TECHNICAL NOTE

Temperature Stability of PCR Quantification Standards

Introduction

Temperature stability of nucleic acid samples such as DNA standards for PCR-based quantifications is an important issue, especially due to its implications for proper transport and storage. Low stability of DNA samples can result in DNA amount or quality loss, as well as significant degradation.

Miverva Biolabs' PCR Quantification Standards contain a certain amount of genomic DNA (in genome copies number) extracted from defined microorganisms at a low culture passage number. Protective additives help stabilize the DNA material during the process of freeze-drying and further add to the storage stability of the freeze-dried mix.

We have tested the stability of these DNA standards by storing them for a maximum of 7 days at various temperatures, in order to mimic transport-related, "stress"-related storage conditions.

Procedure

Lyophilized PCR Quantification Standards of *Mycoplasma hyorhinis* (Cat. No. 52-01300) were stored at 4 °C, 18-25 °C (room temperature, RT), 37 °C, or 60 °C for 7 days. At the end of this incubation time, the vials were resuspended in 100 µl Tris-HCl buffer, as indicated in the corresponding Instructions for Use. In order to fully characterize the potential effect of the storage conditions on the PCR Quantification Standards, we then serially diluted the resuspended reagents to obtain the following final concentrations of DNA, expressed in genome copies: 10000, 1000, 100, 10 genome copies per PCR reaction. Each concentration was prepared in duplicate and no template controls were included. Subsequently, the stored standards were amplified by qPCR using Microsart® ATMP Mycoplasma (Sartorius, SMB95-1003/1004) to assess potential losses in terms of quantity or quality of the DNA standards, depending on the storage temperature. Every rehydrated and stored PCR Quantification Standard at a defined concentration was analyzed by qPCR in duplicate using a BioRad CFX96™ Real-Time Detection System.

Results

Stress- and storage-testing

qPCR-based testing was applied to evaluate potential alterations of the stability of PCR Quantification Standards due to their prolonged (7 days) storage in progressively higher thermal stress conditions. Sensitivity, C_t-values and amplification curves after storage at various temperatures were compared. The C_t values of the stored PCR Quantification Standards revealed positive results (<<40) for all DNA concentrations and temperatures tested. No sensitivity loss was observed for any of the tested temperatures (shifts in C_t-values ≤ 1, s. Table 1 and 2 and Figure 1). The NTCs showed exclusively amplification of the internal control and negative results for the target (see Figure 1E., grey traces).

Table 1. Average C_t values from FAM™ (Target DNA) and ROX™ (internal control DNA) channels for our PCR Quantification Standard (*M. hyorhinis*), after storage at 4 °C, 18-25 °C (room temperature, RT), 37 °C, or 60 °C for 7 days. Serially diluted standards were then amplified in duplicates by qPCR using Microsart® ATMP Mycoplasma. Standard Error Mean values range from 0.01 to 0.56, as minimum and maximum deviations. NTC, No template Control; GU, Genome copies/units.

Sample	4 °C FAM™ / ROX™	RT (18-25 °C) FAM™ / ROX™	37 °C FAM™ / ROX™	60 °C FAM™ / ROX™
10000 GU/PCR	24.3 / 31.6	24.2 / 31.4	24.0 / 31.4	23.9 / 31.2
1000 GU/PCR	27.6 / 31.3	26.8 / 31.3	27.5 / 31.4	27.4 / 31.2
100 GU/PCR	31.1 / 31.6	30.7 / 31.7	31.0 / 31.6	30.8 / 31.9
10 GU/PCR	34.3 / 31.8	34.0 / 32.0	34.2 / 31.8	33.9 / 31.9

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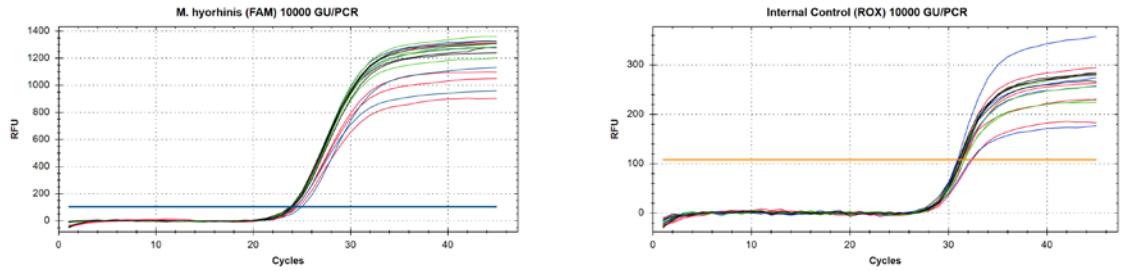
Temperature Stability of PCR Quantification Standards

A.



