



**PCRBIO SYSTEMS**  
simplifying research

## Lyo-Ready Probe Mix

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

### Product description

Lyo-Ready Probe Mix is specially formulated for reliable qPCR-based detection of DNA targets and is ideal for the development of lyophilised diagnostic assays.

The glycerol-free 4x qPCR mix contains hot start Taq polymerase, dNTPs, MgCl<sub>2</sub> and a blend of excipients to ensure reliable lyophilisation, without loss of activity. The kit is suitable for lyophilisation into beads or cakes.

PCR Biosystems real-time PCR probe mixes have been designed for use on a wide range of probe technologies including TaqMan®, Scorpions® and molecular beacon probes.

### 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	4x Lyo-Ready Probe Mix
600 Reactions	3 x 1 mL
2 000 Reactions	2 x 5 mL
10000 Reactions	1 x 50 mL
100000 Reactions	1 x 500 mL

### 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 primers and probes to the 4x Lyo-Ready Probe 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.
3. We have tested the following conditions in 2 mL glass vials containing 500 µL of the 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 <sub>2</sub>
	-	+20	N/A	Half Atmospheric	Stopper
	-	+20	N/A	Atmospheric	Aerate

## Reaction setup

1. To test the wet mix, prepare a master mix based on the following table:

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	
DNA template	2-5 µL	Variable	<100 ng cDNA, <1 µg genomic DNA
PCR grade dH <sub>2</sub> O	Up to 20 µL final volume		

2. Program the instrument using the following conditions, acquiring data on the appropriate channel:

Cycles	Temperature	Time	Notes
1	95 °C	2 minutes	Polymerase activation
40-50	95 °C	5-15 seconds	Denaturation
	55 °C to 65 °C	20-30 seconds	Anneal/Extension
Melt analysis	Refer to instrument instructions		Optional melt profile analysis, available for hybridisation probes only