



PCRBIOSYSTEMS
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Lyo-Ready Probe 1-Step Kit

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, MgCl₂ 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.

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.

Component	2000 reactions	10000 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 -20 °C. 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.

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

Product Use: Unless we agree otherwise in writing, the Goods we supply are provided:

1. For research purposes only and you should not use or rely on the Goods for diagnostic purposes. If you wish to use the Goods in a regulatory approved medical device, please contact us so that we may consider this and discuss it further with you.
2. Subject to our standard terms and conditions found at <https://pcrbio.com/terms-conditions/>.

Product setup

1. Before starting, thaw and briefly vortex the bottle of 4x Lyo-Ready Probe Mix.
2. Add 1.25 µL 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 °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 °C and -20 °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 °C, onset of glass transition temperature (T_g): 68.9 °C, mid-point T_g : 75.1 °C, and end point T_g : 81.3 °C.

We have tested the following conditions in 2 mL glass vials containing 500 µL 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 (°C)	Time (minutes)	Pressure (µBar)	Description
Thermal Treatment Stage	-	+2 to +6	N/A	Atmospheric	Loading
	1	+5	10	Atmospheric	Hold
	2	-50	110	Atmospheric	Ramp (0.5 °C/min)
	3	-50	180	Atmospheric	Hold
Primary Drying Stage	4	-45	10	30	Ramp (0.5 °C/min)
	5	-45	5400	30	Hold
Secondary Drying Stage	6	+20	130	30	Ramp (0.5 °C/min)
	7	+20	600	30	Hold
Actions at end of cycle	-	+20	N/A	Half Atmospheric	Backfill with 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 µL reaction	Final conc.	Notes
4x Lyo-Ready Probe 1-Step Mix	5 µL	1x	This is the 4x Lyo-Ready Probe Mix combined with x3200 UltraScript® RTase (see product setup above)
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	
RNA template	2-5 µL	Variable	4 to 1x10 ⁸ viral copies per reaction
PCR grade dH ₂ O	Up to 20 µL 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 °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	95 °C	15 seconds	Denaturation
	55 °C to 65 °C	58 °C	30 seconds	Anneal/Extension
Melt analysis	Refer to instrument instructions			Optional melt profile analysis, available for hybridisation probes only