Custom Portfolio

Unravelling DNA to Enable Your Discovery

Ben Jackson PhD
Global Marketing Manager

Meridian Bioscience, Inc.
Inspired Science. Trusted Solutions.

BIOLINE
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Custom Portfolio

- SensiFAST qPCR Mix
- SensiFAST One-Step RT-qPCR Mix
- SensiMix qPCR Mix
- SensiMix One-Step RT-qPCR Mix
- MyTaq PCR Mix
- MyTaq One-Step RT-PCR Mix
- Glycerol-free, lyo-ready SensiFAST qPCR Mix
- High-concentration Taq DNA Polymerase
- Reverse transcriptase
- Lyo-ready One-Step RT-qPCR Mix (*in development*)
- Glycerol-free reverse transcriptase (*in development*)

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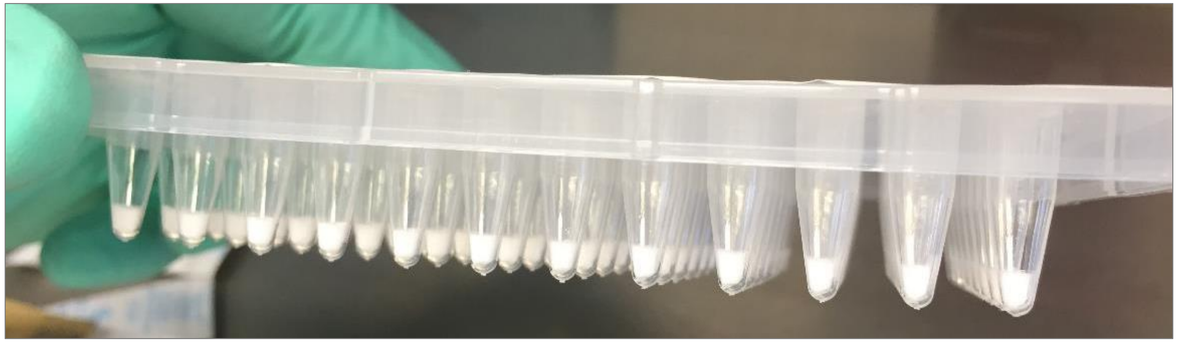
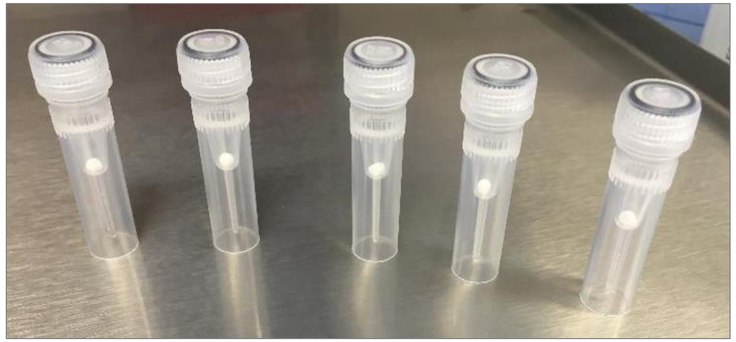
Lyophilization; Advantages

- Maximization of reagent quality and performance
- Simplification of workflow
- Provision of room temperature stability
- Enabling incompatible reagents to co-exist
- Reducing manufacturing and delivery costs
- Streamlining product time to market
- Increasing storage capacity without increasing overheads





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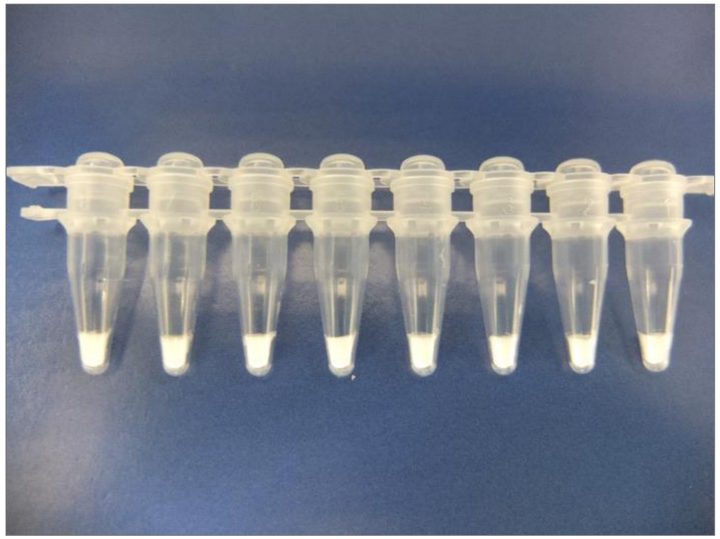
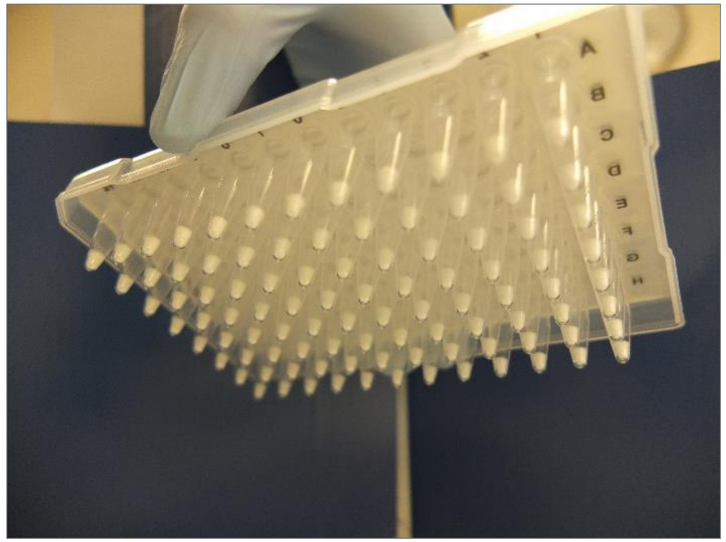
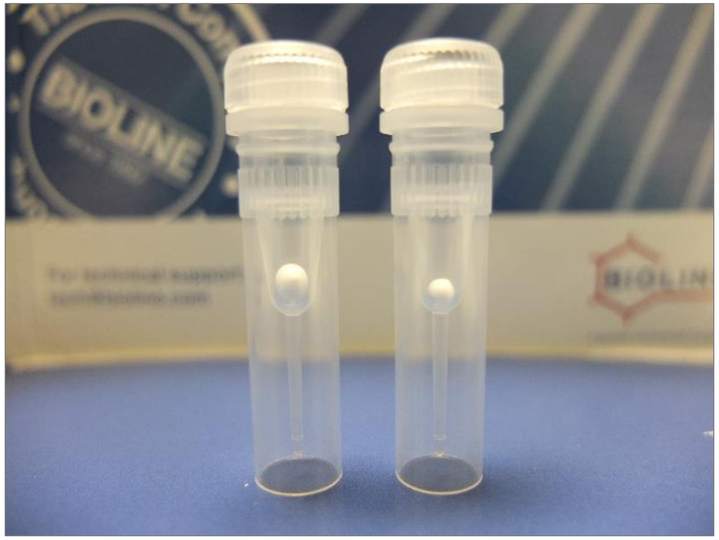
Lyophilization; Formats

