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qPCR BIO Probe 1-Step Virus Detect Separate-ROX

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 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, qPCR BIO Probe 1-Step Virus Detect enables robust, reproducible and high throughput detection of RNA viruses.

Detection of SARS-CoV-2

qPCR BIO 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 qPCR BIO Probe 1-Step Virus Detect No-ROX	1 x 1 mL	3 x 1 mL	1 x 5 mL
50 µM ROX Additive	1 x 200 µL	1 x 200 µL	1 x 200 µL
20x UltraScript RTase (with RNase inhibitor)	1 x 200 µL	1 x 600 µL	1 x 1 mL

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. 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.

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 qPCR BIO Selection Tool to determine which ROX concentration your instrument requires (<https://pcrbio.com/resources/qpcr-selection-tool/>).

ROX additive protocol: The 50 µM ROX Additive supplied is formulated to be added directly to the tube or bottle of 4x qPCR BIO 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 qPCR BIO Probe 1-Step Virus Detect No-ROX	1.0 mL	4x	1x
50 µM ROX Additive	40.0 µL	2 µM	500 nM

Lo-ROX instruments	Reagent volume	Final concentration	Reaction concentration
4x qPCR BIO Probe 1-Step Virus Detect No-ROX	1.0 mL	4x	1x
50 µM ROX Additive	4.0 µL	200 nM	50 nM

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 SARS-CoV-2 diagnostic assays.

Reaction setup

1. Before starting, briefly vortex 4x qPCR BIO 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 PCR BIO Probe 1-Step Virus Detect mix	5 µL	1x	
Forward primer (10 µM)	1-2 µL	400 nM-1 µM	
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 ₂ 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