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Pre-Lyo Probe 1-Step Evaluation Kit

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Product description

Pre-Lyo Probe 1-Step Evaluation Kit is a version of the Lyo-Ready Probe 1-Step Kit (PB90.14) that has been designed for evaluation purposes.

The kit includes a glycerol-free 4x qPCR mix containing hot start Taq polymerase, dNTPs, MgCl₂ and a blend of excipients to ensure reliable lyophilisation, without loss of activity.

UltraScript Reverse Transcriptase provided in this kit is for evaluation only. Due to its high glycerol content, it is not suitable for lyophilisation. A highly concentrated version of UltraScript Reverse Transcriptase is available as part of the Lyo-Ready Probe 1-Step Kit.

Detection of SARS-CoV-2

Lyo-Ready Probe 1-Step Kit 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.

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.

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	600 rxns
4x Lyo-Ready Probe Mix	3 x 1 mL
20x UltraScript RTase (with RNase inhibitor)	1 x 600 µL

Shipping and storage

On arrival the kit should be stored between -30 °C and -15 °C. 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

This product has been manufactured under an ISO 13485 certified Quality Management System and is suitable for further manufacturing use as a component, reagent or reagent assembly for molecular biology diagnostics.

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

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 Lyo-Ready Probe 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 Lyo-Ready Probe 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	1-2 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