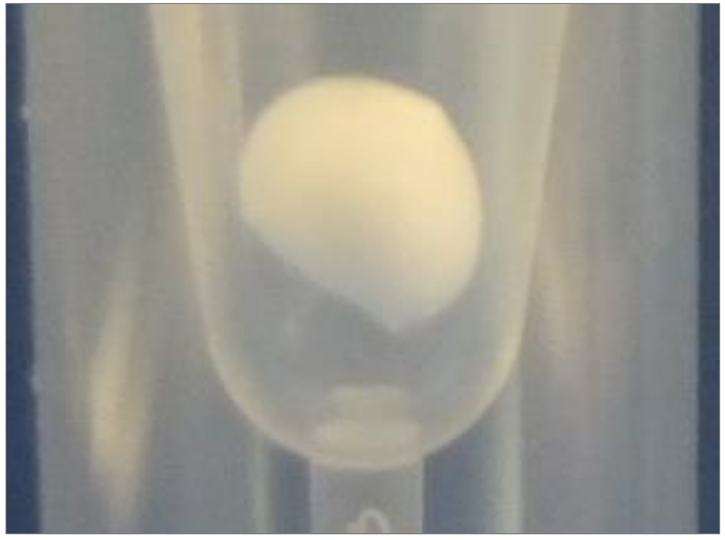
Cake

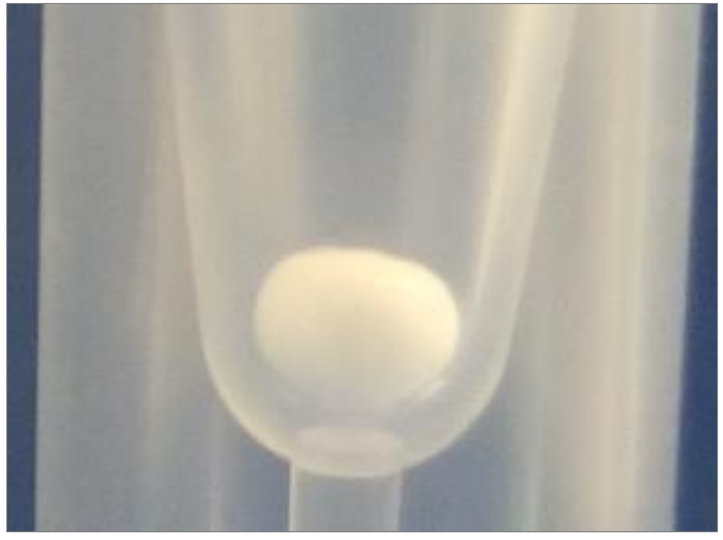




Beads







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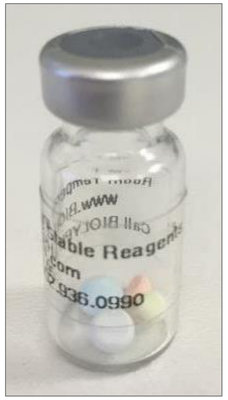
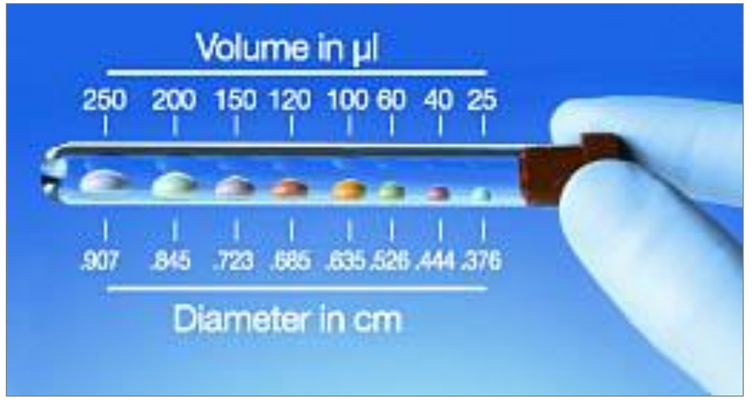
Lyophilization; Partnerships

- Collaborate with lyophilization experts
- Assays can be lyophilized with primers / probes
- Low up-front cost for proof of principle
- Ultra-dry LyoSpheres™ dissolve instantly
- Picoliter to microliter capabilities





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Internal Lyophilization Study

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[1]. 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).

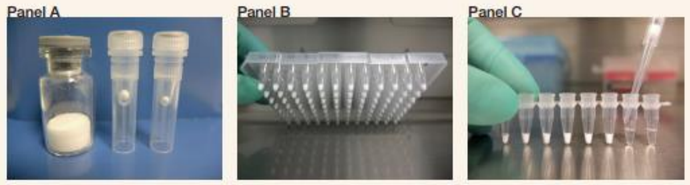

Figure 1. SensiFAST Lyo-Ready No-ROX Mix Lyophilised formats
Lyophilised SensiFAST Lyo-Ready No-ROX Mix in cake (glass vial) and bead (polypropylene screw-cap tubes) formats (Panel A); Lyophilised cakes in PCR plate and strip (Panel B & C, respectively).

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).

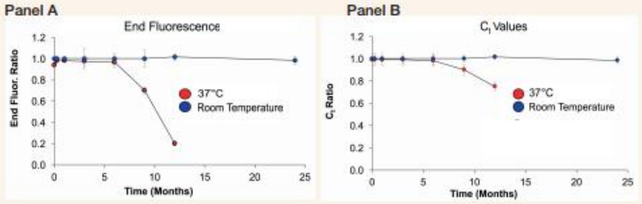

Figure 4. Storage and accelerated stability test
Lyophilised SensiFAST Lyo-Ready No-ROX mix was tested for stability at room temperature and 37 °C up to 24 months and 12 months, respectively. End fluorescence (Panel A) and Ct values (Panel B) were calculated as a ratio of the dry to the wet mix.

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).

wet mix with qPCR efficiencies of

apdh

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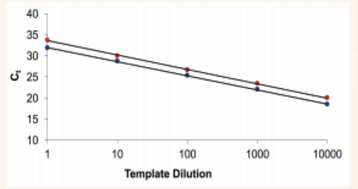

Figure 5. Compatibility of SensiFAST Lyo-Ready No-ROX Mix with RT-qPCR
The mix showed high efficiencies for *Rps18* (99 %) and *B2m* (97 %) amplicons in multiplex reactions.

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).

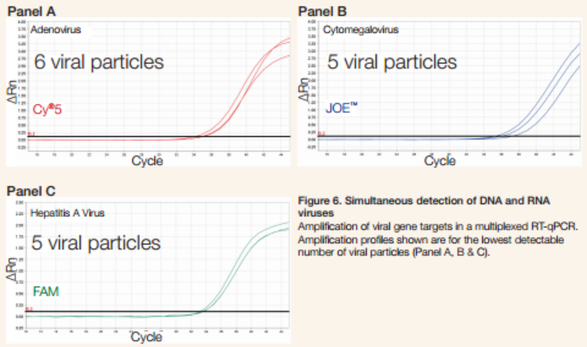

Figure 6. Simultaneous detection of DNA and RNA viruses
Amplification of viral gene targets in a multiplexed RT-qPCR. Amplification profiles shown are for the lowest detectable number of viral particles (Panel A, B & C).

