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qPCRBIO Probe 1-Step Virus Detect Separate-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. Combined with antibody-mediated hot start technology and smart screen buffer chemistry, qPCRBIO Probe 1-Step Virus Detect enables robust, reproducible and high throughput detection of RNA viruses.

Detection of SARS-CoV-2

qPCRBIO Probe 1-Step Virus Detect has been validated for qualitative detection of SARS-CoV-2 nucleic acid using the Charité (Berlin, Germany) recommended primer-probe sequences (RdRp and E genes)[1], and CDC (Atlanta, USA) primer-probe sequences (N gene)[2]. For further information please email technical@pcrbio.com.

[1] Diagnostic detection of 2019-nCoV by real-time RT-PCR (https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf)

[2] 2019-Novel Coronavirus (2019-nCoV) Real-time rRT-PCR Panel Primers and Probes (https://www.cdc.gov/coronavirus/2019-ncov/downloads/rt-pcr-panel-primer-probes.pdf)

Component	200 rxns	600 rxns	1000 rxns
4x qPCRBIO Probe 1-Step Virus Detect No-ROX	1 x 1mL	3 x 1mL	1 x 5mL
50µM ROX Additive	1 x 200µL	1 x 200µL	1 x 200µL
20x UltraScript RTase (with RNase inhibitor)	1 x 200µL	1 x 600µL	1 x 1mL

Shipping and storage

On arrival the kit should be stored between -30°C and -15°C. Avoid prolonged exposure to light. If stored correctly the kit will retain full activity for 12 months. The kit can go through 30 freeze/thaw cycles with no loss of activity.

Limitations of product use

For research use only. This product alone does not provide any diagnostic result.

Technical support

Help and support is available on our website at https://pcrbio.com/resources/ including answers to frequently asked technical questions. For technical support and troubleshooting please email technical@pcrbio.com with the following information:

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

Important considerations

Instrument compatibility: Different real-time PCR instruments require different levels of ROX passive reference. Generally, modern instruments do not need passive reference but include the option to use it for normalisation. Please use our qPCRBIO Selection Tool to determine which ROX concentration your instrument requires (https://pcrbio.com/resources/qpcr-selection-tool/).

ROX additive protocol: The 50µM ROX Additive supplied is formulated to be added directly to the tube or bottle of 4x qPCRBIO mix supplied. Once the ROX is added, the reagent may be used straight away or stored between -30˚C and -15˚C for future use. Please use the following 2 charts to add the correct amount of ROX for your instrument. Vortex thoroughly after ROX addition.

Hi-ROX instruments	Reagent volume	Final concentration	Reaction concentration
4x qPCRBIO Probe 1-Step Virus Detect No-ROX	1.0mL	2x	1x
50µM ROX Additive	20.0µL	1µM	500nM

Lo-ROX instruments	Reagent volume	Final concentration	Reaction concentration
4x qPCRBIO Probe 1-Step Virus Detect No-ROX	1.0mL	2x	1x
50µM ROX Additive	2.0µL	100nM	50nM

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 5µL volumes will improve assay precision. 5µL of swab extract is recommended for SARS-CoV-2 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	20µL reaction	Final conc.	Notes
4x PCRBIO Probe 1-Step Virus Detect mix	5µL	1x	
Forward primer (10µM)	1-2µL	400nM-1µM	
Reverse primer (10µM)	1-2µL	400nM-1µM	
Probe (10µM)	0.25-1µL	125-500nM	
20x UltraScript RTase	1µL	1x	
RNA template	2-5µL	Variable	4 to $1x10^8$ viral copies per reaction. See above for further template considerations.
PCR grade dH_2O	Up to 20µL 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 minutes singleplex 10 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