Lucigen
Reagent Components

Enabling room temperature shipping and storage through compatibility of RapiDxFire qPCR 5X Master Mix GF with lyophilisation

Application Note

Introduction

Lyophilisation, or freeze drying, is a low temperature dehydration that involves freezing a reagent at a low temperature, lowering pressure, and then removing any ice by sublimation. The resultant product is either a bead or a cake-like substance and, after rehydration, can be used the same way as before lyophilisation.

Lyophilisation is helpful in a few ways: it eliminates the need for cold chain storage and simplifies shipping conditions, extends shelf life at ambient temperature, and enables over the counter (OTC) and point of care testing (POCT) in specialised devices.

RapiDxFire™ qPCR 5X Master Mix GF can be combined with gene-specific primers and hydrolysis probes to be used both in high-throughput environments as well as in point of care devices. The master mix is provided at a 5X concentration without a passive reference dye which allows greater room for the samples within the reaction and facilitates detection of up to five targets at one time. RapiDxFire qPCR 5X Master Mix GF is also designed for detection of DNA down to ten genomic DNA copies with a wide dynamic range for multiplexing.

Here LGC Biosearch Technologies™, working with a specialised lyophilisation partner, illustrate the suitability of our RapiDxFire qPCR 5X Master Mix GF for lyophilisation, thus expanding the downstream applicability of this mix to a broader scope of molecular diagnostics workflows.

This application note will demonstrate three things:

- Sensitive detection of bacterial pathogens with pre- and post-lyophilised RapiDxFire qPCR 5X Master Mix GF
- Similar or enhanced performance with lyophilised RapiDxFire qPCR 5X Master Mix GF versus lyophilised competitor qPCR master mix products
- Stability of lyophilised RapiDxFire qPCR 5X Master Mix GF at both room temperature and elevated temperature

Materials and methods

Lyophilisation

RapiDxFire qPCR 5X Master Mix GF was lyophilised by Biofortuna Limited (UK). Several excipient formulations were tested, and one formulation selected and used in all presented studies. For the selected formulation, 2200 µL of RapiDxFire qPCR 5X Master Mix GF was combined with 1925 µL of excipient to generate approximately 500 beads with an average bead diameter of 3.01 mm.

Prior to being used in the evaluation experiments, individual spheres were resuspended in 10 µL of 2X Assay Mix consisting of primers and probes for the appropriate target(s) as well as nuclease-free water.

Gram negative bacterial DNA template preparation

For the liquid vs lyophilised and the competitor comparison experiments DNA template comprised of four gram-negative bacterial species: Salmonella typhi (14028™, ATCC), Pseudomonas aeruginosa (35554™, ATCC), Escherichia coli (K12, MG1655) and Serratia liquefaciens (27592™, ATCC). Bacteria were isolated on TSA blood agar plates and a single colony grown overnight in 5 mL of TSA broth. Nucleic acid purification was subsequently performed using the MasterPure Complete DNA and RNA Purification Kit (LGC Biosearch Technologies). Purified DNA was quantified using PicoGreen on the Quant-iT (Thermo Fisher Scientific).

Liquid vs lyophilised RapiDxFire qPCR 5X Master Mix GF

The performance of liquid RapiDxFire qPCR 5X Master Mix GF was compared with the lyophilised version using a 4-plex gram-negative bacteria qPCR assay (detecting E. coli, S. typhi, P. aeruginosa, and S. liquefaciens). A dilution series of the prepared gram-negative bacteria DNA template was performed (10, 102, 103, 104, 105, 106 copies/reaction) to generate standard curves. The qPCR experiments were performed following the manufacturer's protocol for RapiDxFire qPCR 5X Master Mix GF, using both a CFX96™ System (Biorad) and a QuantStudio™ 5 Real-Time PCR System (Applied Biosystems) to run the qPCR.

Competitor comparison

Two competitor qPCR master mixes that are provided lyophilised (Competitor A and Competitor B) were resuspended according to the manufacturer's protocols. The competitor qPCR master mixes were evaluated through qPCR using a 4-plex gram negative bacteria assay (detecting E. coli, S. typhi, P. aeruginosa, and S. liquefaciens). A dilution series of the prepared gram-negative bacteria DNA template was performed (10-106 copies/reaction) to generate standard curves. The qPCR experiments were performed following the manufacturers' protocols for each master mix, using both a CFX96 and a QuantStudio 5 to run the qPCRs. For each master mix, 6 samples were analysed for each copy number.