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
diagnostic devices. The capacity of the mix to multiplex in qPCR and RT-qPCR to a high standard highlights its broad applicability.

al of Pharmaceutical Sciences 103, 9 (2009)

Trademarks: ROX™, FAM™, JOE™ (Life Technologies, Inc.); Cy®5 (Thermo Fisher Scientific Inc.); Quasar® 670, Quasar® 705, CAL Fluor® Orange 560 (Biosearch Technologies)

For more information please email info@bioline.com

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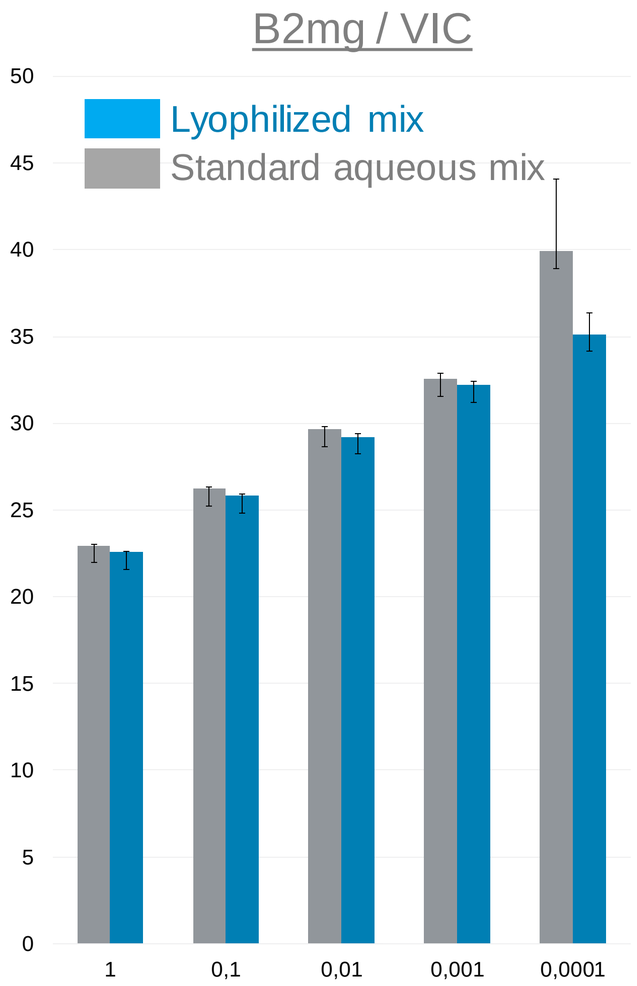
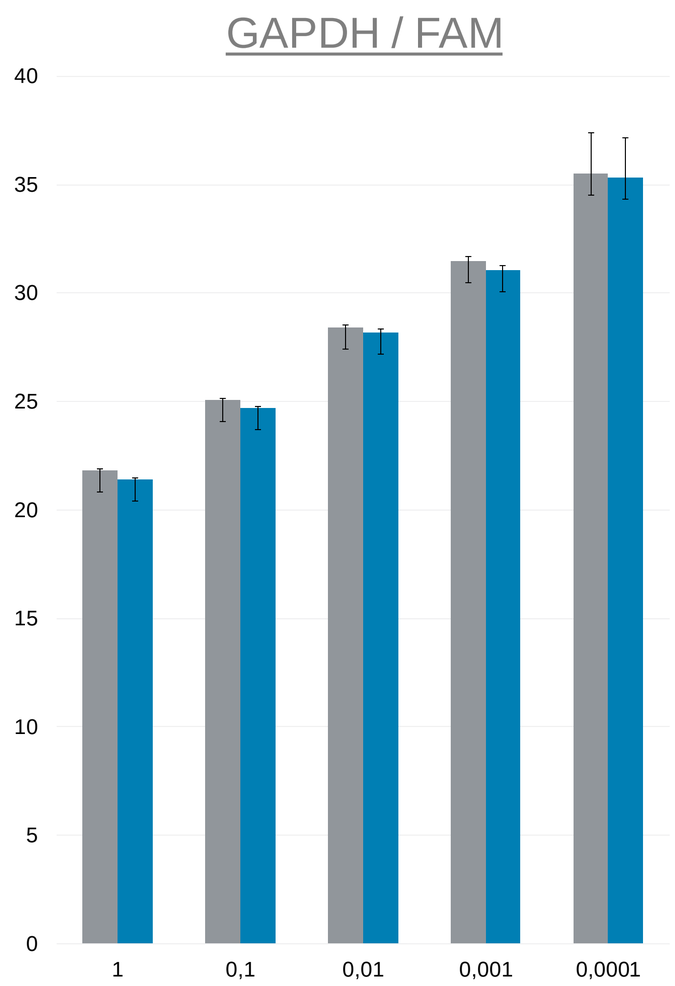
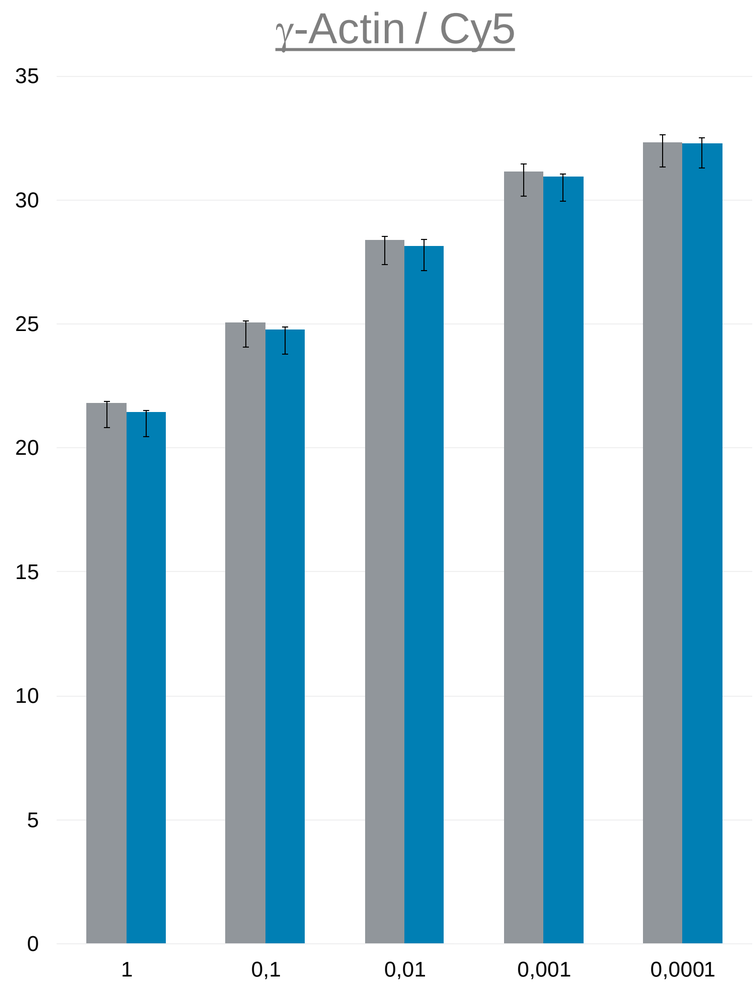
SensiFAST Lyo-Ready Mix; Performance







Lyophilized mix stored for 20 months at room temperature delivers a comparable performance to the standard aqueous mix

