

## Introduction

**Every PCR assay is unique - Choose the right buffer for your application.**

**PhoenixDx® ABC qPCR Mastermixes** contain everything needed for a successful qPCR or RT-qPCR experiment. The 2X qPCR Mixes contain dNTPs, MgCl<sub>2</sub> and an optimized buffer formulation to provide the right environment for successful reverse transcription and PCR amplification. The 20X Enzyme Mix provides: VitaTaq® DNA Polymerase for fast and reliable amplification of your sequence of interest and Procomcure's proprietary anti-Taq HotStart antibody to grant full control over the reaction start and protects the enzymes under harsh reaction conditions. The 20X RT Enzyme additionally contains the VitaScript® Reverse Transcriptase for precise and sensitive reverse transcription of RNA to cDNA and a reliable RNase inhibitor for best sample integrity.

**PhoenixDx® qPCR Mastermix Alpha** is 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 and can be used for many qPCR-based applications such as gene expression studies, pathogen detection and many more.

**PhoenixDx® qPCR Mastermix Bravo** is optimized for fast amplification and multiplex applications making it the best option when high throughput and performance is required.

**PhoenixDx® qPCR Mastermix Charlie** is the "all-around" which can be used for any PCR related application amplifying short and longer sequences reliably.

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® qPCR ABC Mastermixes** are 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 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).

The qPCR Kits do not contain primer or fluorescent probes of any kind, these need to be provided by the user. **PhoenixDx® qPCR Mastermix Bravo** requires previous DNA/RNA isolation; materials for isolation need to be provided by the user. Depending on the sample's matrix, **PhoenixDx® qPCR Mastermix Alpha and Charlie** can be used without prior RNA isolation.

## PhoenixDx® ABC qPCR Mastermixes

Component	Quantity	Notes
20X Enzyme Mix	1 x 500 µl	Contains HotStart Antibody and VitaTaq® DNA Polymerase
20X RT Enzyme Mix	1 x 500 µl	Contains HotStart Antibody, VitaScript® Reverse Transcriptase, RNase Inhibitor and VitaTaq® DNA Polymerase
100X ROX Passive Reference Dye	1 x 500 µl	Use Passive Reference Dye as 1X if the device requires ROX normalization
PhoenixDx® qPCR 2X Mastermix Alpha	1 x 1.25 ml	Contains dNTPs, MgCl <sub>2</sub> and an optimized buffer formulation
PhoenixDx® qPCR 2X Mastermix Bravo	1 x 1.25 ml	
PhoenixDx® qPCR 2X Mastermix Charlie	1 x 1.25 ml	

### 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<sub>2</sub>O
- materials for RNA Isolation

### Before Starting

When working with RNA targets, 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 (only for RNA detection)	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 <sup>1</sup>	30 sec <sup>1</sup> <b>enable data collection</b>
Extension		72°C	20 sec

<sup>1</sup> 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 (only for RNA detection)	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 <sup>1</sup>	30 sec <sup>1</sup> <b>enable data collection</b>

Prepare mastermix and set up the reactions on ice.

It is strongly recommended to prepare  $\geq 2$  replicates per reaction and to always include a control reaction with dH<sub>2</sub>O instead of RNA. A water control helps to detect a contamination of the reagents with RNA/cDNA/DNA previously amplified target sequences or cross-contamination during isolation of nucleic acids.

### Reaction Setup

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

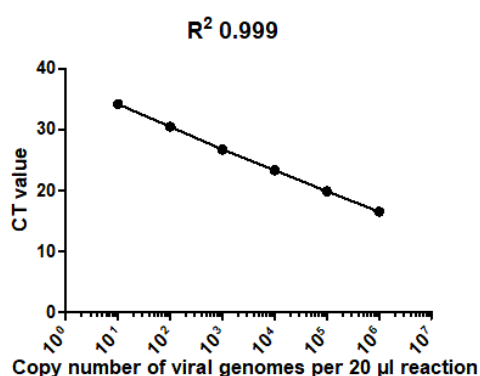
It is recommended to add ROX Passive Reference Dye to a larger volume of 2X qPCR Mix in advance. For example, 20  $\mu$ l of ROX can be added to 1 ml of 2X qPCR Mix. In this way, fluctuation of the ROX signal due to pipetting inaccuracies are minimized.

Component	Volume	Final Concentration
2X qPCR Mix	10 $\mu$ l	1X
20X RT Enzyme Mix	1 $\mu$ l	1X
Forward Primer (10 $\mu$ M)	0.4-0.8 $\mu$ l	0.2-0.4 $\mu$ M <sup>2</sup>
Reverse Primer (10 $\mu$ M)	0.4-0.8 $\mu$ l	0.2-0.4 $\mu$ M <sup>2</sup>
Probe (10 $\mu$ M)	0.2-0.4 $\mu$ l	0.1-0.2 $\mu$ M <sup>2</sup>
100X ROX Reference dye (optional)	0.2 $\mu$ l	1X
Sample DNA/RNA		to 20 $\mu$ l

<sup>2</sup> 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® qPCR Mastermix Bravo 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 than 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 DNA/RNA to determine the right concentration for your sequence of interest.

## Support

If you have any questions concerning PhoenixDx® ABC qPCR Mastermixes and their application, do not hesitate to contact us at:

[sales@traxconnects.com](mailto:sales@traxconnects.com)

## Ordering Information

Kit	Content	SKU
PhoenixDx® ABC qPCR Mastermixes	1.25 ml 2X Mastermix each	TRAXSKU12011
PhoenixDx® qPCR Mastermix Alpha	5 ml 2X Mastermix	TRAXSKU12012
PhoenixDx® qPCR Mastermix Bravo	5 ml 2X Mastermix	TRAXSKU12013
PhoenixDx® qPCR Mastermix Charlie	5 ml 2X Mastermix	TRAXSKU12014
PhoenixDx® RT-qPCR Mastermix Alpha	5 ml 2X Mastermix	TRAXSKU12015
PhoenixDx® RT-qPCR Mastermix Bravo	5 ml 2X Mastermix	TRAXSKU12016
PhoenixDx® RT-qPCR Mastermix Charlie	5 ml 2X Mastermix	TRAXSKU12017

You can order all Products via

Trax Lab Systems

[www.traxlabsystems.com](http://www.traxlabsystems.com)

[sales@traxconnects.com](mailto:sales@traxconnects.com) 833-548-8378

**PhoenixDx® ABC qPCR Mastermixes**  
Are also available as **bulk product**  
for assembling companies or users with substantial reaction numbers. If you are  
interested in bulk solutions, contact us at  
**[sales@traxconnects.com](mailto:sales@traxconnects.com)**