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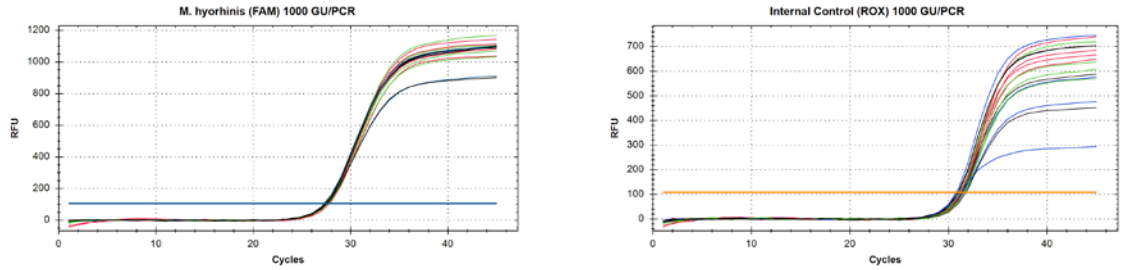

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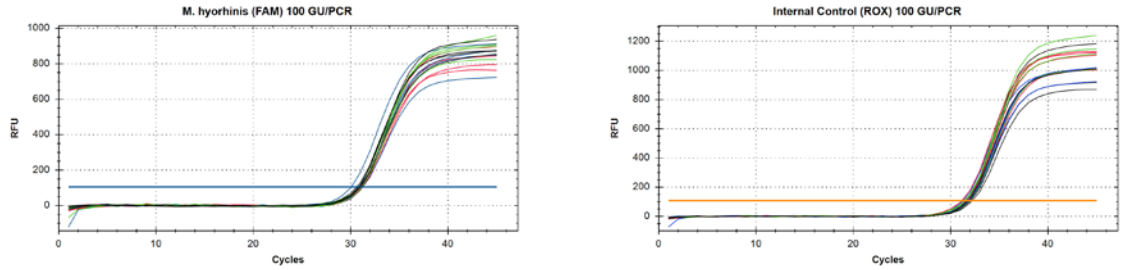

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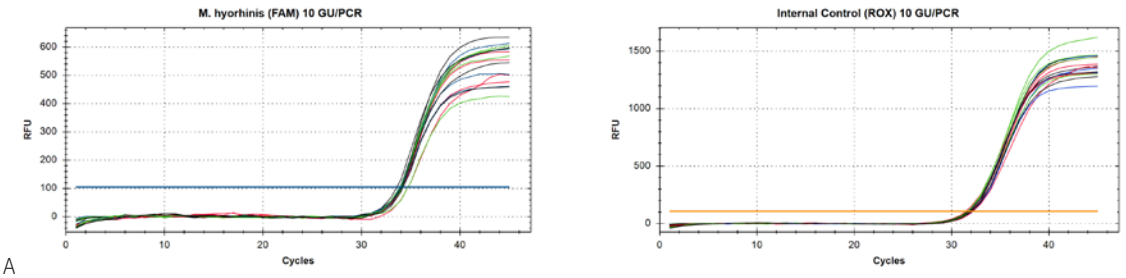

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E.

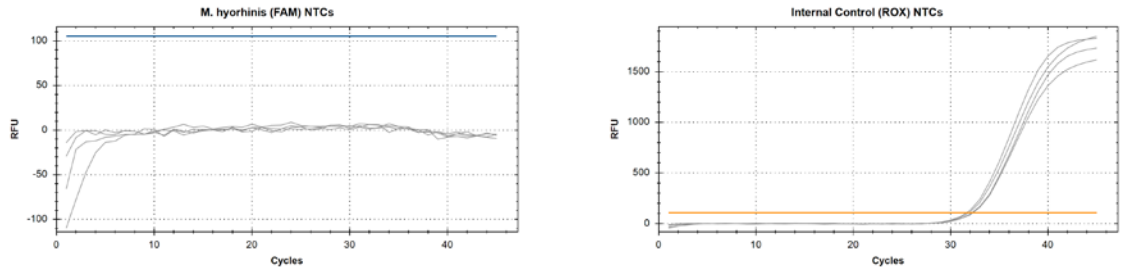

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Figure 1. qPCR amplification curves obtained with Microsart® ATMP Mycoplasma of PCR Quantification Standards (*M. hyorhinis*) after incubation at various temperatures and serial dilution to (**A.**) 10000, (**B.**) 1000, (**C.**) 100 and (**D.**) 10 genome units (GU) per PCR tube and prepared in duplicate. Each tube was then incubated at the given temperatures (4 °C, red curves, 18-25 °C (RT), blue curves, 37 °C, green curves or 60 °C, black curves) for 7 days and processed in parallel using Microsart® ATMP Mycoplasma kit for real-time PCR. Specific amplification of the target DNA was detected in the FAM™ (left) whereas the internal control to evaluate the performance of the PCR reactions was measured in the ROX™ channel (right). Negative controls (NTCs, **E.**) were negative for mycoplasma and positive for the internal control, indicating successful PCR reactions (grey curves).

Table 2. Summary of the assessment of stability-related parameters (C_t value or sensitivity, based on the results with serial dilutions) for the PCR Quantification Standard *M. hyorhinis*, stored at various temperatures for 7 days. The symbol = indicates no significant difference compared to the values obtained at the reference storage temperature.

Temperature	C_t	Sensitivity
4 °C	reference	< 10 genome copies / PCR
RT (18-25 °C)	=	< 10 genome copies / PCR
37 °C	=	< 10 genome copies / PCR
60 °C	=	< 10 genome copies / PCR

Conclusions

Temperature stability testing of the PCR Quantification Standard of *M. hyorhinis* revealed high stability of the product at room temperature, 37 °C or even 60 °C for 7 days. These results indicate that these reagents require regular shipping at ambient temperatures without the need for dry ice or cool packs. For long-term storage, however, lyophilized PCR Quantification Standards should ideally be stored at +2 – +8 °C.

References

This Technical Note also applies to Genomic DNA Extracts (e.g. Cat. No. 51-0111).

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