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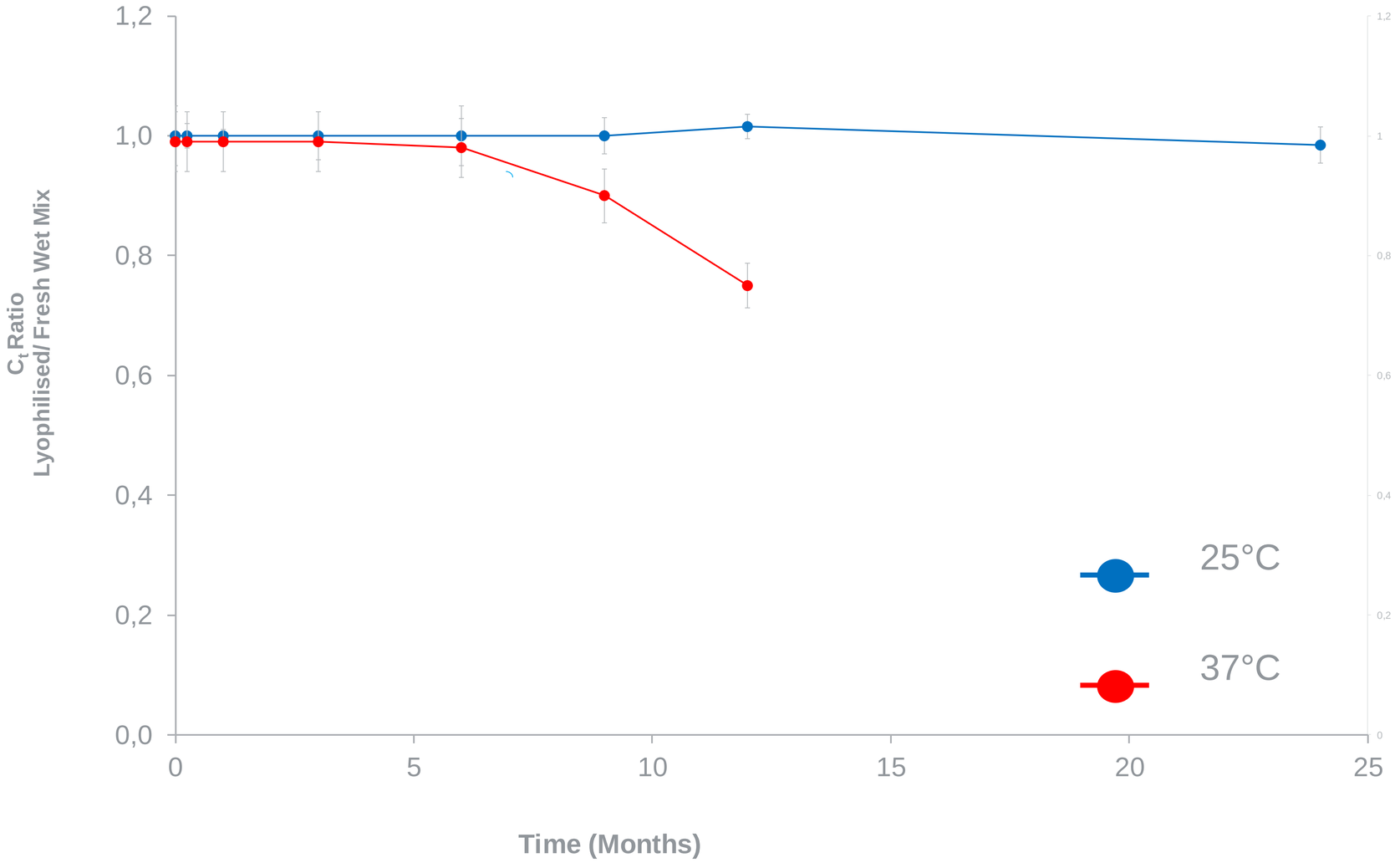
SensiFAST Lyo-Ready Mix; Stability



- Up to 2 years at 25°C
- Up to 1 month at 37°C

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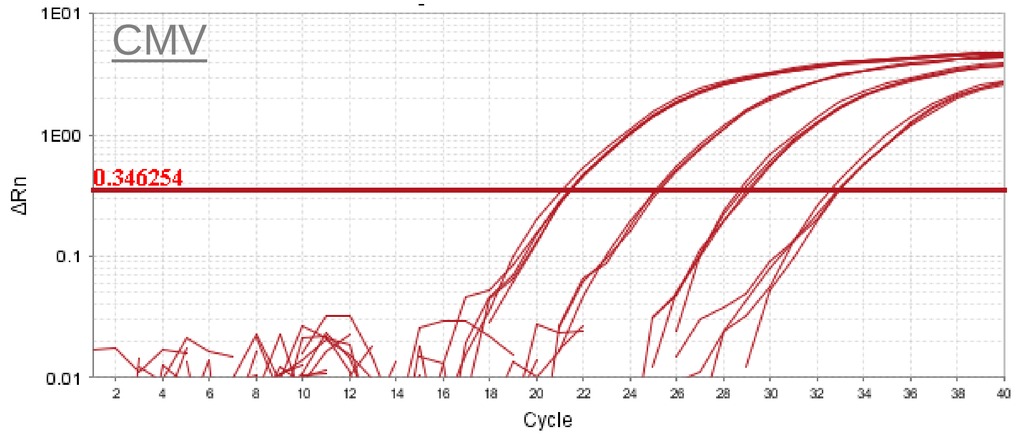
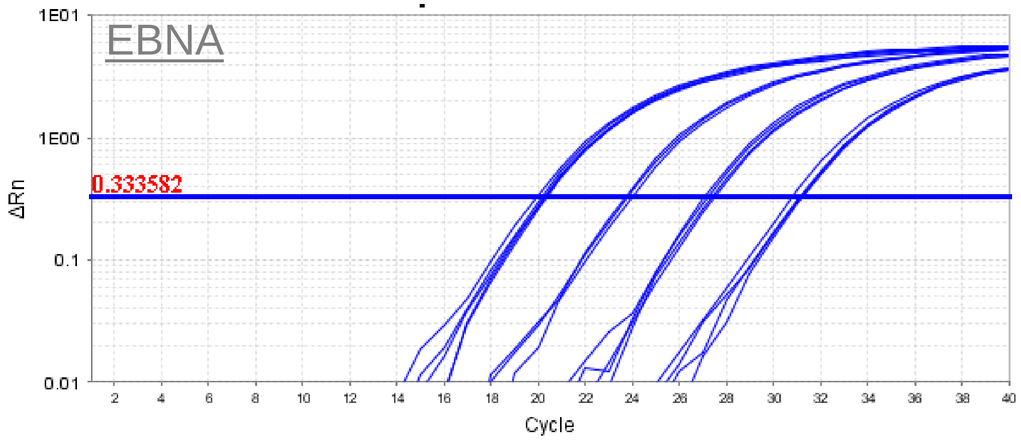
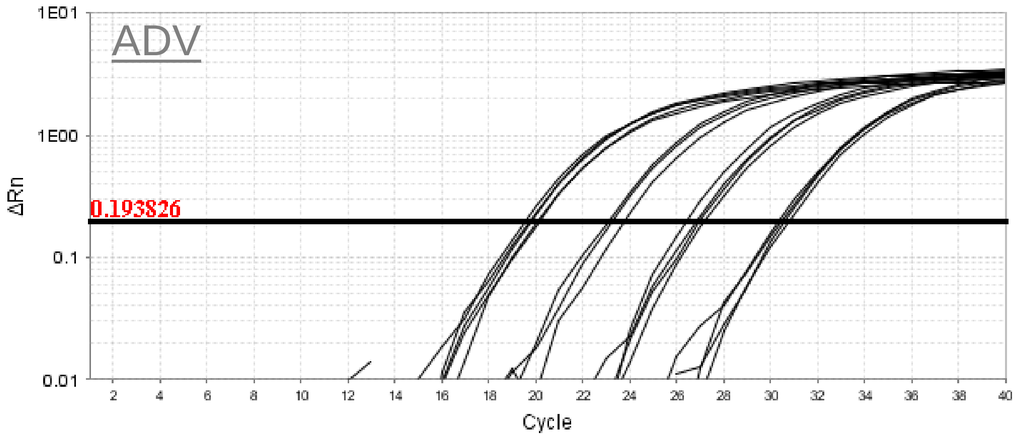
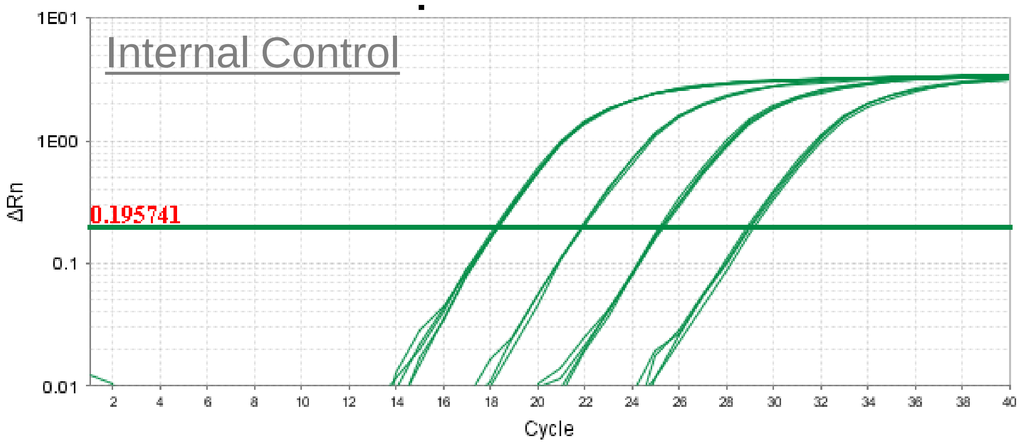
SensiFAST Lyo-Ready Mix; Multiplex









