qPCR Kit UNG w High ROX
PanProbes™ One-Step RT-qPCR Kit UNG w Low ROX

Catalog Number	Size	Concentration
QPR02-0100	100 reactions (30 µl vol)	2X
QPR02-H100	100 reactions (30 µl vol)	2X
QPR02-L100	100 reactions (30 µl vol)	2X

Storage Conditions

Stable for up to 24 months at -20°C.

Description

The PanProbes™ One-Step RT-qPCR Kit UNG is a one-step multiplex qRT-PCR test cassette. This kit has been specially optimized to effectively complete 1-4 qRT-PCR tests with high sensitivity and specificity. During the experiment, cDNA synthesis and qPCR reactions were done in the same reaction system, simplifying the experimental operation and reducing the risk of contamination. In addition, the dUTP/Heat-sensitive UNG anti-contamination system is used in this kit to effectively prevent gas-soluble contamination, and the heat-sensitive UNG can be quickly inactivated during reverse transcription, ensuring the expansion efficiency of qRT-PCR. This product is suitable for multiplex detection of RNA viruses and trace RNA samples, with sensitivity up to 1 pg of total RNA or < 10 copies of RNA samples.

Kit Content(s)

QPR02-0100	RT-qPCR Enzyme Mix (UNG)	150 µl x 1 vial
	2X Universal qPCR Master Mix UNG (dUTP)	1.5 ml x 1 vial
QPR02-H100	RT-qPCR Enzyme Mix (UNG)	150 µl x 1 vial
	2X Universal qPCR Master Mix UNG (dUTP)	1.5 ml x 1 vial
	High ROX Reference Dye	40 µl x 1 vial
QPR02-L100	RT-qPCR Enzyme Mix (UNG)	150 µl x 1 vial
	2X Universal qPCR Master Mix UNG (dUTP)	1.5 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

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

Component	Volume		
	QPR02-0100	QPR02-H100	QPR02-L100
RNA Template [a]	1-5 µl		
Primer & Probe Mix [b]	X µl		
RT-qPCR Enzyme Mix (UNG)	15 µl		
2X Universal qPCR Master Mix UNG (dUTP)	1.5 µl		
High ROX Reference Dye	-	0.4 µl	-
Low ROX Reference Dye	-	-	0.4 µl
Nuclease-free Water	Add to 30 µl		
Total volume	30 µl		

[a] qPCR is extremely sensitive, and it is recommended to dilute the template with a Ct value between 20 and 35.

[b] The Primer&Probe Mix can contain multiple pairs of primers and probes, typically at a primer concentration of 0.2 µM, which can be adjusted in the range of 0.1-1.0 µM depending on amplification, and a final probe concentration in the range of 0.05-0.5 µM.

2 Reaction setting

2.1 Two-step reaction setting

Procedure	Time and Temperature	
cDNA Synthesis	10 minutes at 55°C	
Initial Denaturation	2 minutes at 95°C	
Denaturation	15 seconds at 95°C	35-45 cycles
Annealing- Extension [c]	45-60 seconds at 60°C	

[c] The extension time should be adjusted according to the minimum time limit required for the information you are using with the Real-Time PCR instrument: at least 30 seconds with ABI 7700 and 7900HT, at least 31 seconds with ABI 7000 and 7300, and at least 34 seconds with ABI 7500.

2.2 Three-step reaction setting

Procedure	Time and Temperature	
cDNA Synthesis	10 minutes at 55°C	
Initial Denaturation	2 minutes at 95°C	
Denaturation	15 seconds at 95°C	35-45 cycles
Annealing	15 seconds at 55-65°C	
Extension	30 seconds at 72°C	
Melting curve		

[d] When the two-step amplification efficiency is not good, it is recommended to choose the three-step method for qPCR reactions.

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

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

Template

Purified high-quality RNA is needed for a successful RT-qPCR reaction. For the final concentration of the RNA template please refer to Reaction Setup step 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. This product can only use gene-specific primers, and cannot use Random Primer and Oligo18 (dT) for reverse transcription reactions.
4. When performing multi-tube qRT-PCR reactions, it is recommended to prepare a mix of premixed reagents and then aliquot into each reaction tube to reduce experimental errors.
5. The ROX reference dye keeps away from light.

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. 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.