Product Bulletin

Developing Air-Dried, Inhibitor-Tolerant qPCR Assays: A simple, fast and low-cost option for point-of-care diagnostics

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Introduction

Air-drying has emerged as a simple, fast, and cost-effective option for developing dried-format qPCR-based diagnostic kits. With the new Air-Dryable Inhibitor-Tolerant qPCR mixes from PCR Biosystems, kit developers can now confidently create room temperature-stable molecular tests capable of sensitive detection on even the most challenging sample types.

qPCR and RT-qPCR assays developed with the Air-Dryable Inhibitor-Tolerant Probe Mix and Air-Dryable Inhibitor-Tolerant Probe 1-Step Mix can be dried in-house in just 80-90 minutes, with a simple laboratory oven and without extensive training. Organisations are able to retain full control over the drying process and quality control steps, without incurring significant infrastructure or outsourcing costs.

Once suitable primer and probe concentrations for the desired application are identified, these air-dryable mixes can be directly dried in reaction tubes or 96-well plates. This dried mix can be stored at ambient temperature for at least 12 months, eliminating the costs and logistics of cold-chain shipping and storage. The dried reaction mixes can be reconstituted by simply adding samples and PCR-grade water to the tube. Simple resuspension can be followed with a standard qPCR or RT-qPCR thermocycling program, as would be carried out with a normal wet mix.

Benefits of air-drying your assay

- Air-dry in as little as 80-90 minutes
- Drying can be carried out in-house with a simple laboratory oven
- Air-dried products are less susceptible to humidity, reducing the requirement for strict humidity control during manufacturing
- Air-drying incurs far fewer costs compared to other drying methods
- Air-drying offers maximum process control and is ideal for small to medium companies

Addressing PCR Inhibition Challenges

Optimal sample extraction techniques can often mitigate issues with inhibition in qPCR workflows and diagnostic processes. However, certain sample types—such as those containing blood, tissue, or cellular debris—pose persistent challenges due to their inhibitory compounds. Additionally, there is a growing trend towards conducting PCR on crude samples such as saliva and urine, which are inherently rich in PCR inhibitors.

To meet this challenge, the Air-Dryable Inhibitor-Tolerant mixes have been developed with enhanced tolerance to a broad spectrum of chemical compounds. This includes common laboratory chemicals like

SDS, guanidine, and ethanol, as well as biological sample inhibitors found in blood, saliva, and urine. The mixes have demonstrated robust performance in the presence of inhibitors such as hemin, hematin, haemoglobin, heparin, immunoglobulins, lactoferrin, sodium citrate, urea, humic acid, catechin, quercetin, tannic acid, cellulose, and chlorophyll.

Results

Air-Dryable Inhibitor-Tolerant Probe 1-Step Mix is designed as a versatile, all-in-one, 4x mix for sensitive detection of RNA (and DNA) sequences. It contains an optimised blend of excipients for reliable drying without loss of activity and has been extensively validated against crude (or diluted) saliva and blood, along with other pure chemical inhibitors listed above.

Because Air-Dryable Inhibitor-Tolerant Probe 1-Step Mix is primarily geared towards diagnostic applications, we tested its performance in a multiplex assay against four common winter viruses in the presence of 2.5% crude saliva. The RNA targets chosen were Influenza A, Influenza B, Respiratory Syncytial Virus, and SARS-CoV-2, as representative of a winter respiratory disease test panel. Dilutions from 4000 down to 4 copies were used as template and the assay was set up using both wet and reconstituted (after drying) Air-Dryable Inhibitor-Tolerant Probe

Key features of Air-Dryable Inhibitor-Tolerant Mixes

- Sensitive detection of DNA and RNA targets, down to 4 copies per reaction
- Single-tube format (with UltraScript® Reverse Transcriptase included in the 1-step mix)
- Easy reaction setup – add only primers & probes before drying
- Rapid air-drying protocol (80-90 minutes)
- Same high performance before & after drying
- Room-temperature shipping and storage of dried reactions
- Enhanced tolerance to inhibitors found in:

  Biological samples: saliva, blood (hemin, hematin, haemoglobin, heparin, immunoglobulins, lactoferrin, sodium citrate) and urine (urea)