Stability study

To assess long-term stability, lyophilised RapiDxFire qPCR Master Mix was stored at both 20-25 °C and at 40 °C and was evaluated at 0, 1, 2, 3, 6, 9, 12, 18, 24, 30, and 36 months. Product performance was evaluated using qPCR. Individual gBlocks (IDT) of mouse DNA sequence were blended based on copy number. A dilution series of prepared mouse DNA was performed (3*10, 3*102, 3*103, 3*104, 3*105, 3*106 copies/reaction) and amplified using an in-house 3-plex mouse assay to generate standard curves on the CFX96 (Biorad). To continue with subsequent timepoints in the stability test, each target's standard curve had to display 90-110% efficiency and an R2 value of over 0.98.

Results and discussion

Liquid vs lyophilised RapiDxFire qPCR 5X Master Mix GF

The results obtained demonstrate is that there is no discernible difference in performance between liquid and lyophilised RapiDxFire qPCR 5X Master Mix GF. Performance was assessed using qPCR, and the dilution series results illustrate that the Cq and RFU values are comparable between the liquid and lyophilised versions. These results confirm that the lyophilisation process did not negatively affect the RapiDxFire qPCR 5X Master Mix GF.

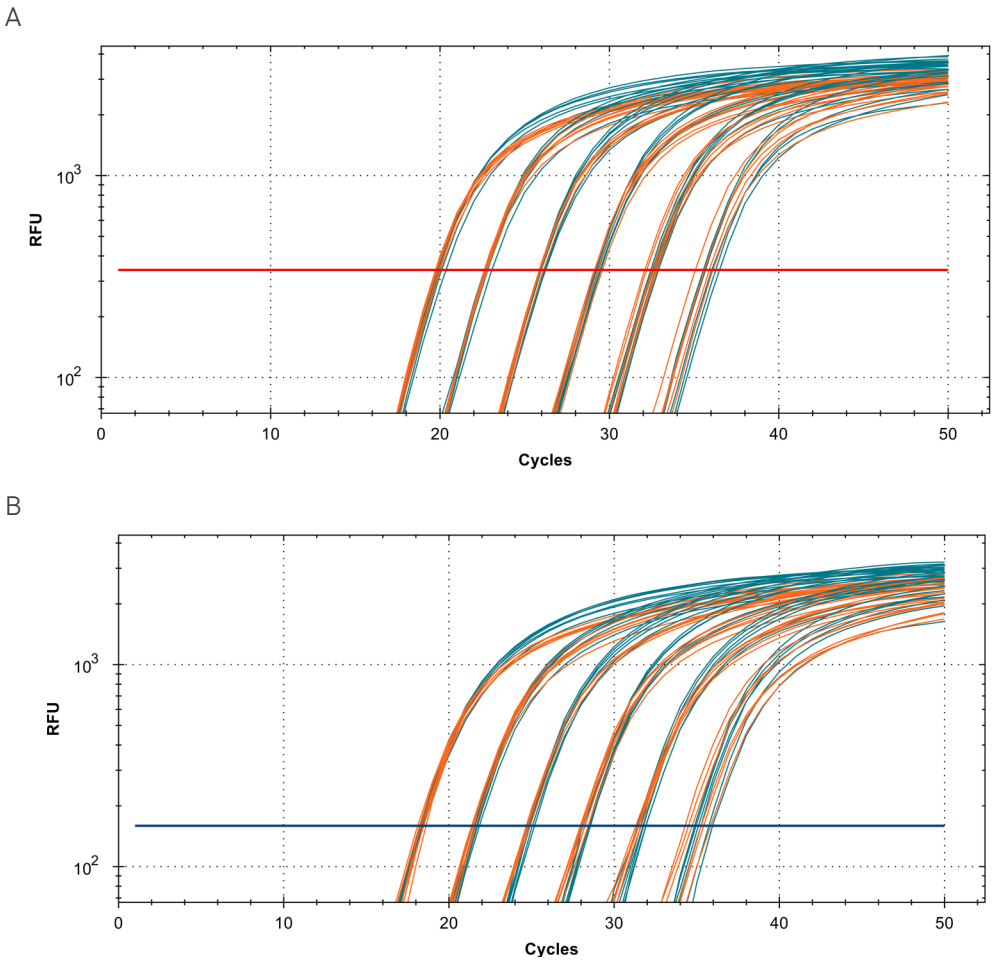

