PhoenixDx® RT-qPCR Kit – Performance & Robust

for research use

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Introduction

PhoenixDx® RT-qPCR Kit – Performance & Robust contain everything needed for a successful RT-PCR experiment. The 2X qPCR Mix contains dNTPs and an optimized buffer formulation to provide the right environment for successful reverse transcription and PCR amplification in one reaction. The 20X RT Enzyme Mix provides the enzymes for RT-PCR: VitaScript® Reverse Transcriptase for precise and sensitive reverse transcription of RNA to cDNA and VitaTaq® DNA Polymerase for fast and reliable amplification of your sequence of interest. Procomcure's proprietary HotStart antibody grants full control over the reaction start and protects the enzymes under harsh reaction conditions.

PhoenixDx® RT-qPCR Kit – Robust is also highly resistant to a broad field of PCR inhibitors such as heparin (blood samples), serum or humic acid (soil / plant samples). This makes it a flexible solution compatible with many different sample types. **RT-qPCR Kit – Robust** can be used for many qPCR-based applications such as gene expression studies, RNA virus detection and many more.

PhoenixDx® RT-qPCR Kit – Performance is optimized for fast amplification and multiplex applications making it the best option when high throughput is required.

The reverse transcription reaction uses a novel VitaScript® Reverse Transcriptase with increased performance, specificity and reduced RNAse H activity.

In 1Step-RT-PCR, reverse transcription and PCR amplification are combined in 1 reaction granting several advantages:

- Minimal handling steps reduce the risk of errors
- Experiment setup and running time are reduced
- A high sample number can be screened easily

PhoenixDx® RT-qPCR Kit – Performance is designed for probe-based qPCR and therefore dye-free. If normalization via the ROX channel is required by the device in use, ROX Passive Reference Dye is provided separately as a 100X solution. ROX is added when using PCR cyclers that perform fluorescence signal correction between wells such as a real-time PCR machines made by Applied Biosystem, e.g. the 7500 Real-Time PCR System. It is not necessary for devices that perform a baseline normalization e.g. the CFX series (Bio-Rad) or the LightCycler Series (Roche Diagnostics). **PhoenixDx® RT-qPCR Kit – Robust** already contains the ROX Passive Reference Dye in a universal concentration compatible with low and high ROX cyclers.

The qPCR Kits do not contain primer or fluorescent probes of any kind, these need to be provided by the user. **PhoenixDx® RT-qPCR Kit – Performance** requires previous RNA isolation, materials for isolation need to be provided by the user. Depending on the sample's matrix, **PhoenixDx® RT-qPCR Kit – Robust** can be used without prior RNA isolation.

PhoenixDx® RT-qPCR Kit – Performance

Component	Volume		Notes
	200 rxn	10 000 rxn	
2X qPCR Mix Performance	2 x 1 ml	1 x 100 ml	Contains dNTPs and qPCR Buffer
20X RT Enzyme Mix	1 x 200 µl	1 x 10 ml	Contains HotStart Antibody, VitaScript® Reverse Transcriptase and VitaTaq® DNA Polymerase
100X ROX Passive Reference Dye	1 x 100 µl	1 x 5 ml	Use Passive Reference Dye as 1X if the device requires ROX normalization

PhoenixDx® RT-qPCR Kit – Robust

Component	Volume		Notes
	200 rxn	10 000 rxn	
2X qPCR Mix Robust	2 x 1 ml	1 x 100 ml	Contains dNTPs and qPCR Buffer
20X RT Enzyme Mix	1 x 200 µl	1 x 10 ml	Contains HotStart Antibody, VitaScript® Reverse Transcriptase and VitaTaq® DNA Polymerase

Storage

Store all components at -20°C and avoid repeated freezing and thawing. Protect ROX Passive Reference Dye from light.

Additional Materials Required

- qPCR device equipped and calibrated for your fluorophore of choice
- suitable PCR plastics for your device
- Adjustable pipettes and filtered tips
- nuclease-free tubes
- nuclease-free dH_2O
- materials for RNA Isolation

Before Starting

Design primers to span an intron in your gene of interest to make sure that only cDNA but no genomic DNA is amplified.

Before starting the experiment, thaw all components on ice, mix gently but thoroughly and spin down quickly to collect all liquid at the bottom of the tube.

Program your PCR device with a suitable program for your application. An example for a 1Step-RT-PCR protocol is given below. Please note that individual adjustments will be necessary depending on your experiment.

