

# Lyophilisation-compatible qPCR mix for low-copy number target detection

Clare Nevin, Gianpiero Spedale, Rory O'Neill, Simon Baker  
Bioline Reagents Limited, Unit 16 The Edge Business Centre, Humber Road, London NW2 6EW, UK

## Introduction

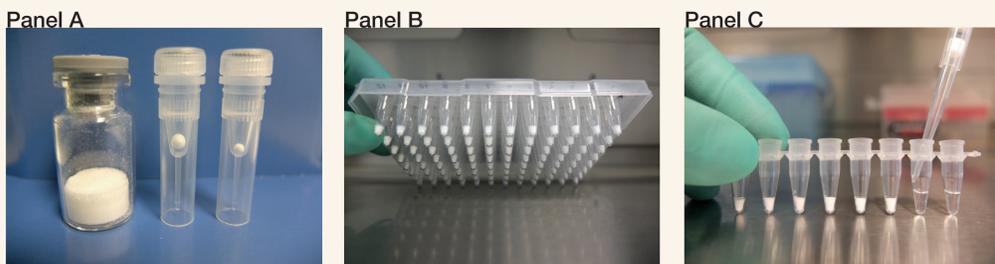
Glycerol-free, lyophilisation-compatible qPCR mixes provide a number of advantages over the standard 'wet' mix formulations that make them ideal for incorporation into high-throughput, diagnostic systems and point-of-care devices<sup>1</sup>. The reconstitution of a lyophilised qPCR mix, containing target-specific primers and probes, with template alone is key to reducing handling time, while ambient temperature stability permits transportation and storage under a range of conditions.

We sought to develop a robust and lyophilisable probe-based qPCR mix to meet the requirements of our research partners. Here we describe the product development and properties of SensiFAST™ Lyo-Ready No-ROX Mix, a glycerol-free, readily-lyophilisable qPCR mix.

## Development Strategy

High-throughput screening of master mixes was conducted with many different classes of lyo-excipients and PCR enhancers to determine the most effective combinations. Key responses were volume and uniformity of the lyophilised 'cake' (Figure 1), and activity recovery, which inferred stability and functional properties respectively. A multifactorial design approach was used to develop the top candidates further and optimize for rehydration speed, multiplex-qPCR efficiency, limit of detection (LOD) and ambient temperature stability.

The resultant formulation, SensiFAST Lyo-Ready No-ROX Mix, was validated in lyophilised form in different vessels and in cake and 'bead' formats (Figure 1).

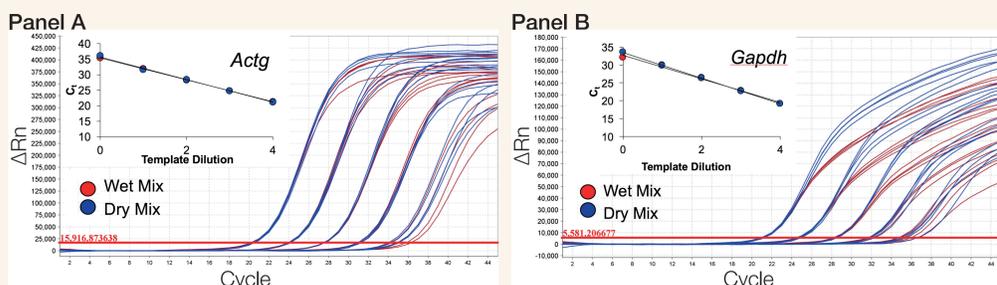


**Figure 1. SensiFAST Lyo-Ready No-ROX Mix Lyophilised formats**  
Lyophilised SensiFAST Lyo-Ready No-ROX Mix in cake (glass vial) and bead (polypropylene screw-cap tubes) formats (Panel A); Lyophilised cakes in PCR plate and strip (Panel B & C, respectively).

