Product Bulletin

Air-Dried Assay Development

A simple, fast and cost-effective option to dry molecular assays in-house.

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Introduction

Assay developers are increasingly moving towards dried formats to alleviate the costly cold-chain shipping and storage demands of traditional 'wet' qPCR-based diagnostic tests. Air-drying has emerged as a simple, fast and cost-effective option for drying down molecular assays.

With the new air-dryable qPCR and RT-qPCR reagents from PCR Biosystems, drying can be carried out in just 80-90 minutes with a simple laboratory oven, and without extensive training. As a result, organisations can now create high-performance dried assays in-house, with full control over the drying process and quality control steps, and without significant infrastructure or outsourcing costs.

Furthermore, air-dried assays are less susceptible to moisture and so do not require the strict humidity control during manufacturing that is needed with lyophilised tests. This is particularly useful in high-humidity regions and allows local diagnostic kit developers to more confidently expand their portfolio into dried formats.

Once suitable primer and probe concentrations for the desired application are identified, these new 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. These 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

Results

Air-Dryable Probe 1-Step Mix is designed as a versatile, all-in-one, air-dryable 4x reagent mix for sensitive detection of RNA and DNA sequences. During development, Air-Dryable Probe 1-Step Mix was put through a stringent set of assays to ensure the highest possible performance of the mix.

Because Air-Dryable Probe 1-Step Mix is primarily geared towards diagnostic applications, we tested its performance in a multiplex assay against four common winter viruses. 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 40 000 down to 4 copies were used as template and the assay was set up using both wet and reconstituted (after drying) Air-Dryable Probe 1-Step Mix, in order to test

Figure 1. Reliable performance after air-drying in a multiplex setup

Multiplex amplification of common winter viruses (Influenza A, Influenza B, Respiratory Syncytial Virus, and SARS-CoV-2) using Air-Dryable Probe 1-Step Mix before (blue bars) or after drying (purple bars). Ct values are shown in the top panels and efficiency in the bottom panels. 5 serial dilutions of RNA template were used, corresponding to 40000, 4000, 400, 40, and 4 copies of each viral genome. The total reaction volume was 20 µL. Cycle conditions were 45 ˚C 20 min, 95 ˚C 3 min and 50 cycles of 95 ˚C 15 s, 60 ˚C 30 s. Air-Dryable Probe 1-Step Mix shows no significant loss of efficiency, speed or sensitivity after drying.

whether the performance of the mix was affected by drying.

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

The impact of the drying process was also tested in singleplex reactions to detect common mammalian housekeeping genes β-Actin and γ-Actin from RNA, using equivalent amounts of wet and air-dried Air-Dryable Probe 1-Step Mix. Results of this assay (Figure 2) clearly demonstrate no loss of activity of the reaction mix after drying and reconstitution.

Finally, to test the stability of the dried Air-Dryable Probe 1-Step Mix, the same β- and γ-Actin singleplex assays were set by adding samples at the same concentration to reaction tubes containing dried Air-Dryable Probe 1-Step Mix stored at -20 ˚C for 12 weeks, and to an equivalent amount of dried Air-Dryable Probe 1-Step Mix that had been stored at 37 ˚C for the same time period. Results of this experiment (Figure 3) demonstrate no loss of performance of Air-Dryable Probe 1-Step Mix after storage for at least 12 weeks at 37 ˚C, thereby demonstrating the extreme robustness

Key features of Air-Dryable Probe 1-Step Mix

- Unbiased, sensitive detection of both DNA and RNA targets
- Sensitivity down to 4 copies per reaction
- Single-tube format with modified UltraScript™ Reverse Transcriptase included in the 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 storage and shipping of dried reactions, with reduced weight and volume
- Increased sample volume input

of the dried mix.

Conclusion

Air-Dryable Probe 1-Step Mix is a powerful enzyme mix designed to solve the difficulties and cost of cold-chain storage and shipping associated with point-of-care diagnostics. There is no loss of activity or impact on performance of the mix 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. This ensures that accurate qPCRs can be set up in preparation for point-of-care diagnostic testing, even under extreme temperature conditions, without the need for cold-chain shipping and storage.