Figure 1. Amplification plot curves for liquid and lyophilised RapiDxFire qPCR 5X Master Mix GF. Gram-negative bacterial template DNA was amplified by qPCR using an in-house 4-plex assay. Figures A and B demonstrate equivalent performance of liquid (teal) and lyophilised (orange) master mix for E. coli and S. typhi respectively. Data for P. aeruginosa and S. liquefaciens is not shown.

Competitor comparison

The performance of two market-leading competitor lyophilised qPCR master mixes was compared against the liquid and lyophilised versions of RapiDxFire qPCR 5X Master Mix GF using qPCR. Figure 2 illustrates the standard curves for liquid and lyophilised RapiDxFire qPCR 5X Master Mix GF (A and B) and for both competitor master mixes (C and D) using the 4-plex gram-negative bacterial assay. As shown in figure 2A and 2B, the liquid and lyophilised versions of RapiDxFire qPCR 5X Master Mix GF demonstrated strong linearity with R2 values over 0.98 and high reaction efficiencies (between 90-110%) across all four gram-negative bacterial assays. Competitor A performed similarly to RapiDxFire qPCR 5X Master Mix GF mix, demonstrating strong linearity and high efficiencies for all four gram-negative bacterial assays (figure 2C). Results for competitor B master mix (figure 2D) are indicative of E. coli contamination in the lyophilised mix, shown by an R2 value below 0.98 and a particularly high efficiency value for the E. coli assay. Comparable results were obtained for these experiments when run on the CFX96 and the QuantStudio qPCR instruments (data generated on the QuantStudio not shown).

