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simplifying research

## Lyo-Ready Probe 1-Step Kit

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

### Product description

Lyo-Ready Probe 1-Step Kit is specially formulated for reliable RT-qPCR-based detection of viral RNA sequences and is ideal for the development of lyophilised diagnostic assays.

The kit includes a glycerol-free 4x qPCR mix containing hot start Taq polymerase, dNTPs, MgCl<sub>2</sub> and a blend of excipients to ensure reliable lyophilisation, without loss of activity. UltraScript Reverse Transcriptase is provided separately in a highly concentrated format, with RNase inhibitor included. The kit is suitable for lyophilisation into beads or cakes.

### 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)<sup>1</sup>, and CDC (Atlanta, USA) primer-probe sequences (N gene)<sup>2</sup>.

### 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.

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

<sup>2</sup> 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	2 000 reactions	10 000 reactions	100 000 reactions
4x Lyo-Ready Probe Mix	2 x 5 mL	1 x 50 mL	1 x 500 mL
3200x UltraScript RTase (with RNase inhibitor)	1 x 12.5 µL	1 x 62.5 µL	1 x 625 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.

### 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](mailto:technical@pcrbio.com) with the following information:

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

## Product setup

1. Before starting, thaw and briefly vortex the bottle of 4x Lyo-Ready Probe Mix.
2. Add 1.25  $\mu\text{L}$  of 3200x UltraScript RTase for each mL of 4x Lyo-Ready Probe Mix used, taking care as the RTase solution is viscous.
3. Briefly vortex to mix the components. The 4x Lyo-Ready Probe 1-Step Mix is now ready to use or can be stored for up to 3 days at 4  $^{\circ}\text{C}$ . If less than the entire bottle is required, a smaller amount of 4x Lyo-Ready Probe 1-Step Mix can be prepared, and the remaining separate components (4x Lyo-Ready Probe Mix and 3200x UltraScript RTase) returned to storage at between -30  $^{\circ}\text{C}$  and -15  $^{\circ}\text{C}$ .
4. Add primers and probes to the 4x Lyo-Ready Probe 1-Step Mix, then start the freeze-drying cycle. We suggest diluting to 1x or 2x with these extra components and water to facilitate the lyophilisation process. The mix has the following critical temperatures: onset of collapsing temperature ( $T_c$ ): -35.1  $^{\circ}\text{C}$ , onset of glass transition temperature ( $T_g$ ): 68.9  $^{\circ}\text{C}$ , mid-point  $T_g$ : 75.1  $^{\circ}\text{C}$ , and end point  $T_g$ : 81.3  $^{\circ}\text{C}$ .

We have tested the following conditions in 2 mL glass vials containing 500  $\mu\text{L}$  of the 1-step mix. Shorter drying times will be required if standard PCR tubes and plates are used. Further custom optimisation may be needed depending on the lyophilisation instrument.

Stage	Step	Shelf Temperature ( $^{\circ}\text{C}$ )	Time (minutes)	Pressure ( $\mu\text{Bar}$ )	Description
	-	+2 to +6	N/A	Atmospheric	Loading
Thermal Treatment Stage	1	+5	10	Atmospheric	Hold
	2	-50	110	Atmospheric	Ramp (0.5 $^{\circ}\text{C}/\text{min}$ )
	3	-50	180	Atmospheric	Hold
Primary Drying Stage	4	-45	10	30	Ramp (0.5 $^{\circ}\text{C}/\text{min}$ )
	5	-45	5400	30	Hold
Secondary Drying Stage	6	+20	130	30	Ramp (0.5 $^{\circ}\text{C}/\text{min}$ )
	7	+20	600	30	Hold
Actions at end of cycle	-	+20	N/A	Half Atmospheric	Backfill with $\text{N}_2$
	-	+20	N/A	Half Atmospheric	Stopper
	-	+20	N/A	Atmospheric	Aerate

## Reaction setup

1. To test the wet mix (obtained after step 3 of product setup), prepare a master mix based on the following table. We also recommend setting up a no-RTase control:

Reagent	20 $\mu\text{L}$ reaction	Final conc.	Notes
4x Lyo-Ready Probe 1-Step Mix	5 $\mu\text{L}$	1x	This is the 4x Lyo-Ready Probe Mix combined with x3200 UltraScript RTase (see product setup above)
Forward primer (10 $\mu\text{M}$ )	1-2 $\mu\text{L}$	400 nM-1 $\mu\text{M}$	
Reverse primer (10 $\mu\text{M}$ )	1-2 $\mu\text{L}$	400 nM-1 $\mu\text{M}$	
Probe (10 $\mu\text{M}$ )	0.25-1 $\mu\text{L}$	125-500 nM	
RNA template	2-5 $\mu\text{L}$	Variable	4 to 1x10 <sup>8</sup> viral copies per reaction
PCR grade dH <sub>2</sub> O	Up to 20 $\mu\text{L}$ final volume		

2. 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 $^{\circ}\text{C}$ to 55 $^{\circ}\text{C}$	55 $^{\circ}\text{C}$	5-10 minutes singleplex 10-20 minutes multiplex	Reverse transcription
1	95 $^{\circ}\text{C}$	95 $^{\circ}\text{C}$	3 minutes	Polymerase activation and RTase inactivation
50	95 $^{\circ}\text{C}$ 55 $^{\circ}\text{C}$ to 65 $^{\circ}\text{C}$	95 $^{\circ}\text{C}$ 58 $^{\circ}\text{C}$	15 seconds 30 seconds	Denaturation Anneal/Extension
Melt analysis	Refer to instrument instructions			Optional melt profile analysis, available for hybridisation probes only