  Plant and environmental samples: humic acid, catechin, quercetin, tannic acid, cellulose, and chlorophyll

  Standard laboratory chemicals: SDS, guanidine, and ethanol

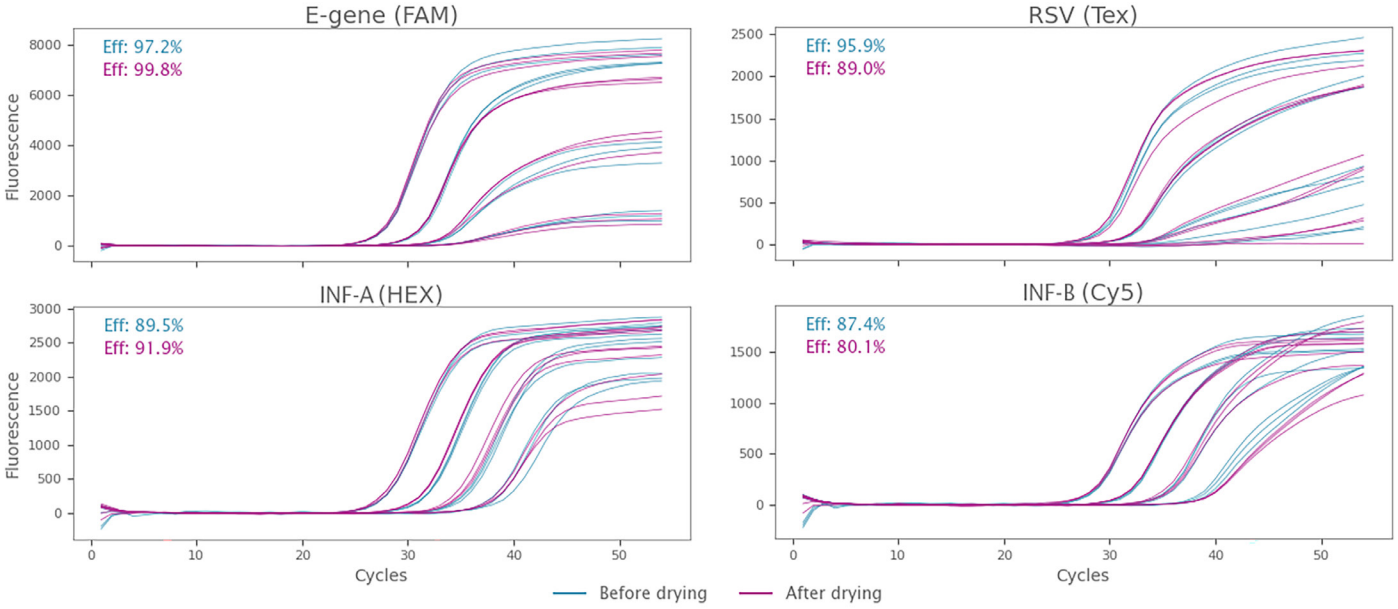

Figure 1. Reliable performance after drying even in the presence of saliva

Four RNA targets, SARS-CoV-2 E-gene (E-gene), Respiratory Syncytial Virus (RSV), Influenza-A (INF-A), and Influenza-B (INF-B) were amplified, with human saliva, in multiplex 1-step RT-qPCR reactions with Air-Dryable Inhibitor-Tolerant Probe 1-Step Mix before (blue curves) and after (purple curves) drying (80 min at 40 ˚C). Four template dilutions (4000, 400, 40, and 4 copies) with three technical replicates for each target were used in 20 µL reactions. 5 µL of saliva diluted 1/10 in universal transport medium, corresponding to 2.5% human saliva, were added per reaction. Cycling conditions were: 47 ˚C for 10 min, 95 ˚C for 2 min, followed by 50 cycles of 95 ˚C for 10 s, and 60 ˚C for 30 s.

1-Step Mix, in order to test whether the performance of the mix was affected by drying.

The results (Figure 1) indicate the mix is capable of reliably detecting down to at least four copies of all target molecules tested, that there is no significant impact on performance of the mix after drying, and that the mix robustly tolerates saliva.