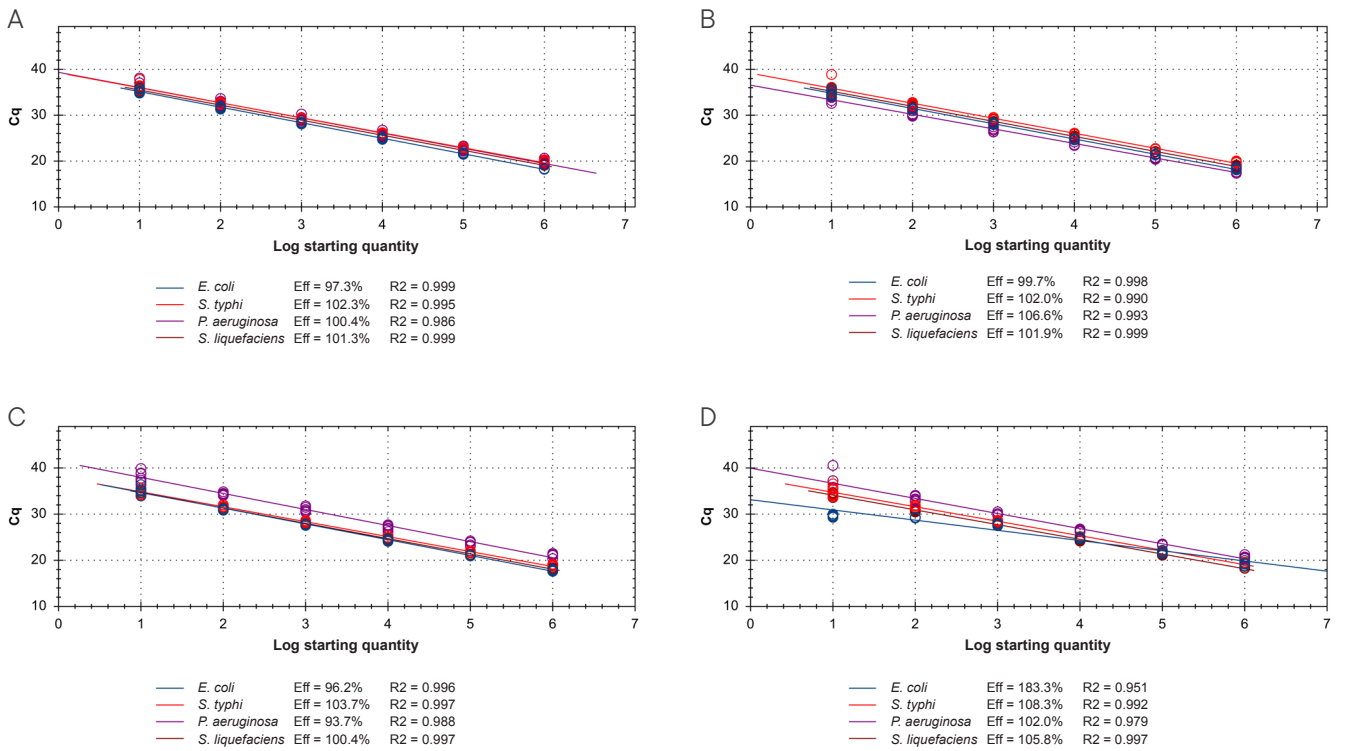

Figure 2. qPCR standard curves for liquid (A) and lyophilised (B) RapiDxFire qPCR 5X Master Mix GF and two lyophilised competitor master mixes (C and D). Standard curves were generated using a 4-plex gram-negative bacterial assay on the CFX96 instrument.

Figure 3 compares the range of Cq values obtained for the E. coli assay for RapiDxFire qPCR 5X Master Mix GF (liquid and lyophilised) and the two competitor mixes. The range of Cq values (shown by the standard deviation) is significantly greater for Competitor A (green) at the 10-copy number concentration than for the wet (teal) or lyophilised (orange) RapiDxFire qPCR 5X Master Mix. There is clear evidence of E. coli contamination in Competitor B master mix as illustrated by the presence of an amplification signal shown in the no template control (NTC) wells (circled).

RapiDxFire qPCR 5X Master Mix GF performs more consistently than the competitor master mixes at lower copy numbers and is devoid of contaminating E. coli DNA.

