

Temperature Stability of qPCR Kits

ABSTRACT

Temperature stability of qPCR systems is an important transport-related issue. We have tested the stability of our freeze-dried qPCR products at various ambient temperatures over a period of 14 days covering most transport related situations. While transport is usually done on cool packs, our products performed without any functional loss even if stored at room temperature for 14 days or at 37 °C for up to 7 days. In addition, we have tested the functionality and performance of freeze-dried qPCR products after freezing at -20 °C for 1 or 8 days, whereupon no functionality loss was observed.

INTRODUCTION

Our qPCR based *Mycoplasma* detection systems contain freeze-dried reaction mixes composed of primers, probes, nucleotides and Taq polymerase. Protective additives help stabilizing the most sensitive component, Taq polymerase, during the process of freeze-drying and further add to the storage stability of the freeze-dried mix. We have tested the product specifications under conditions of “stress” storage as might be expected during product transport or after accidental freezing.

PROCEDURE

Results presented are based on the same qPCR Kit using the identical reaction concentrations. Due to minor differences in absolute concentration and volumes, data presented for the first assay of temperature stability testing (room temperature, 37 °C and 60 °C) are means of 3 lots (Table 1 and 2). For testing the temperature stability of qPCR kits after storage of freeze-dried components at -20 °C for 1 or 8 days, qPCR amplification of a dilution series (10000 – 10 GU/PCR) and DNA extract of 10CFU™ Sensitivity Standard of *Mycoplasma fermentans* was tested and compared with the qPCR amplification using fresh freeze-dried qPCR mix (stored according to Instructions for Use at 2 – 8 °C).

RESULTS

Visually, freeze-dried mixes show highly similar consistency, color and distribution within the storage tubes: majority of the freeze-dried mix forms a dry, firmly attached scaffold-like structure at the bottom of the conical tube. These characteristics are stable at cool-pack, room temperature and 37 °C storage for up to 14 days and at -20 °C for up to 8 days. Storage at 60 °C quickly decreases stability and leads to color and consistency changes of the product. Functional testing was done comparing sensitivity, C_t -values and amplification curves (incl. fluorescence levels) after storage at various temperatures. The freeze-dried product shows stability after at least 7 days at 37 °C or 14 days at temperatures \leq room temperature (see Table 1 and 2) and stability quickly decreasing at storage \geq 60 °C. Product is also stable after at least 8 days at -20 °C, where for all dilutions and the 10CFU™ Sensitivity Standard the shifts in C_t -value are \leq 1, and fluorescence intensity \geq 70 % of positive control (see Table 3 and Graphics 1 and 2).

Table 1. Representative C_t values from FAM™ (sample) and ROX™ (internal control) channels for our qPCR-Kits, after storage at 37 °C for the indicated time periods.

| Sample | Reference FAM™/ROX™ | Storage time | | |
|---------------|------------------------|---------------|---------------|----------------|
| | | 1 d FAM™/ROX™ | 7 d FAM™/ROX™ | 14 d FAM™/ROX™ |
| NTC | No C_t / 34 | No C_t / 34 | No C_t / 33 | No C_t / 33 |
| 100000 GU/PCR | 23 / 32 | 23 / 32 | 23 / 29 | 22 / 31 |
| 10000 GU/PCR | 26 / 30 | 26 / 30 | 27 / 32 | 26 / 30 |
| 1000 GU/PCR | 29 / 32 | 29 / 32 | 30 / 30 | 29 / 30 |
| 100 GU/PCR | 33 / 32 | 33 / 32 | 33 / 32 | 32 / 32 |
| 10 GU/PCR | 36 / 32 | 37 / 32 | 44 / 31 | 36 / 32 |

