PCRBIOSYSTEMS
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qPCRBIO Probe 1-Step Virus Detect No-ROX

Product description

qPCRBIO 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.

qPCRBIO 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 µL).

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

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.

Pack size	4x qPCRBIO Probe 1-Step Virus Detect No-ROX	20x UltraScript® RTase (with RNase Inhibitor)
200 reactions	1 x 1 mL	1 x 200 µL
600 reactions	3 x 1 mL	1 x 600 µL
1000 reactions	1 x 5 mL	1 x 1 mL
10000 reactions	1 x 50 mL	2 x 5 mL
100000 reactions	1 x 500 mL	1 x 100 mL

Shipping and storage

On arrival the kit should be stored between -30 °C and -20 °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 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/).

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 Tm 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 $1x10^8$ viral copies per reaction. See above for further template considerations.
PCR grade dH_2O	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