Amplification efficiency of ≥96% for individual assays in a 4-plex assay - as shown by a 10-fold serial dilution

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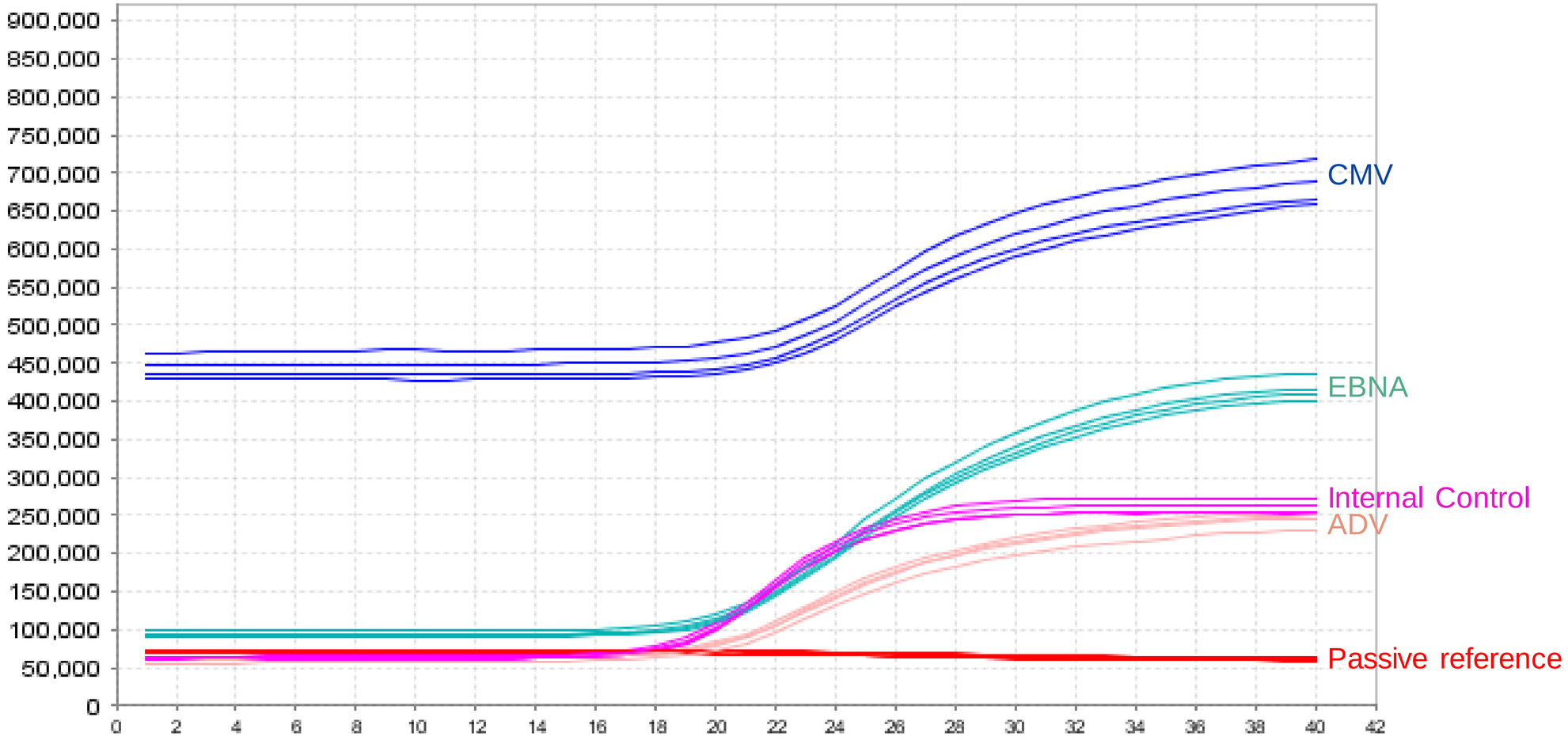
SensiFAST Lyo-Ready Mix; Multiplex



Individual assays shows a comparable performance in a 4-plex assay – for each dilution

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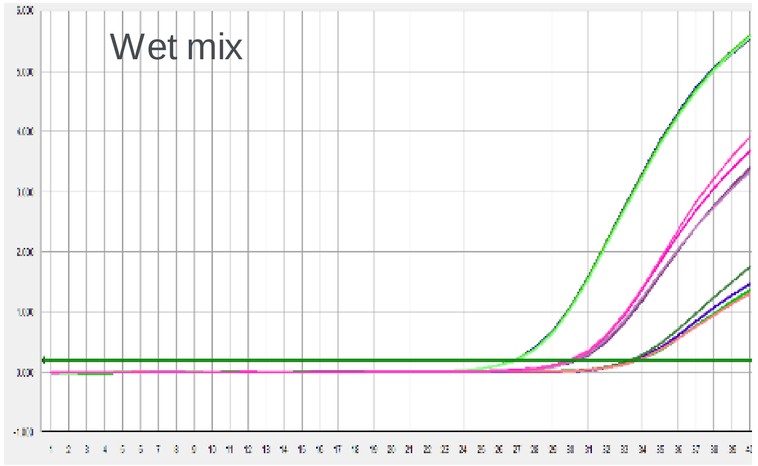
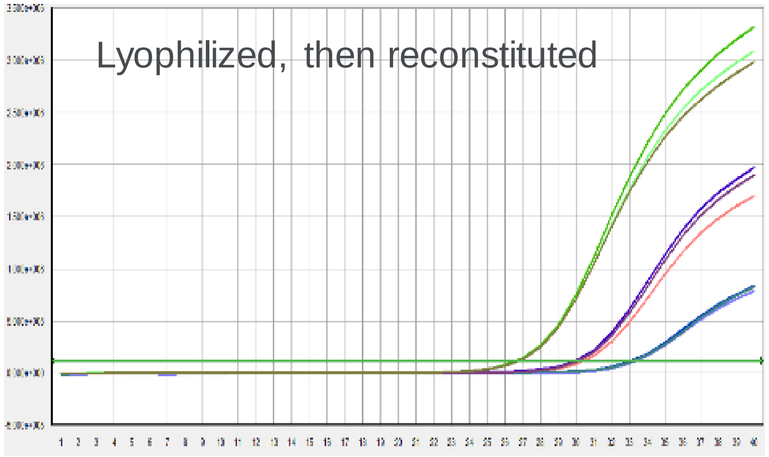
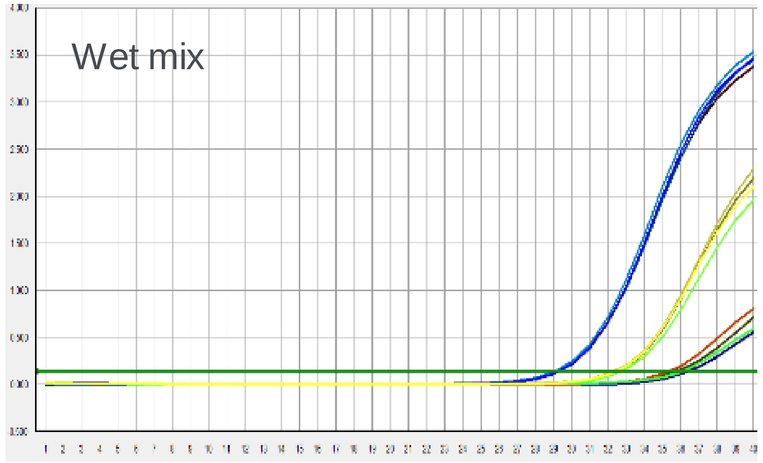
SensiFAST Lyo-Ready Mix; Customer Data

