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Air-Dryable Probe 1-Step Mix

Product description

The Air-Dryable Probe 1-Step Mix is specially formulated for the development of air-dried diagnostic assays offering reliable qPCR-based detection of both RNA and DNA target sequences.

The kit includes a glycerol-free 4x qPCR mix containing hot start Taq polymerase, dNTPs, MgCl₂ and a blend of excipients to ensure reliable drying, without loss of activity. An enhanced version of UltraScript Reverse Transcriptase, modified for increased stability upon drying, and our RiboShield™ RNase inhibitor are also blended in this complete 1-step mix.

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.

Pack size	4x Air-Dryable Probe 1-Step Mix
600 reactions	3 x 1 mL
2000 reactions	2 x 5 mL
10000 reactions	1 x 50 mL
100 000 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 are 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

When air-drying, we recommend getting a loss of weight, referred to as Loss on Drying (**LoD**), of approximately **70%**, which results in a dried gel containing only 5-10% water.

LoD is calculated using the following formula:

$$\text{LoD} = (\text{W2}-\text{W3})/(\text{W2}-\text{W1}) \times 100\%$$

where **W1** is the weight of the tube, **W2** the weight of the wet product, and **W3** the weight of the air-dried product.

If primers, probes, and eventually ROX (if the PCR instrument requires it) are added before the process, stock solutions at high concentrations should be used, in order to add the smallest volume possible to the 4x mix. We recommend not exceeding 1 extra μL in volume. Larger volumes would require longer drying times and higher LoD to reach the same percentage of remaining water in the dried product.

The time to obtain the desired LoD can vary using different drying instruments. We routinely achieve an **LoD of $70 \pm 1\%$** when drying 5 μL of the 4x Air-Dryable Probe 1-Step Mix in 96 well plates or PCR tubes and an **LoD of $74.5 \pm 0.5\%$** when drying 6 μL of the 4x Air-Dryable Probe 1-Step Mix plus oligos **at 40°C for 80-90 min**. In both cases, the amount of remaining water was **approximately 8%**.

When using this product for the first time with a specific drying instrument, we recommend measuring the LoD using multiple tubes at regular time intervals (e.g., after 60, 70, 80, 90 min) in order to define the best protocol to achieve a LoD of $70 \pm 1\%$ or $74.5 \pm 0.5\%$ based on the mix (without or with oligos) and the drying instrument used.

Reaction setup

1. Before starting, thaw and briefly vortex the bottle of 4x Air-Dryable Probe 1-Step Mix
2. Add primers, probes and eventually ROX, then distribute the mix in the well and start the drying process. See Important considerations above for more details on this stage.

Reagent	20 μL reaction	Final concentration	Notes
4x Air-Dryable Probe 1-Step Mix	5 μL	1x	
Forward primer (0.1 - 1 mM)		400 nM - 1 μM	Total volume of primers, probes, and ROX should not exceed 1 μL . See 'Important considerations' above.
Reverse primer (0.1 - 1 mM)		400 nM - 1 μM	
Probe (0.1 - 1 mM)		125 - 500 nM	
ROX (50 μM) <i>Optional</i>		Depending on instrument requirements	ROX Additive is available separately, catalogue number PB20.14-P6

3. Once ready, add 20 μL RNA or DNA template and water to each well.
4. Program the instrument using the following conditions, acquiring data on the appropriate channel(s):

Cycles	Temperature General	Temperature SARS-CoV-2 Detection	Time	Notes
1 <i>Optional</i>	52 $^\circ\text{C}$	52 $^\circ\text{C}$	5-10 minutes singleplex 10-20 minutes multiplex	Reverse transcription. Required only for RNA templates.
1	95 $^\circ\text{C}$	95 $^\circ\text{C}$	3 minutes	Polymerase activation and RTase inactivation
50	95 $^\circ\text{C}$	95 $^\circ\text{C}$	15 seconds	Denaturation
	55 $^\circ\text{C}$ to 65 $^\circ\text{C}$	58 $^\circ\text{C}$	30 seconds	Anneal/Extension
Melt analysis	Refer to instrument instructions			Optional melt profile analysis, available for hybridisation probes only