The stability of the dried mix was also tested using singleplex reactions to detect common mammalian housekeeping genes β-microtubulin, γ-Actin and GAPDH. Assays were set by adding RNA samples at the same concentration to reaction tubes containing dried mix stored at -20 ˚C for 12 weeks, and to an equivalent amount of dried mix that had been stored at 37 ˚C for the same time period.

Results of this experiment (Figure 2) demonstrate minimal impact on performance after storage for at least 12 weeks at 37 ˚C, thereby demonstrating the extreme robustness of Air-Dryable Inhibitor-Tolerant Probe 1-Step Mix.

Conclusion

Assays with better resistance to inhibitors allow for improved diagnostic accuracy, fewer extraction steps, faster results, and ultimately lower testing costs. The Air-Dryable Inhibitor-Tolerant mixes from PCR Biosystems are designed to meet this need. They combine precise qPCR with broad-spectrum inhibitor tolerance, and provide a simple, low-cost route for kit manufacturers to develop dried assay formats.

These powerful enzyme mixes show no significant impact on performance or loss of activity in the presence of saliva, or as a result of air-drying, under the conditions tested. Moreover, the dried mix is stable at 37 ˚C for a minimum of 12 weeks after air-drying. Room-temperature stable qPCR assays for point-of-care detection on the most challenging samples are now more accessible than ever.

Learn More

To learn more about our air-dryable and lyophilisable reagents, or to discuss which of our products are best suited to your application, contact our team of experts at info@pcrbio.com.

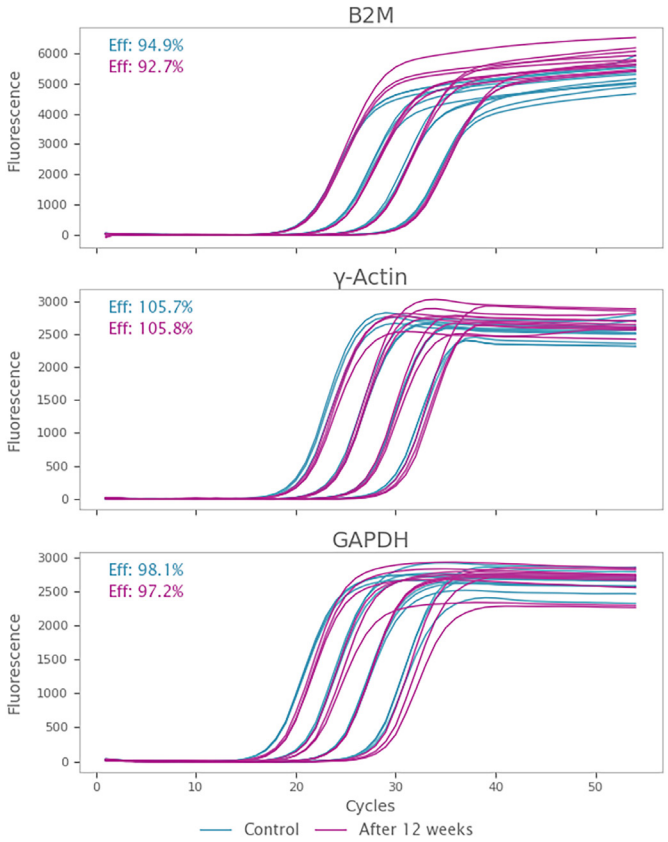

Figure 2. Stability of the dried gel after 12 weeks at 37 ˚C

Three RNA targets, B2M (β-microtubulin), γ-Actin, and GAPDH) were amplified in singleplex RT-qPCR reactions with Air-Dryable Inhibitor-Tolerant Probe 1-Step Mix. The mix was dried and then stored at -20 ˚C (blue curves) or incubated at 37 ˚C for 12 weeks (purple curves). Efficiency of the reactions before and after drying are shown as insets in each graph. Four serial dilutions of 1.25 ng/µL, 125 pg/µL, 12.5 pg/µL, and 1.25 pg/µL of mouse RNA were used. Cycling conditions were: 45 ˚C for 20 min, 95 ˚C for 2 min, followed by 54 cycles of 95 ˚C for 10 s, and 60 ˚C for 30 s.

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 for molecular biology diagnostics. 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.