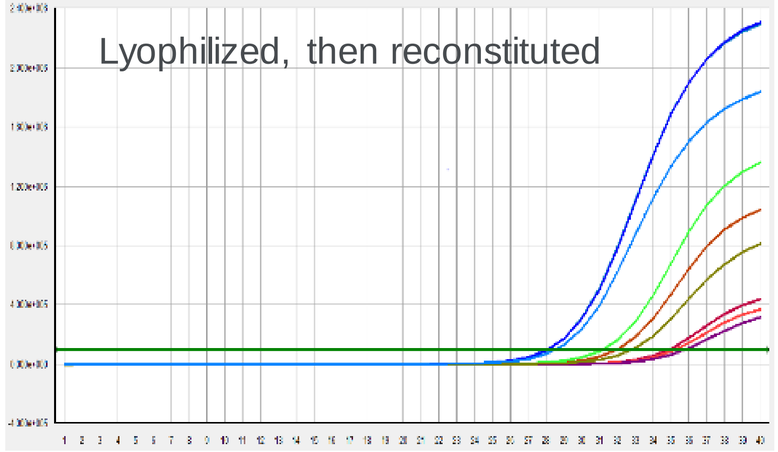
Target 1





Target 2





- Sample: Vet / Human
- Assay: Multiplexed pathogen RNA assay

Comparable performance following lyophilization then reconstitution

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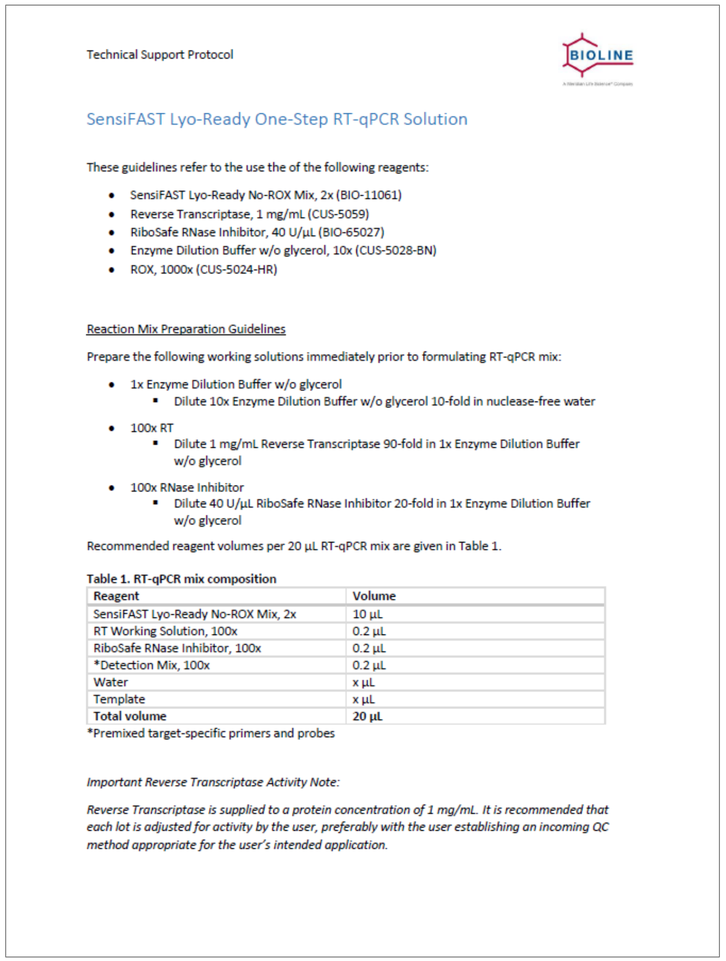
One-Step RT-qPCR; Protocol



Technical Support Protocol

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SensiFAST Lyo-Ready One-Step RT-qPCR Solution

These guidelines refer to the use the of the following reagents:

- SensiFAST Lyo-Ready No-ROX Mix, 2x (BIO-11061)
- Reverse Transcriptase, 1 mg/mL (CUS-5059)
- RiboSafe RNase Inhibitor, 40 U/µL (BIO-65027)
- Enzyme Dilution Buffer w/o glycerol, 10x (CUS-5028-BN)
- ROX, 1000x (CUS-5024-HR)

Reaction Mix Preparation Guidelines

Prepare the following working solutions immediately prior to formulating RT-qPCR mix:

- 1x Enzyme Dilution Buffer w/o glycerol
  - Dilute 10x Enzyme Dilution Buffer w/o glycerol 10-fold in nuclease-free water
- 100x RT
  - Dilute 1 mg/mL Reverse Transcriptase 90-fold in 1x Enzyme Dilution Buffer w/o glycerol
- 100x RNase Inhibitor
  - Dilute 40 U/µL RiboSafe RNase Inhibitor 20-fold in 1x Enzyme Dilution Buffer w/o glycerol

Recommended reagent volumes per 20 µL RT-qPCR mix are given in Table 1.

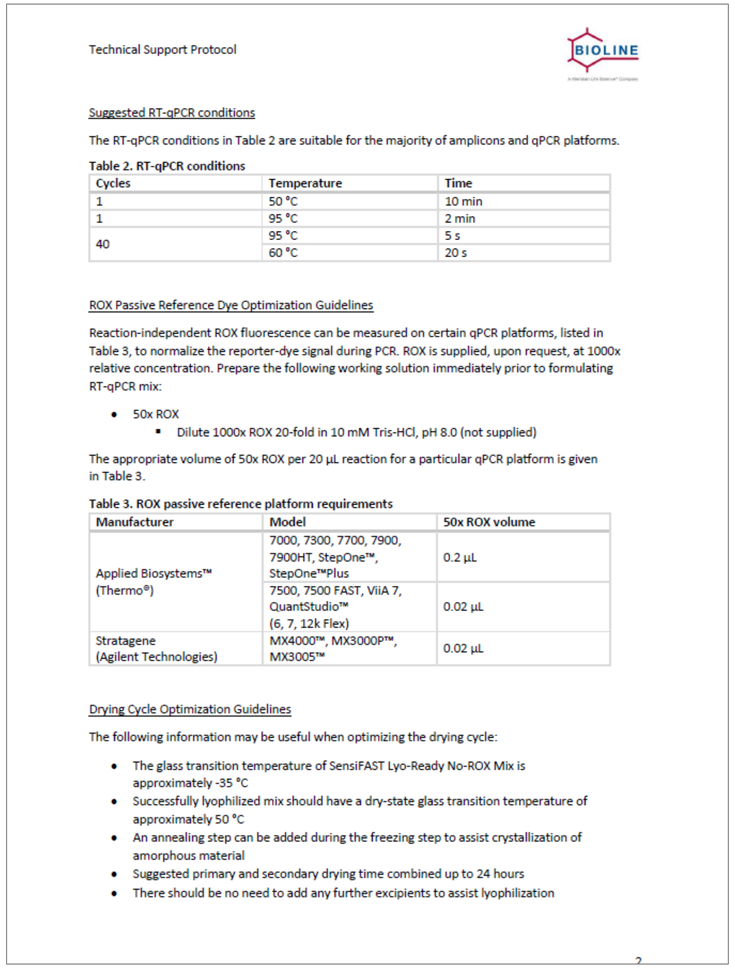
Table 1. RT-qPCR mix composition

Reagent	Volume
SensiFAST Lyo-Ready No-ROX Mix, 2x	10 µL
RT Working Solution, 100x	0.2 µL
RiboSafe RNase Inhibitor, 100x	0.2 µL
*Detection Mix, 100x	0.2 µL
Water	x µL
Template	x µL
Total volume	**20 µL**

