Revolutionizing Point-of-Care Molecular Diagnostics with Cold-Chain-Free, Rapid-Dissolving, Single-Reaction Lyophilized qPCR Master Mix Bead

Abstract

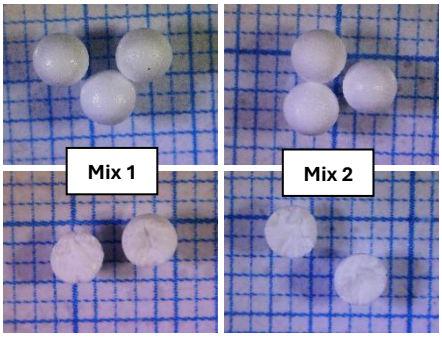
Lyophilization of qPCR master mix (MM) formulations significantly enhances stability, allowing for ambient storage and transport without cold chain logistics.[1] This study shows that optimized freeze-drying conditions, along with careful selection of excipients, preserves the biochemical integrity and performance of the MM, enabling rapid rehydration and consistent amplification. Accelerated stability tests confirm that lyophilized MM retains efficiency across temperatures, underscoring its suitability for long-term preservation in point-of-care diagnostics.

Excipient and freeze-drying parameters selection

Excipient selection and freeze-drying parameters are both critical for achieving high-quality lyophilized reagents.[2] Factors like robustness, residual moisture, bead consistency, and dissolution rates rely on stabilizing excipients and precise cycle settings. Optimized excipient choices and drying conditions together ensure particle uniformity, rapid rehydration, and extended stability; all essential for reliable diagnostic performance.

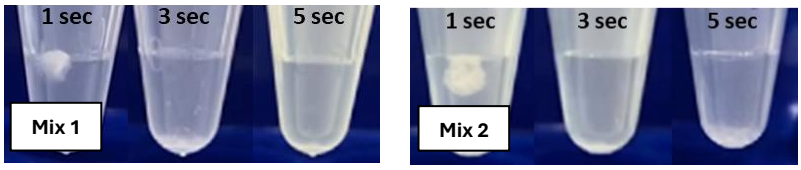
In this study, two different excipient matrices (Mix 1 and Mix 2), comprising both carbohydrate-based and non-carbohydrate components, were evaluated for the lyophilization of a qPCR MM. A relatively short and highly efficient 16-hour freeze-drying cycle was designed to optimize both production time and product quality. qPCR formulations mainly consisted of at least 10% total excipient(s), MM and reverse transcriptase (RT).

Physical characteristics of lyophilized qPCR MM beads



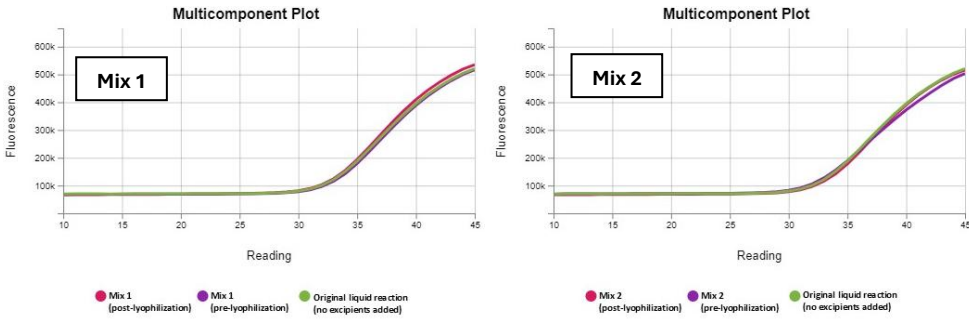
Formulations containing a complete qPCR MM, RT, buffer, and specific excipient mixtures, were dispensed into liquid nitrogen at a volume of 12.5 µL and lyophilized. The morphology of the lyophilized beads was evaluated using microscopy, revealing uniform internal and external structures across all samples. Residual moisture content analysis showed that both formulations maintained acceptable levels (<4%), with Mix 1 having lower residual moisture (1.9%) compared to Mix 2 (2.9%). Robustness was assessed through crush force testing with a force gauge, where all beads

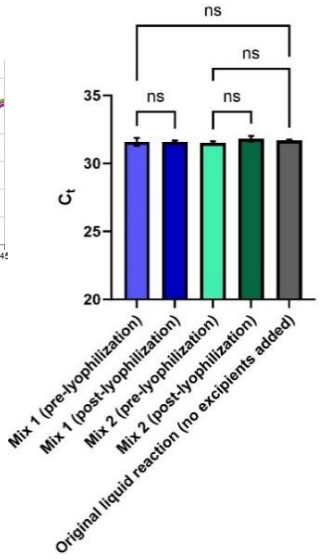
demonstrated a crush force exceeding 0.4 N, indicating suitable hardness for handling and assembly. Both formulations exhibited a rapid dissolution rate, achieving complete dissolution within 5 seconds without the need for any agitation.



Do excipients and/or lyophilization process affect qPCR performance?

While excipients can sometimes influence qPCR assay performance, if their type and concentration are selected carefully, they serve to stabilize reagents during lyophilization and storage and may even enhance amplification efficincy.[3] If excipients and/or lyophilization parameters are not properly selected, effects such as inhibition of enzymatic activity, disruption of primer and probe binding affinity, and alteration of reaction conditions such as pH and osmolarity may negatively impact amplification.