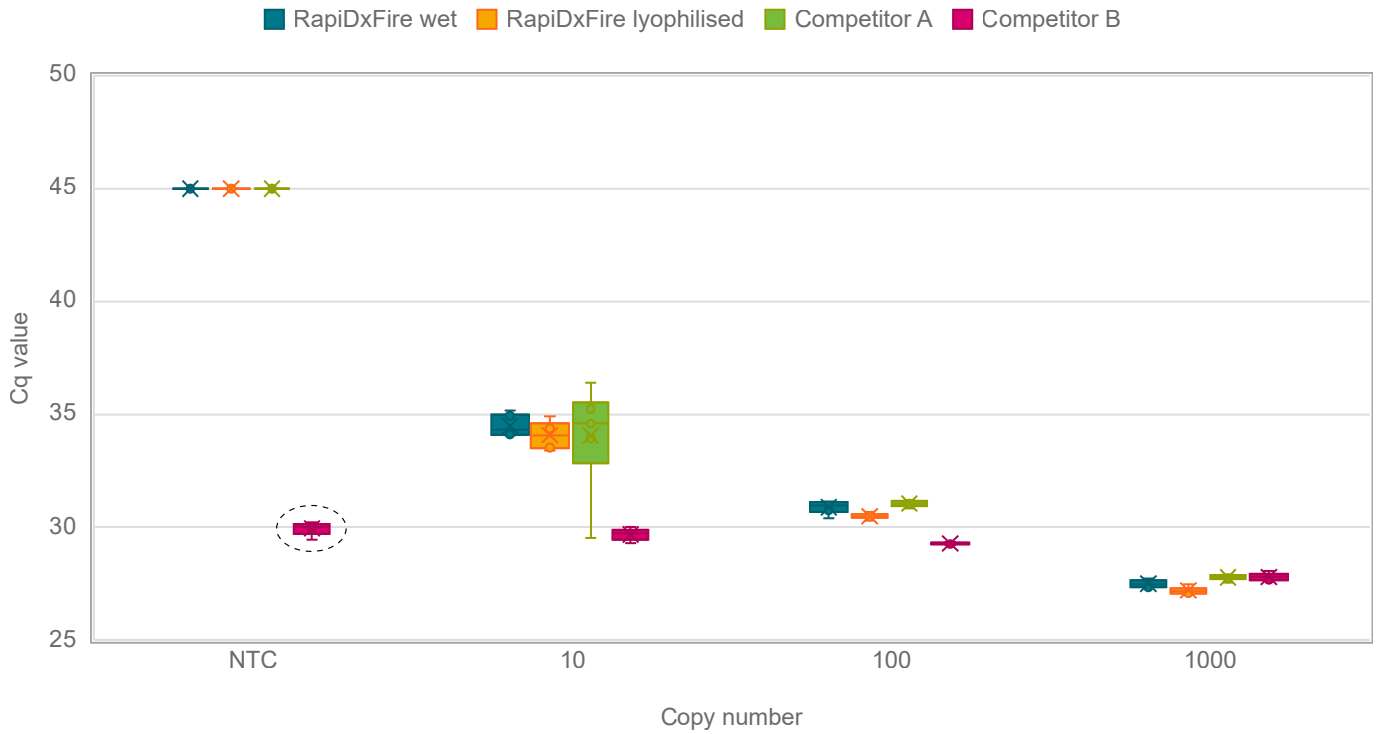

Figure 3. Comparison of Cq value variability for RapiDxFire qPCR 5X Master Mix (liquid and lyophilised) with competitor lyophilised qPCR master mixes. Data is shown for the E. coli assay for NTC, 10, 100 and 1000 copy numbers (n=6 for each copy number). qPCR master mixes are represented as follows: teal = liquid RapiDxFire qPCR 5X Master Mix , orange = lyophilised RapiDxFire qPCR 5X Master Mix, green = Competitor A master mix, pink = competitor B master mix. Both liquid and lyophilised RapiDxFire qPCR 5X Master Mix demonstrate consistent Cq values across all starting concentrations including the NTC reactions. Competitor A and Competitor B demonstrate increased variability in Cq values for all starting concentrations. Reactions were run on the CFX (BIO-RAD).

Stability study

Figure 4 illustrates the Cq values for each dye obtained for the multiplex mouse assay using lyophilised RapiDxFire qPCR 5X Master Mix GF that has been stored at both 25 °C and 40 °C. The range of Cq values obtained (shown by the standard deviation) is consistent throughout the 36-month test period, demonstrating that lyophilised RapiDxFire qPCR 5X Master Mix GF can be stored and is stable at both 25 °C and 40 °C.