*Premixed target-specific primers and probes

Important Reverse Transcriptase Activity Note:

Reverse Transcriptase is supplied to a protein concentration of 1 mg/mL. It is recommended that each lot is adjusted for activity by the user, preferably with the user establishing an incoming QC method appropriate for the user's intended application.



Technical Support Protocol

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Suggested RT-qPCR conditions

The RT-qPCR conditions in Table 2 are suitable for the majority of amplicons and qPCR platforms.

Table 2. RT-qPCR conditions

Cycles	Temperature	Time
1	50 °C	10 min
1	95 °C	2 min
40	95 °C	5 s
	60 °C	20 s

ROX Passive Reference Dye Optimization Guidelines

Reaction-independent ROX fluorescence can be measured on certain qPCR platforms, listed in Table 3, to normalize the reporter-dye signal during PCR. ROX is supplied, upon request, at 1000x relative concentration. Prepare the following working solution immediately prior to formulating RT-qPCR mix:

- 50x ROX
  - Dilute 1000x ROX 20-fold in 10 mM Tris-HCl, pH 8.0 (not supplied)

The appropriate volume of 50x ROX per 20 µL reaction for a particular qPCR platform is given in Table 3.

Table 3. ROX passive reference platform requirements

Manufacturer	Model	50x ROX volume
Applied Biosystems™ (Thermo®)	7000, 7300, 7700, 7900, 7900HT, StepOne™, StepOne™Plus	0.2 µL
	7500, 7500 FAST, ViiA 7, QuantStudio™ (6, 7, 12k Flex)	0.02 µL
Stratagene (Agilent Technologies)	MX4000™, MX3000P™, MX3005™	0.02 µL

Drying Cycle Optimization Guidelines

The following information may be useful when optimizing the drying cycle:

- The glass transition temperature of SensiFAST Lyo-Ready No-ROX Mix is approximately -35 °C
- Successfully lyophilized mix should have a dry-state glass transition temperature of approximately 50 °C
- An annealing step can be added during the freezing step to assist crystallization of amorphous material
- Suggested primary and secondary drying time combined up to 24 hours
- There should be no need to add any further excipients to assist lyophilization

2

Components

- SensiFAST Lyo-Ready No-ROX Mix
- Reverse Transcriptase (1 mg/mL)
- RiboSafe RNase Inhibitor (40 U/µl)
- Enzyme Dilution Buffer (w/o glycerol)
- ROX, 1000x

Guidelines

- Reaction mix preparation
- Suggested RT-qPCR conditions
- ROX passive reference dye optimization
- Drying cycle optimization

Protocol for addition of high concentration RT to SensiFAST Lyo-Ready No-ROX Mix followed by lyophilization

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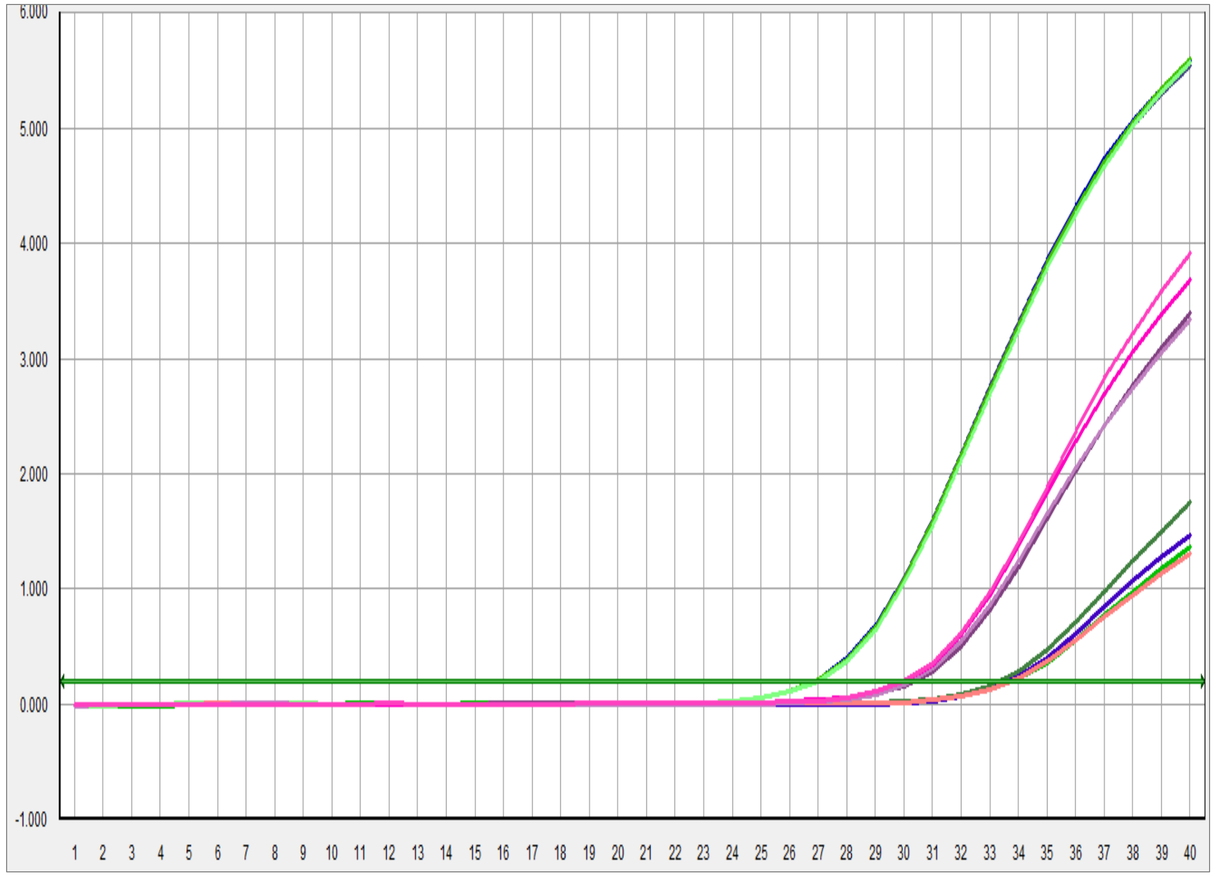
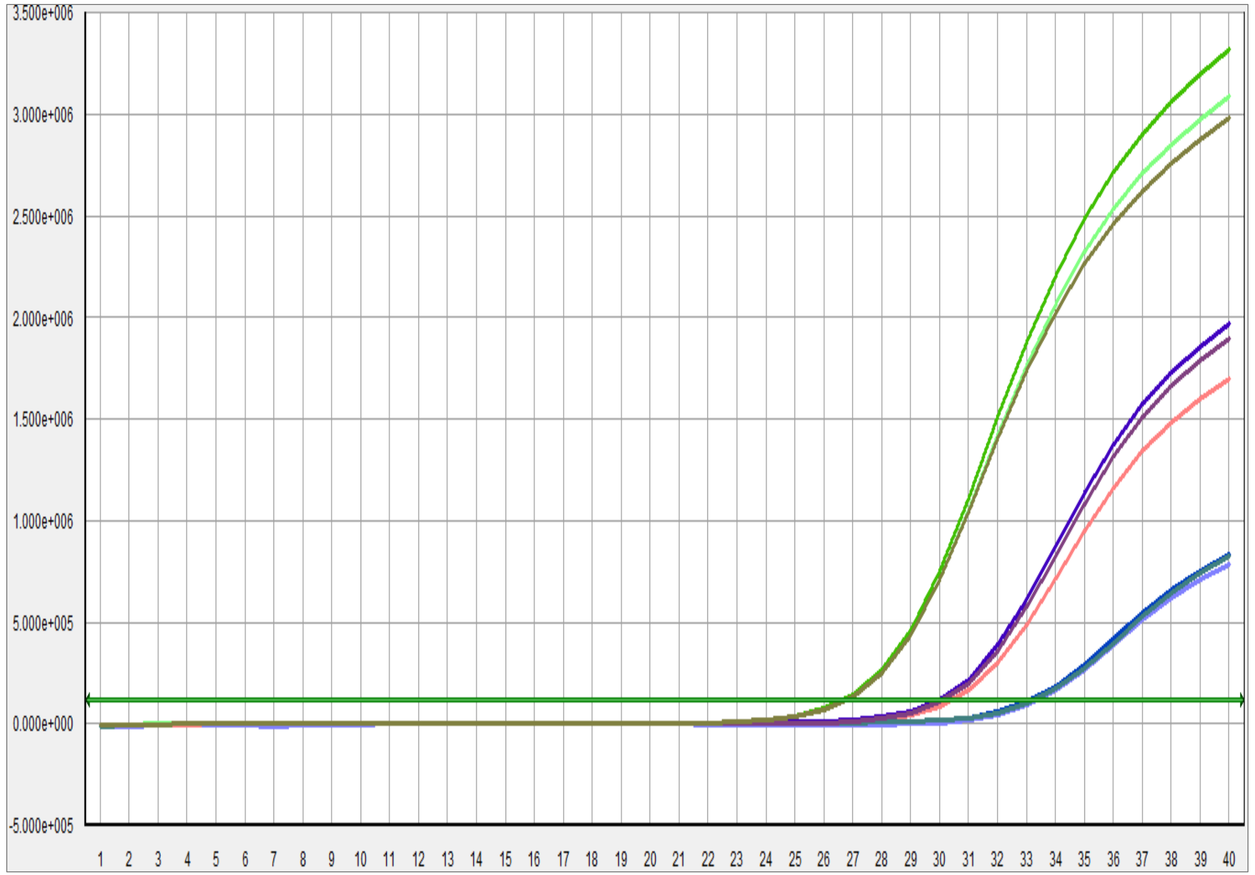
One-Step RT-qPCR; Customer Data