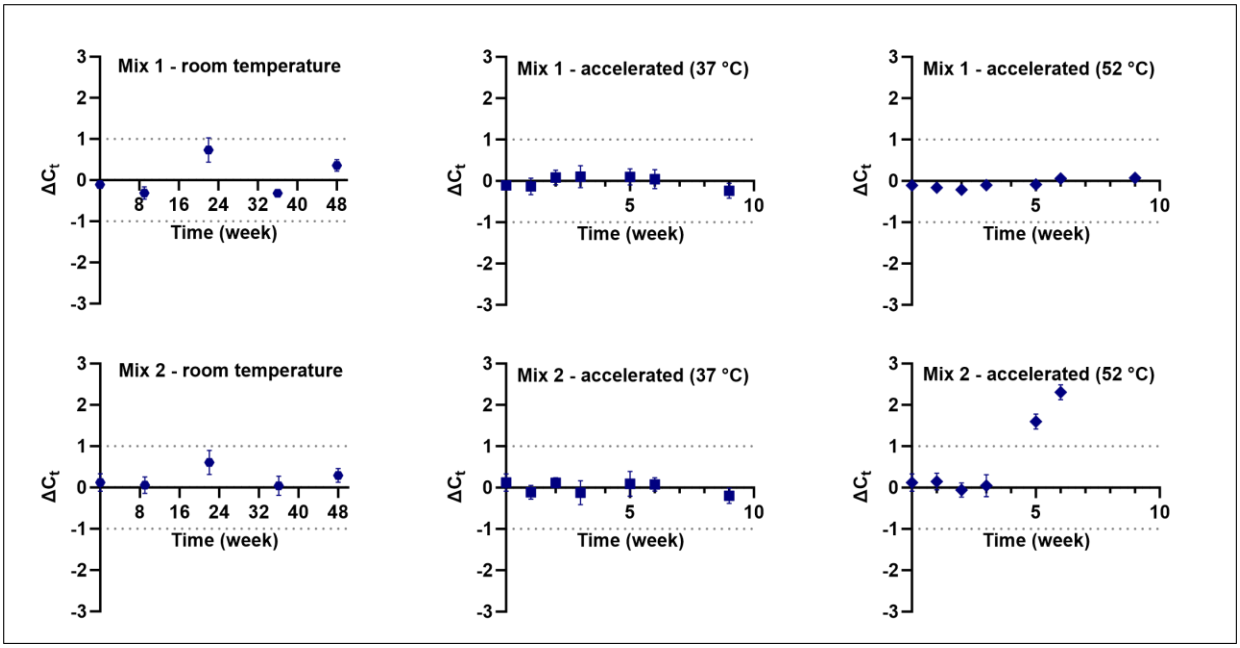
To assess the impact of excipients and lyophilization on amplification efficiency, qPCR reactions were conducted and compared across three formulations: freshly prepared original liquid reactions, pre-lyophilization liquid reactions containing excipients, and lyophilized single-reaction beads containing excipients. Upon reconstitution, SARS-CoV-2-N2-specific primers, probes, and the RNA template were added to the reaction. All reactions, including both lyophilized and non-lyophilized controls, contained SARS-CoV-2 RNA template and were brought to a final volume of 20 µL for amplification. No significant differences were observed in amplification efficiency or C_t values across the tested conditions. Neither the addition of excipients nor the lyophilization process caused any significant increase in C_t values compared to the original liquid reactions. This result suggests that both excipient addition and lyophilization were compatible with maintaining qPCR performance in this assay.

Are lyophilized qPCR MM beads stable overtime?

Lyophilized qPCR beads were stored under both real time (ambient temperature for up to a year) and accelerated conditions (37 ºC and 52 ºC for up to 9 weeks). To evaluate retention of reaction activity

in the freeze-dried qPCR mixes, amplification performance was compared to that of freshly prepared liquid reagents. ΔC_t values, calculated as [C_t (lyophilized bead) - C_t (original fresh liquid reaction)], were monitored over time, with a ΔC_t variation of up to ±1 considered an acceptable threshold for stability.

The real-time stability study at ambient temperature shows that after about 12 months, both Mix 1 and Mix 2 lyophilized beads maintain amplification efficiency with no significant deviations in C_t values. Under accelerated conditions at 37 °C, ΔC_t values for both Mix 1 and Mix 2 remained within



the acceptable range throughout the 9-week period, indicating robust performance at moderately elevated temperatures. At 52 °C, Mix 2 exhibited a transient increase in ΔC_t at approximately 5 weeks, suggesting a slight reduction in amplification efficiency under these more extreme conditions; however, Mix 1 remained stable with no statistically significant loss in performance.

Conclusion

This study confirms that lyophilized qPCR MM beads, optimized with specific excipients and lyophilization cycle, maintain structural integrity, rapid dissolution, and stable amplification performance, comparable to fresh liquid reagents. Stability testing under real-time (ambient) and accelerated (37 °C and 52 °C) conditions demonstrated robust performance, with both Mix 1 and Mix 2 remaining stable for long term at room temperature and tolerating moderate to extreme heat exposure. Mix 1 exhibited superior stability even at 52 °C, with no significant C_t deviations, while Mix 2 showed minor ΔC_t fluctuations under extreme conditions.

Lyophilized beads offer multiple advantages over traditional liquid master mixes, particularly for point-of-care diagnostics. Their long-term stability at room temperature allows for cold chain-free storage and distribution, significantly reducing logistical challenges and costs. The pre-measured, single-use format minimizes user error and ensures batch-to-batch consistency, while also lowering

contamination risks through reduced handling. Rapid rehydration and ready-to-use convenience further streamline workflows, enabling faster, more reliable testing in diverse and resource-limited environments. This approach supports the development of accessible, shelf-stable diagnostic reagents that are well-suited for remote and decentralized testing, advancing molecular diagnostics in varied settings worldwide.

References

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