## Results

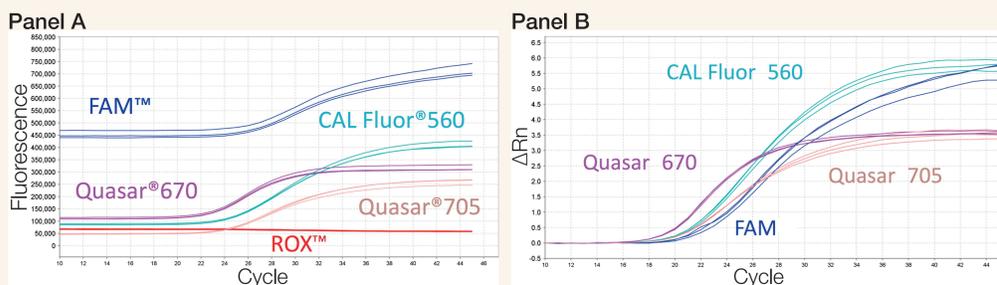
### Multiplex efficiency and sensitivity

Lyophilised SensiFAST Lyo-Ready No-ROX Mix (dry mix) maintained activity comparable to the wet mix with qPCR efficiencies of >90 % (Figure 2).



**Figure 2. Comparison of the efficiency and sensitivity of wet and dry mixes**  
The dry (blue) and wet (red) mix amplification profiles for *Actg* (Panel A) and *Gapdh* (Panel B) amplicons are shown. The calculated efficiencies for *Actg* and *Gapdh* were 95 % and 95 %, for the wet mix and 93 % and 94 % for the dry mix (Panel A & B; inset graphs).

The SensiFAST Lyo-Ready No-ROX Mix also showed high multiplex-qPCR efficiency with viral amplicons. Figure 3 shows a multiplex qPCR consisting of four viral targets, including one internal control, with and without ROX passive reference normalization. The qPCR efficiencies for each amplicon were >95 % with a detection limit of two target copies.



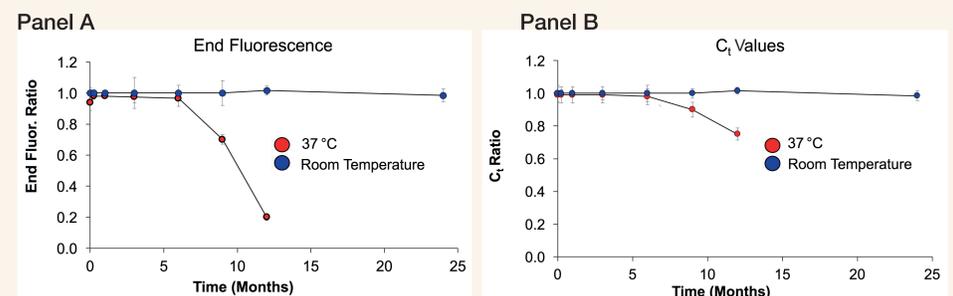
**Figure 3. Viral target detection in a multiplexed qPCR assay**  
Raw (Panel A) and normalized (Panel B) fluorescence profiles for 4 target amplicons using compatible fluorophores and passive reference. Efficiencies for FAM, CAL Fluor 560, Quasar670 and Quasar705 were 90 %, 95 %, 96 % and 91 % respectively.

## Conclusion

SensiFAST Lyo-Ready No-ROX Mix is a glycerol-free qPCR mix which can be employed as a user-driven lyophilisable wet mix, or alternatively it can be provided as a customized lyophilised detection kit with primers and probes. The dry mix is stable at ambient temperatures, therefore providing a beneficial tool for point-of-care and diagnostic devices. The capacity of the mix to multiplex in qPCR and RT-qPCR to a high standard highlights its broad applicability.

## Stability

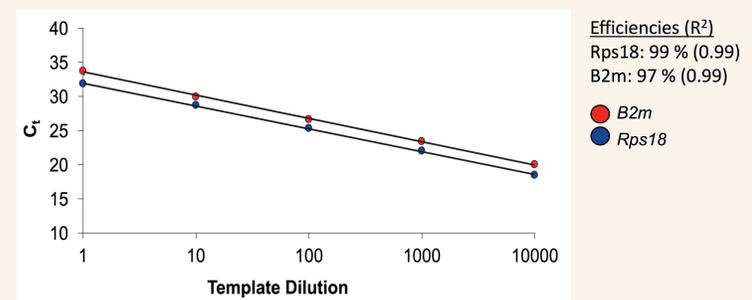
Functional tests showed that the dry mix, pre-mixed with or without primers and probes, is stable for a minimum of 24 months at room temperature (17-23 °C) and for up to three months at 37 °C, compared to the freshly-made wet mix (Figure 4; data shown are without pre-mixed primers and probes).