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.

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.

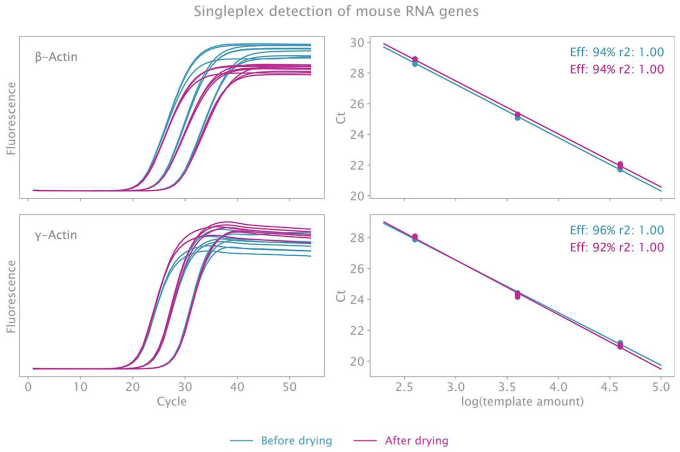

Figure 2. Reliable performance after air-drying in a singleplex setup

Amplification of common housekeeping genes (β-Actin, γ-Actin) in singleplex setup using Air-Dryable Probe 1-Step Mix before (blue curves) or after drying (purple curves). Amplification curves are shown in the left panels and efficiency in the right panels. 3 serial dilutions of mouse total RNA template were used, corresponding to 5 ng/µL, 500 pg/µL, and 50 pg/µL. The total reaction volume was 20 µL. Cycle conditions were 45˚C 10 min, 95 ˚C 3 min and 50 cycles of 95 ˚C 15 s, 58 ˚C 30 s. Air-Dryable Probe 1-Step Mix shows no significant loss of efficiency, speed or sensitivity after drying.

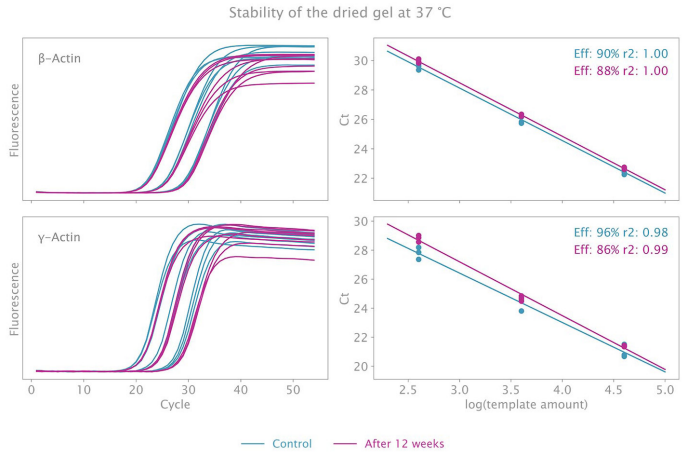

Figure 3. Stability of the dried gel after 12 weeks at 37 ˚C

Amplification of common house-keeping genes (β-Actin, γ-Actin) in singleplex setup using Air-Dryable Probe 1-Step Mix. The mix was dried and stored at -20 ˚C (blue curves) or incubated at 37 ˚C for 12 weeks (purple curves). Amplification curves are shown in the left panels and efficiency in the right panels. 3 serial dilutions of mouse total RNA template were used, corresponding to 5 ng/µL, 500 pg/µL, and 50 pg/µL. The total reaction volume was 20 µL. Cycle conditions were 45 ˚C 20 min, 95 ˚C 3 min and 50 cycles of 95 ˚C 15 s, 58 ˚C 30 s. Air-Dryable Probe 1-Step Mix shows no significant loss of efficiency (less than 10% difference), speed (less than 1 Ct delay) or sensitivity after incubation at 37 ˚C for 12 weeks.