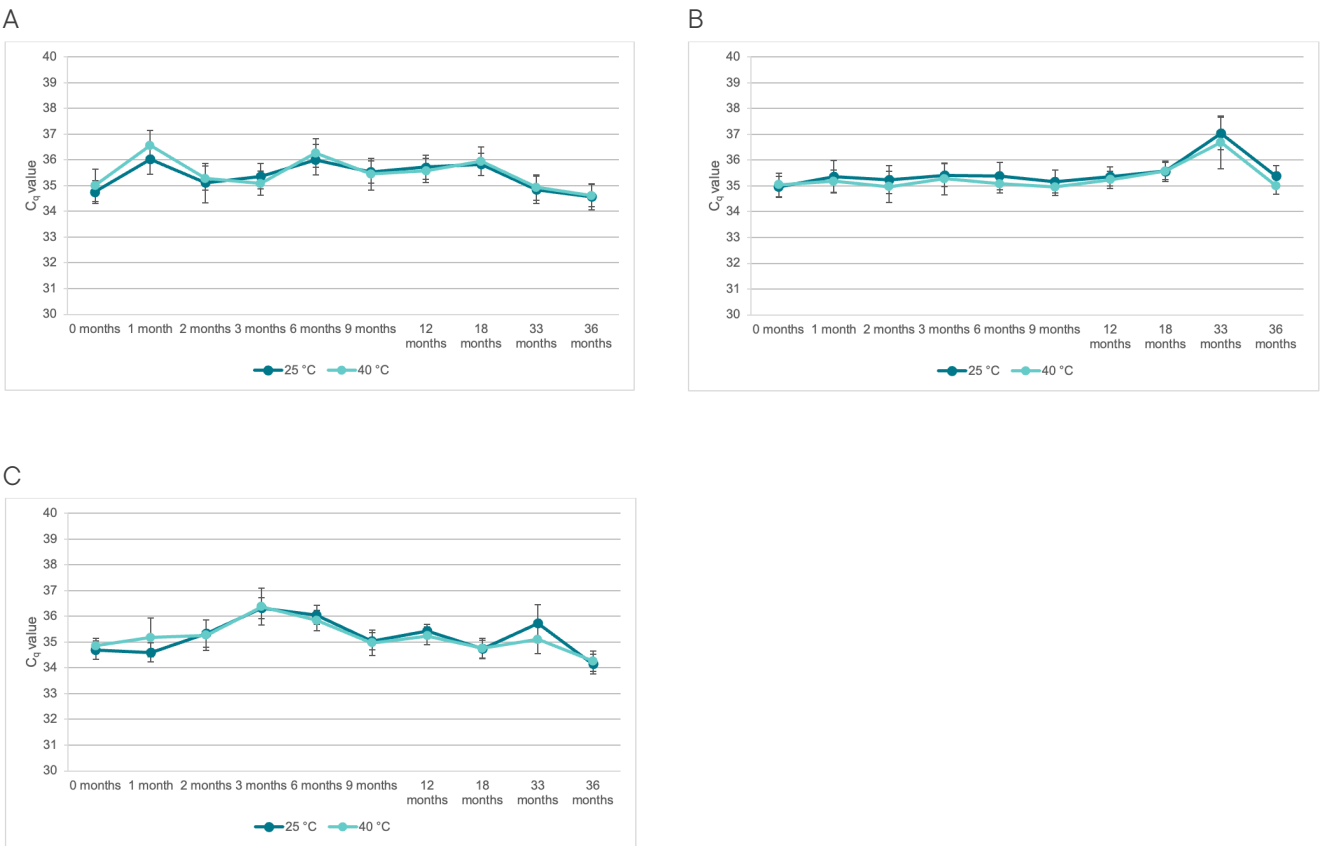

Figure 4. C_q values obtained using lyophilised RapiDxFire qPCR 5X Master Mix stored at 25 °C and 40 °C over a 36-month test period. Data is shown for a multiplex mouse assay (3 dyes) used for QC at a low target level (30 copies/reaction). Temperature storage conditions are represented as follows: blue = 25 °C storage, teal = 40 °C storage. Both temperatures demonstrate consistent C_q values over the 36-month period for all three dyes (A = HEX; B = Quasar 670, and; C = Texas Red). Error bars represent standard deviation. Reactions were run on the CFX (BIO-RAD).

In figure 5 the RFU values obtained using lyophilised RapiDxFire qPCR 5X Master Mix GF for each storage temperature are shown. The range of RFU values obtained (shown by the standard deviation) is consistent across each dye for the 36-month test period at both of the storage temperatures.

This provides further evidence that lyophilised RapiDxFire qPCR 5X Master Mix GF can be stored and is stable at both 25 °C and 40 °C.