**Figure 4. Storage and accelerated stability test**  
Lyophilised SensiFAST Lyo-Ready No-ROX mix was tested for stability at room temperature and 37 °C up to 24 months and 12 months, respectively. End fluorescence (Panel A) and Ct values (Panel B) were calculated as a ratio of the dry to the wet mix.

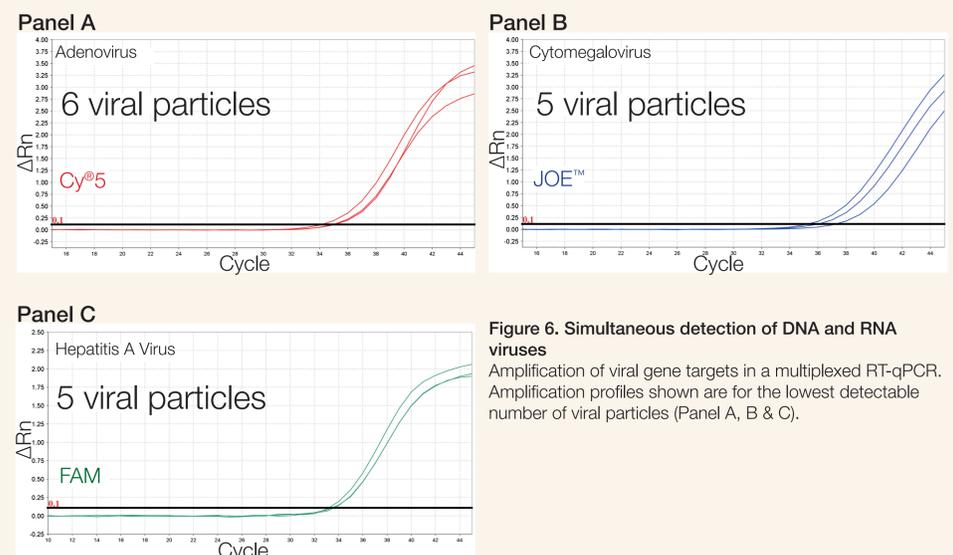
## RT-qPCR compatibility

Further testing of the SensiFAST Lyo-Ready No-ROX Mix with reverse transcriptase (RT) showed high efficiency in multiplex RT-qPCR assays (Figure 5). Supporting data from external collaborators confirmed that the mix showed high sensitivity detection of low copy number RNA targets (data not shown).



**Figure 5. Compatibility of SensiFAST Lyo-Ready No-ROX Mix with RT-qPCR**  
The mix showed high efficiencies for *Rps18* (99 %) and *B2m* (97 %) amplicons in multiplex reactions.

A key feature of the RT-qPCR with SensiFAST Lyo-Ready No-ROX Mix is very low RNA copy number detection in both singleplex and multiplex reactions. This high RT-qPCR sensitivity enabled detection of <10 viral particles per sample (Figure 6).



**Figure 6. Simultaneous detection of DNA and RNA viruses**  
Amplification of viral gene targets in a multiplexed RT-qPCR. Amplification profiles shown are for the lowest detectable number of viral particles (Panel A, B & C).

## References:

(1) Waters RH et al., Next Generation Drying Technologies for Pharmaceutical Applications. *Journal of Pharmaceutical Sciences* 103, 9 (2009)  
Trademarks: ROX™, FAM™, JOE™ (Life Technologies, Inc.); Cy5 (Thermo Fisher Scientific Inc.); Quasar® 670, Quasar® 705, CAL Fluor® Orange 560 (Biosearch Technologies)

For more information please email [info@bioline.com](mailto:info@bioline.com)



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