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Air-Dryable Inhibitor-Tolerant Probe 1-Step Mix

Product description

Air-Dryable Inhibitor-Tolerant Probe 1-Step Mix is specially formulated for reliable qPCR-based detection of both RNA and DNA target sequences even in the presence of common inhibitors of PCR reactions. The mix is ideal for the development of air-dried diagnostic assays

This reagent is a 4x qPCR mix containing hot start Taq polymerase, dNTPs, $MgCl_2$, and an enhanced version of UltraScript® Reverse Transcriptase, and our RiboShield® RNase inhibitor, providing a complete 1-step RT-qPCR mix. It is developed to work well with the full range of probe types, including TaqMan, Scorpions and molecular beacons.

The mix additionally contains excipients which ensure reliable performance in crude saliva samples and in the presence of PCR-inhibitory compounds that include but are not limited to: standard laboratory chemicals (SDS, guanidine, and ethanol), and biological sample inhibitors, such as those found in blood samples (hemin, hematin, haemoglobin, heparin, IgG immunoglobulins, lactoferrin, sodium citrate), urine (urea), plant, and environmental samples (humic acid, catechin, quercetin, tannic acid, cellulose, and chlorophyl).

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 Inhibitor-Tolerant Probe 1-Step Mix
600 reactions	3 x 1 mL
2000 reactions	2 x 5 mL
10000 reactions	1 x 50 mL

Shipping and storage

On arrival the kit should be stored between -30 °C and -20 °C. Avoid prolonged exposure to light. 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

Important considerations

Air-drying: 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: **LoD = (W2-W3)/(W2-W1) x 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 made, in order to add the smallest volume possible to the 4x mix. We recommend not exceeding 1 extra µL. Larger volumes would require longer drying times and higher LoD to reach the same percentage of remaining water in the dryed product.

The time to obtain the desired LoD can vary using different drying instruments. We routinely achieve an **LoD of 70 ± 1%** when drying 5 µL of the 4x Air-Dryable Inhibitor-Tolerant Probe 1-Step Mix in 96 well plates or PCR tubes and an **LoD of 74 ± 1%** when drying 6 µL of the 4x Air-Dryable Inhibitor-Tolerant Probe 1-Step Mix plus oligos **at 40 °C for 80 min**. In both cases, the amount of remaining water was **approximately 8%.**

When using this product for the first time with a specific instrument, we recommend measuring the LoD using multiple tubes at regular times (e.g., after 60, 70, 80, 90 min) in order to define the best protocol to achieve a LoD of 70 ± 1% or 74 ± 1% based on the mix (without or with oligos) and the drying instrument used.

Template: The kit can be used with RNA or DNA extracted by most commercial kits or standard extraction methods, provided the amount and quality of template are within an acceptable range. Addition of 2 to 5 µL volumes of sample will improve assay precision.

Reaction setup

1. Before starting, thaw and briefly vortex the 4x Air-Dryable Inhibitor-Tolerant Probe 1-Step Mix.
2. Add primers, probes and, if required, ROX, as described in the table below, then distribute the mix in the wells and start the drying process:

Reagent	20 µL reaction	Final concentration	Notes
4x Air-Dryable Inhibitor-Tolerant 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) *Optional*		Depending on instrument requirements	ROX Additive is available separately, catalogue number PB20.14-P6

3. The mastermix can be dried at this stage (see "Important Considerations" above).
4. Once ready, add 20 µL RNA or DNA template and water to each well.
5. Program the instrument using the following conditions, acquiring data on the appropriate channel(s):

Cycles	Temperature	Time	Notes
1 *Optional*	47 °C	5-10 minutes singleplex 10-20 minutes multiplex	Reverse transcription. Required only for RNA templates.
1	95 °C	2 minutes	Polymerase activation and RTase inactivation
40-50	95 °C 55 °C to 65 °C	15 seconds 30 seconds	Denaturation Anneal/Extension
Melt analysis	Refer to instrument instructions		Optional melt profile analysis, available for hybridisation probes only