# Preparation of dry, stable RT-PCR assay reagents with PCRstable® technology using a vacuum concentrator

## INTRODUCTION

As PCR technologies segue into the global market, PCR-based assay reagents will require on-board storage and stabilization at room temperature. PCRstable® is a service to develop chemical stabilizer alternatives to lyophilization. Upon service completion, customers receive an easy-to-dispense custom stabilizer to be used with assay reagents in production. Drying with the stabilizers produces ambient-stable reagents ready for storage or shipment worldwide. This stability enables assay reagents to be readily available globally. In this study, we demonstrate the feasibility of utilizing a vacuum concentrator to dry stabilize RT-qPCR assay reagents with PCRstable® technology, maintaining the assay's efficiency.

## MATERIALS AND METHODS

### *Reagent Preparation*

Quadruplex influenza RT-qPCR assays were assembled with the components in Table 1. PCRstable® Stabilizer (PSB) was added at a 1:1 (vol:vol) ratio. Each reaction was set up in triplicate, with final reaction volume at 10 µL.

| Quadplex Influenza Assay | Final Concentration |
|---|---|
| Buffer | 1x |
| dNTP | 0.15 mM |
| Primers | 0.4 µM |
| Probes | 0.1 µM |
| SuperScript® III Reverse Transcriptase | 2U |
| GoTaq® Glycerol Free | 0.5U |

***Table 1:*** *Final composition of RT-qPCR assay.*

### *Drying and Storage*

PCRstable®-stabilized reagents (PSB) and No Formulation Controls (NFC) were dried onto 96-well PCR plates using a Vacufuge® vacuum concentrator (Eppendorf) at 30°C for 60 minutes. Dried reactions were stored with desiccants in moisture barrier bags at 45°C for 3 days.

### *Rehydration and Analysis*

All reactions were rehydrated with 10 µL of flu RNA template at 10x LOD. Fresh control reactions were set up at the same reagent and template concentrations as the dried reactions. All samples were amplified on a CFX96 Real-Time PCR Instrument (Bio-Rad) with thermal cycling conditions shown in Table 2. Sequences of primers and probes used in the assay are listed in Table 3.

| Cycle | Temperature | Time |
|---|---|---|
| 1x | 25°C | 5 minutes |
| 1x | 42°C | 1 hour |
| 1x | 95°C | 10 minutes |
| 50x | 95°C | 30 seconds |
| 50x | 60°C | 1 minute |

***Table 2:*** *Thermal cycling parameters.*

| Primer | Sequence |
|---|---|
| InfA Forward | 5- GAC CRA TCC TGT CAC CTC TGA C -3' |
| InfA Reverse | 5'- AGG GCA TTY TGG ACA AAK CGT CTA -3' |
| InfA Probe | 5'- /56-FAM/TGC AGT CCT /ZEN/CGC TCA CTG GGC ACG /3IABkFQ/ -3' |
| InfB Forward | 5'- TCC TCA ACT CAC TCT TCG AGC G -3' |
| InfB Reverse | 5'- CGG TGC TCT TGA CCA AAT TGG -3' |
| InfB Probe | 5'- /5HEX/CCA ATT CGA /ZEN/GCA GCT GAA ACT GCG GTG /3IABkFQ/ -3' |
| InfH1 Forward | 5'- TGA GAT ATT CCC CAA GAC AAG TTC -3' |
| InfH1 Reverse | 5'- TTT GTA GAA GCT TTT TGC TCC AG -3' |
| InfH1 Probe | 5' - /5Cy5/TCA TGA CTC GAA CAA AGG TGT AAC GG/3BHQ_2/ -3' |
| InfH3 Forward | 5'- ACC CTC AGT GTG ATG GCT TCC AAA -3' |
| InfH3 Reverse | 5'- TAA GGG AGG CAT AAT CCG GCA CAT -3' |
| InfH3 Probe | 5' - /5TEX615/ACG CAG CAA AGC CTA CAG CAA CTG T/3BHQ_2/ -3' |

***Table 3:*** *Primer and probe sequences for quadruplex influenza RT-qPCR assay.*

## RESULTS

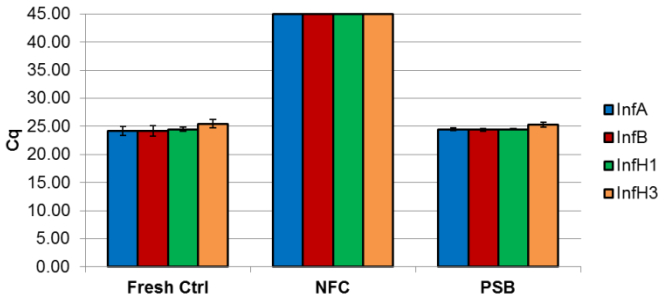


**Figure 1:** *Quantification cycles of stabilized quadruplex influenza RT-PCR reactions. Fresh Ctrl represents the positive control, NFC represents no formulation control, and PSB represent stabilized reactions. Error bars represent triplicate reactions.*

## SUMMARY

The data presented in Figure 1 shows that all the RT-qPCR reactions retain their efficiency to reverse transcribe RNA and amplify DNA after being dried in the presence of a PCRstable® stabilizer using a vacuum concentrator. This demonstrates how one can implement using a vacuum concentrator with the PCRstable® technology to prepare dried reactions.

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