3-Step Standard Protocol

Step	Cycles	Temperature	Time
Reverse Transcription	1	50°C	5 min
Inactivation of VitaScript® Activation of VitaTaq®	1	95°C	5 min
Denaturation	40	95°C	5 sec
Annealing		55°C[1]	20 sec
Extension		72°C	20 sec[1] ***enable data collection***

[1] The Annealing Temperature depends on your Primer/Probe Set, adjust accordingly. The extension time depends on the size of your target sequence, adjust accordingly.

2-Step Standard Protocol

Step	*Cycles*	*Temperature*	*Time*
Reverse Transcription	1	50°C	5 min
Inactivation of VitaScript® Activation of VitaTaq®	1	95°C	5 min
Denaturation	40	95°C	5 sec
Annealing & Extension		60°C[1]	30 sec[1] ***enable data collection***

Prepare mastermix and set up the reactions on ice.

It is strongly recommended to prepare ≥ 2 replicates per reaction and to always include a control reaction with dH_2O instead of RNA. A water control helps to detect a contamination of the reagents with RNA/cDNA/previously amplified target sequences.

Reaction Setup

The right reaction volume depends on the scale of your setup: when using 384-well plates, 10 µl is recommended, when using single tubes or 96-well plates, 20 µl is recommended.

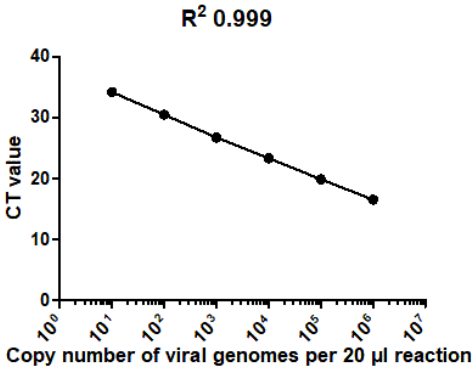
It is recommended to add ROX Passive Reference Dye to a larger volume of 2X qPCR Mix in advance. For example, 20 µl of ROX can be added to 1 ml of 2X qPCR Mix. In this way, fluctuation of the ROX signal due to pipetting inaccuracies are minimized.

Component	*Volume*	*Final Concentration*
2X qPCR Mix	10 µl	1X
20X RT Enzyme Mix	1 µl	1X
Primer 1 (10 µM)	0.4 µl	0.2 µM[2]
Primer 2 (10 µM)	0.4 µl	0.2 µM[2]
Probe (10 µM)	0.2 µl	0.1 µM[2]
Sample RNA		max. 2 µl, 50 fg – 100 ng
dH_2O		to 20 µl

[2] Given concentrations are guide values. Individual adjustments may be necessary depending on the used primers and probes.

Gently mix all components, distribute to your wells and seal the plate. Spin the plate quickly to collect all liquid at the bottom. Place into your device and immediately start the experiment.

Experimental Example



PhoenixDx® RT-qPCR Kit – Performance was used for the detection of SARS-CoV-2 RNA using SARS-CoV-2 specific primers in a probe-based multiplex qPCR with the described 2-Step Standard protocol. The serial dilution of SARS-CoV-2 RNA was detected with a high linearity and less then 5 copies were detected in >95 % of replicas.

Troubleshooting

If your water control produces amplification signal, check for primer dimerization. Prepare fresh reagents for the next experiment as it may also indicate a DNA contamination.

If no fluorescence can be detected, check the instrument settings for correct filter settings and that data collection is enabled during amplification.

If you observe late Ct values for your target sequence, prepare a dilution series of your sample RNA to determine the right concentration for your sequence of interest.

Support

If you have any questions concerning PhoenixDx® RT-qPCR Kit – Robust / Performance and their application, do not hesitate to contact us at:

support@procomcure.com

Ordering Information

Kit	*Content*	*SKU*
PhoenixDx® RT-qPCR Kit – Robust	200 rxn	PCCSKU1206
	10 000 rxn	PCCSKU1208
PhoenixDx® RT-qPCR Kit – Performance	200 rxn	PCCSKU1207
	10 000 rxn	PCCSKU1209

You can order all Procomcure Products via

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PhoenixDx® RT-qPCR Kit – Robust *&* ***PhoenixDx® RT-qPCR Kit – Performance***
are available as ***bulk product***
for assembling companies or users with substantial reaction numbers. If you are interested in bulk solutions, contact us at
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