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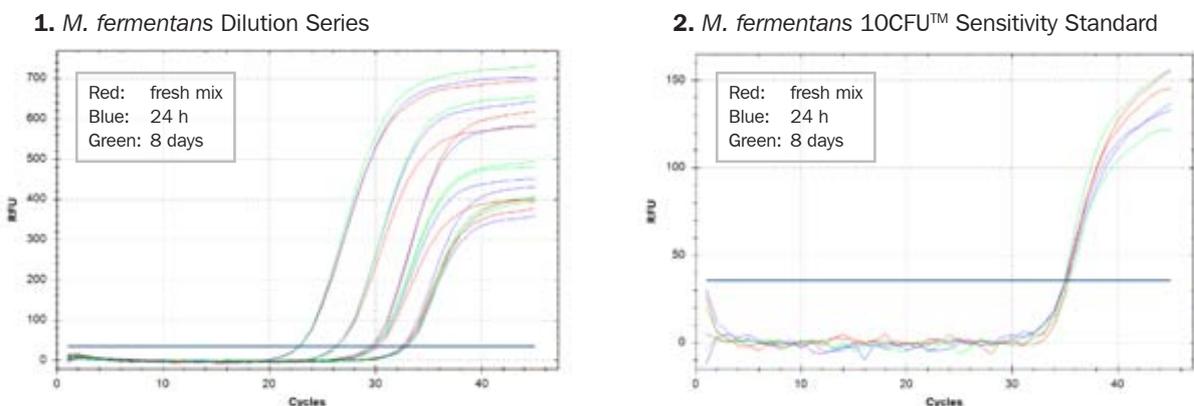
Table 2. Assessment of storage-time (C_t / Sensitivity / Fluorescence level) for our qPCR-Kits stored at various temperatures.

| Temperature | Storage-time (C_t / Sensitivity / Fluorescence level) | | | |
|------------------|--|-----|-----|------|
| | 1 d | 3 d | 7 d | 14 d |
| Cool-pack* | +++ | +++ | +++ | +++ |
| Room temperature | +++ | +++ | +++ | +++ |
| 37 °C | +++ | +++ | +++ | ++- |
| 60 °C | — | — | — | — |

*cool-packs had a temperature of -20 °C in the beginning.

Table 3. C_t values from FAM™ (sample) and HEX™ (internal control) channels for our qPCR-Kits, after storage at -20 °C for the indicated time periods.

| Sample | Reference FAM™/HEX™ | Storage at -20 °C | |
|-----------------|---------------------|-------------------|---------------|
| | | 1 d FAM™/HEX™ | 8 d FAM™/HEX™ |
| NTC | No C_t / 32 | No C_t / 32 | No C_t / 32 |
| 10CFU™ Standard | 35 / 31 | 35 / 31 | 35 / 31 |
| 10000 GU/PCR | 23 / 32 | 23 / 32 | 23 / 32 |
| 1000 GU/PCR | 26 / 31 | 26 / 31 | 26 / 31 |
| 100 GU/PCR | 30 / 32 | 30 / 32 | 30 / 31 |
| 10 GU/PCR | 33 / 32 | 33 / 32 | 33 / 31 |



Graphic 1. Amplification curves for 10000 – 10 GU/PCR dilution series of **1. M. fermentans** and **2. M. fermentans 10CFU™** standard, using lyophilized qPCR mixes stored at -20 °C for 1 day or 8 days, or at 2 – 8 °C.

CONCLUSION

Both functional and visual testing showed that our freeze-dried products are stable for at least 14 days if stored on cool-packs or at room temperature and at least 7 days if stored at 37 °C. Additionally, freezing of lyophilized components for at least 8 days does not affect the stability and performance of the product. We therefore suggest storage temperatures ≤ RT and avoiding storage temperatures > 37 °C. Considering that during shipment, temperatures did not reach 37 °C, qPCR kits can be sent without Cool Pack. For long-term storage, lyophilized components should ideally be stored at 2 – 8 °C.

TRADEMARKS

FAM, ROX, and HEX are trademarks of Applera Corp. or its subsidiaries. 10CFU is a trademark of Minerva Biolabs.