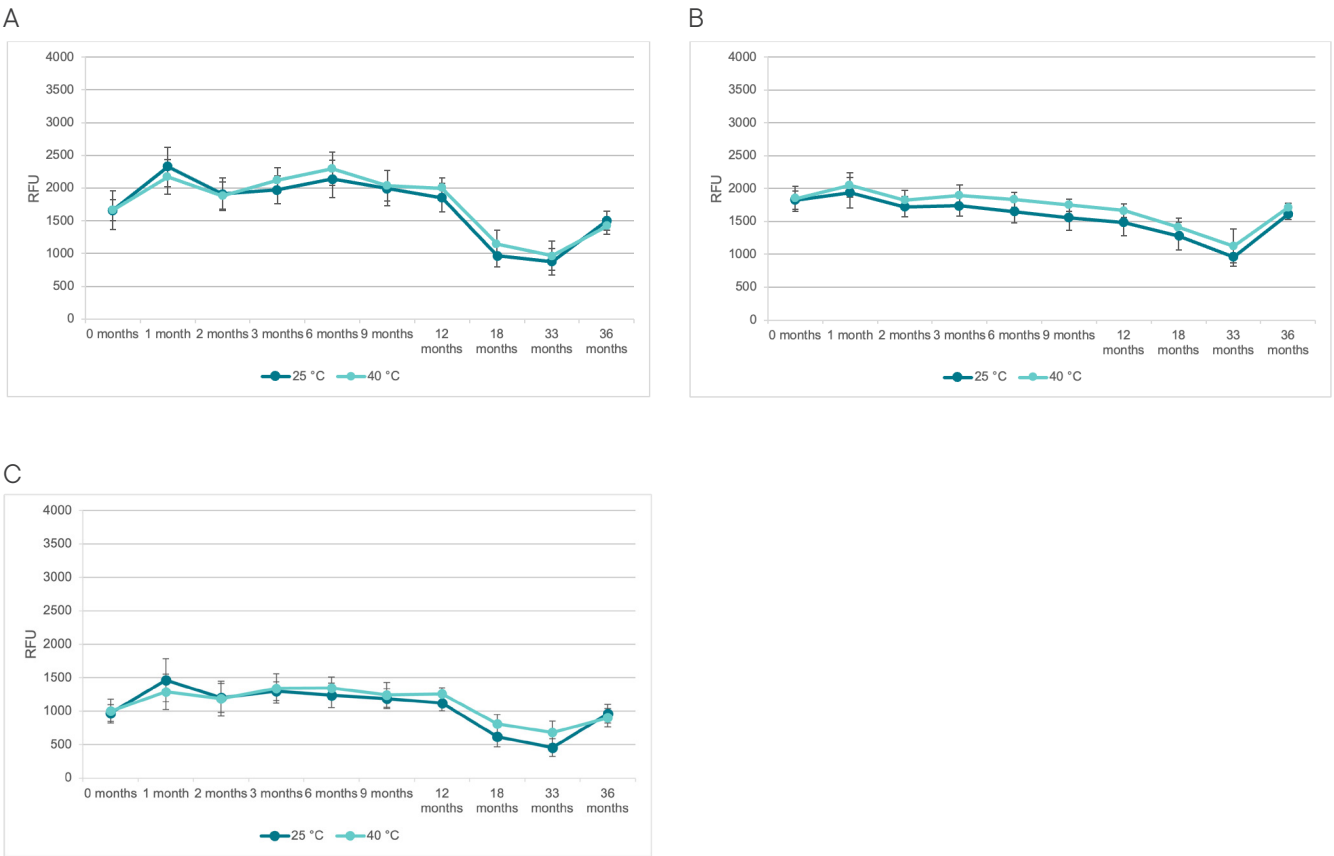

Figure 5. End RFU values obtained using lyophilised RapiDxFire qPCR 5X Master Mix stored at 25 °C and 40 °C over a 36-month test period. Data is shown for a multiplex mouse assay (3 dyes) used for QC at a low target level (30 copies/reaction). Temperature storage conditions are represented as follows: blue = 25 °C storage, teal = 40 °C storage. Both temperatures demonstrate stable RFU values over the 36-month period for all three dyes (A = HEX; B = Quasar 670, and; C = Texas Red). Error bars represent standard deviation. Reactions were run on the CFX (BIO-RAD).

Summary

RapiDxFire qPCR 5X Master Mix GF is suitable for lyophilisation, as demonstrated here by the equivalent performances of both liquid and lyophilised product. We have achieved sensitive multiplex detection of bacterial pathogens with both liquid and lyophilised RapiDxFire qPCR 5X Master Mix GF, using primers and probes from Biosearch Technologies and molecular diagnostics-applicable instrumentation.

The performance of lyophilised RapiDxFire qPCR 5X Master Mix GF is demonstrated as equivalent or better than market-leading lyophilised competitor master mixes. We also illustrate equivalent stability of both liquid and lyophilised product, as shown by the maintenance of Cq and RFU values over time. In addition, lyophilised RapiDxFire qPCR 5X Master Mix GF is shown to be stable at 40 °C, giving equivalent performance to lyophilised product stored at 25 °C.

The suitability of RapiDxFire qPCR 5X Master Mix GF for lyophilisation enables our customers to work with specialist lyophilisation partners to lyophilise this mix for compatibility with their workflow needs. This facilitates storage of RapiDxFire qPCR 5X Master Mix GF at ambient temperature, whilst also enabling its use in a wider range of assays and protocols including the development of diagnostic tests in point of care devices.

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