Wet



Dry



Equivalent performance of a reconstituted 1-step RT-qPCR mix versus non-lyophilized product

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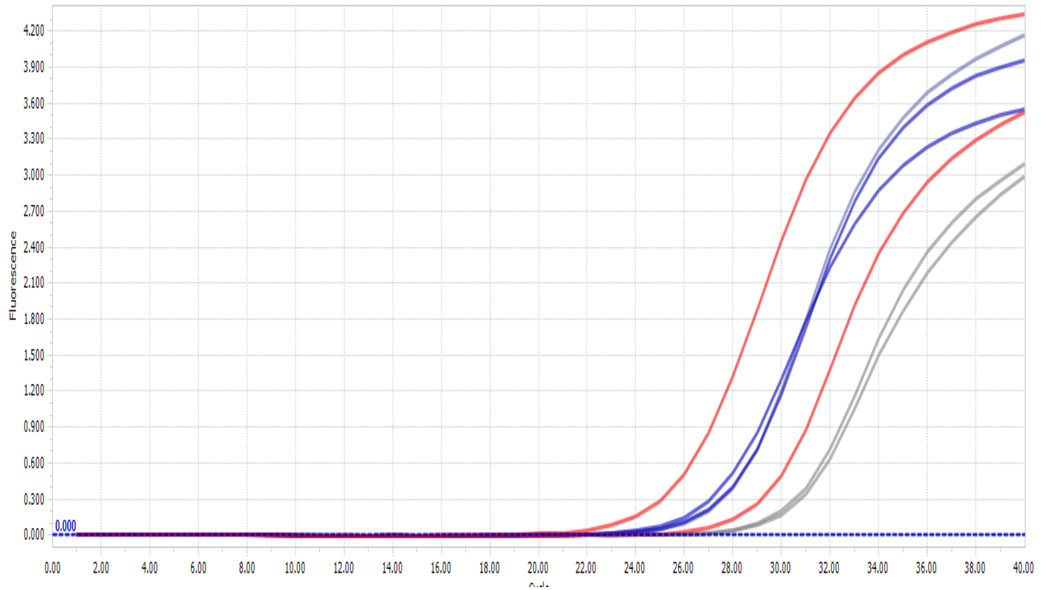
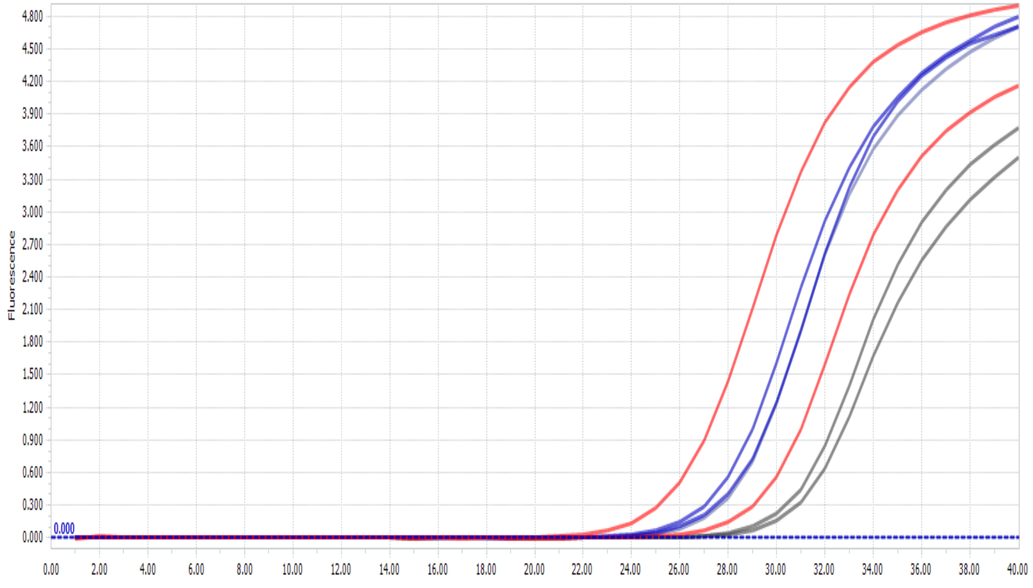
One-Step RT-qPCR; Customer Data

SensiFAST Lyo-Ready No-ROX Mix + RT



SensiFAST Probe Lo-ROX One-Step Kit



Flu A, subtype a & b
Flu A, subtype c & d
Flu A, subtype e & f

SensiFAST Lyo-Ready No-ROX Mix (plus RT) shows equivalent performance to SensiFAST Probe Lo-ROX One-Step Mix for detecting influenza A viruses

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To learn more….

http://www.bioline.com/contact-custom-development-experts

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