



**PCRBIO SYSTEMS**  
simplifying research

## qPCR BIO Probe 1-Step Virus Detect Separate-ROX

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

### Product description

qPCR BIO Probe 1-Step Virus Detect is designed for highly sensitive 1-step RT-qPCR-based detection of viral RNA sequences. The kit has been optimised with a high-concentration 4x mix, enabling greater sample input and increased sensitivity, even when small volume reactions are used.

qPCR BIO Probe 1-Step Virus Detect is engineered for use with a wide range of probe technologies including TaqMan, Scorpions and molecular beacon probes. The kit is compatible with multiplexing assays and can be used to detect viral RNA sequences over a broad range of template concentrations, down to 4 copies per reaction (0.8 copies per  $\mu\text{L}$ ).

The kit includes the thermostable UltraScript® Reverse Transcriptase which is blended with an advanced RNase inhibitor to prevent degradation of RNA by contaminating RNase.

A separate tube of 50  $\mu\text{M}$  ROX additive is provided, enabling use on all real-time instruments.

### Quality control

PCR Biosystems operates under an ISO 13485 certified Quality Management System. Our products are extensively tested and undergo a comprehensive, multi-step quality control process according to ISO 13485 standards, to ensure optimum performance, consistency and traceability.

**Product Use:** Unless we agree otherwise in writing, the Goods we supply are provided:

- For research purposes only and you should not use or rely on the Goods for diagnostic purposes. If you wish to use the Goods in a regulatory approved medical device, please contact us so that we may consider this and discuss it further with you.
- Subject to our standard terms and conditions found at <https://pcrbio.com/terms-conditions/>.

Component	200 rxns	600 rxns	1000 rxns
4x qPCR BIO Probe 1-Step Virus Detect No-ROX	1 x 1 mL	3 x 1 mL	1 x 5 mL
50 $\mu\text{M}$ ROX Additive	1 x 200 $\mu\text{L}$	1 x 200 $\mu\text{L}$	1 x 200 $\mu\text{L}$
20x UltraScript® RTase (with RNase inhibitor)	1 x 200 $\mu\text{L}$	1 x 600 $\mu\text{L}$	1 x 1 mL

### Shipping and storage

On arrival the kit should be stored between  $-30\text{ }^{\circ}\text{C}$  and  $-20\text{ }^{\circ}\text{C}$ . Avoid prolonged exposure to light. If stored correctly, the kit will retain full activity until the indicated expiry date. Avoid exposure of the stock solution to frequent temperature changes and limit handling at room temperature to the necessary minimum. Do not store the mix once it is combined with the RTase.

### Technical support

Scan or click the QR code for troubleshooting help and answers to frequently asked technical questions. For further technical support, please email [technical@pcrbio.com](mailto:technical@pcrbio.com) with the following information:

- Amplicon size
- Reaction setup
- Cycling conditions
- Screen grabs of amplification traces and melting profile



TROUBLESHOOT



FAQS

## Important considerations

**Instrument compatibility:** Different qPCR instruments may require different levels of ROX passive reference for normalisation. Use our qPCR Selection Tool to determine which ROX concentration your instrument requires (<https://pcrbio.com/resources/qpcr-selection-tool/>).

**ROX additive:** The 50 µM ROX Additive can be added directly to the 1 mL tube of mix supplied. Once added, the reagent may be used straight away or stored at -30 °C to -20 °C for future use. Use the table below to add the correct amount of ROX for your instrument. Vortex thoroughly after ROX addition.

Reagent	Lo-ROX instruments	Hi-ROX instruments	Reaction concentration
4x qPCRBIO Probe 1-Step Virus Detect No-ROX	1 mL	1 mL	1x
50 µM ROX Additive	4 µL	40 µL	50 nM (Lo-), 500 nM (Hi-ROX)

**Primer design:** For efficient amplification we recommend amplicon lengths between 80-200 bp and not exceeding 400 bp. Shorter amplicons allow for faster cycling. Primers should have an approximate T<sub>m</sub> of ~60 °C using default Primer 3 settings (<https://bioinfo.ut.ee/primer3/>). To verify the best annealing temperature for your primers in our products, please visit <https://pcrbio.com/resources/tm-calculator/>. For TaqMan probes choose a probe close to the 5' primer, avoiding terminal guanosine residues.

**Template:** The kit can be used with RNA extracted by most commercial kits, provided the amount and quality of template RNA are within an acceptable range. Addition of sample as 2 to 5 µL volumes will improve assay precision. 5 µL of swab extract is recommended for diagnostic assays.

## Reaction setup

1. Before starting, briefly vortex 4x qPCRBIO Probe 1-Step Virus Detect mix
2. Prepare a master mix based on the following table. We also recommend setting up a no-RTase control:

Reagent	20 µL reaction	Final conc.	Notes
4x PCRBIO Probe 1-Step Virus Detect mix	5 µL	1x	
Forward primer (10 µM)	1-2 µL	400 nM-1 µM	See above for optimal primer design
Reverse primer (10 µM)	1-2 µL	400 nM-1 µM	
Probe (10 µM)	0.25-1 µL	125-500 nM	
20x UltraScript® RTase	1 µL	1x	
RNA template	2-5 µL	Variable	4 to 1x10 <sup>8</sup> viral copies per reaction. See above for further template considerations.
PCR grade dH <sub>2</sub> O	Up to 20 µL final volume		

3. Program the instrument using the following conditions, acquiring data on the appropriate channel:

Cycles	Temperature General	Temperature SARS-CoV-2 Detection	Time	Notes
1	45 °C to 55 °C	55 °C	5-10 minutes singleplex 10-20 minutes multiplex	Reverse transcription
1	95 °C	95 °C	3 minutes	Polymerase activation and RTase inactivation
50	95 °C 55 °C to 65 °C	95 °C 58 °C	15 seconds 30 seconds	Denaturation Anneal/Extension
Melt analysis	Refer to instrument instructions			Optional melt profile analysis, available for hybridisation probes only