Microsart® ATMP Mycoplasma Detection Kit

Impact of repetitive freezing cycles on the quality/stability of Microsart® ATMP Mycoplasma detection kit reagents

Technical Note

The Microsart® ATMP Mycoplasma Detection Kit is a Mycoplasma detection system based on qPCR enabling sufficient detection of Mycoplasma contamination in cell cultures or cell culture-derived biologicals. This report summarizes the stability testing of Microsart® ATMP Mycoplasma kit components upon repetitive freezing cycles and long-time storage

Introduction

The Microsart® ATMP Mycoplasma Detection Kit (Item No.: SMB95-1003) is a Mycoplasma detection system based on qPCR. The kit is employed for the detection of Mollicutes (including *Mycoplasma, Acholeplasma, Spiroplasma*) in cell cultures and cell culture- derived biologicals. The kit consists of a Mycoplasma Mix, which includes primers, probes, nucleotides and Taq polymerase. Additionally, the kit contains a Rehydration Buffer, PCR-grade Water, an Internal Control DNA (IC) and a Positive Control DNA (PC). The Mycoplasma Mix, IC and PC are freeze-dried for stabilization, shipping, and improved shelf-life. These components are dissolved before use with the provided Rehydration Buffer or PCR-grade water in the kit. To test the Microsart® ATMP Mycoplasma detection kit for its stability the following experiments were performed:

The stability and shelf-life of the IC and PC was tested after the lyophilized kit components were rehydrated according to the Instruction for Use (IFU). Both the IC and PC were subjected to a range of one to ten freeze-thaw cycles at -18°C. The Mycoplasma Mix was freshly dissolved, and the samples were analyzed by qPCR. Freshly dissolved IC and PC were included as reference values.

For 12-month long-term stability test all Microsart® ATMP Mycoplasma Detection Kit components were dissolved and stored for 12 months at ≤-18 °C prior qPCR test runs.

Procedure

Ten ICs and ten PCs were repetitively frozen and thawed for one to ten times. The Mycoplasma Mix was freshly dissolved in Rehydration Buffer and aliquoted with 90 µl per tube. This corresponds to 6 reactions per aliquot. In preparation for each sample measurement 6 µl IC were pipetted into 90 µl Mycoplasma Mix. 15 µl of each mixture was transferred to PCR tubes. Subsequently, 10 µl sample, PC or a negative control (NC) was added to the respective Mycoplasma Mix. The samples were compared with the freshly dissolved reference IC and PC. The qPCR was performed according to the IFU.

For the long-term stability study, all freeze-dried components of the Microsart® ATMP Mycoplasma Detection Kit (Mycoplasma Mix, IC and PC) were dissolved and stored at ≤ -18 °C for 12 months. A freshly dissolved kit was included as a reference value. The comparison of the long-term stored kit and the freshly dissolved kit is based on the amplification of a Microsart® Mycoplasma fermentas Calibration Reagent (Item No.: SMB95-2026) dilution series. The stability after 12 months storage was analyzed via qPCR. The qPCR was performed according to the IFU.

Results

Testing the stability of the IC and PC:
Repeated freezing and thawing cycles (1x-10x) of the rehydrated PC did not generate an influence on qPCR performance. For both, the freshly rehydrated reference sample and the samples that passed multiple freeze-thaw cycles, cycle threshold (CT) values of about 24 were determined. The tested PC samples show a normal scattering of fluorescence intensity.

The repetitively frozen and thawed ICs showed no major changes in qPCR performance when compared to the freshly rehydrated reference IC (Figure 2). The calculated Ct values range from 32-34. The freshly rehydrated reference IC had a Ct value of 33. A slight loss in fluorescence intensity was observed after the 9th (black curve) and 10th (pink curve) freezing and thawing cycle.

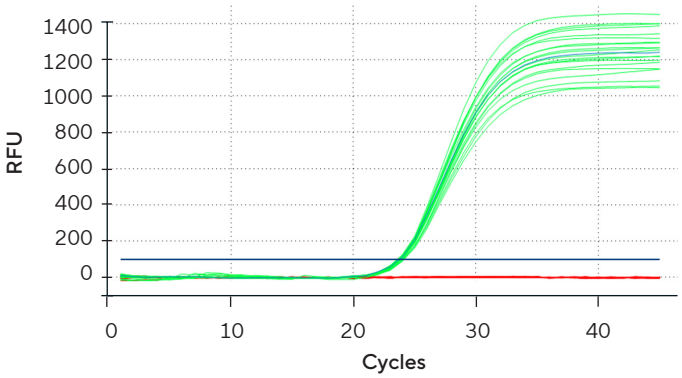

Figure 1: Amplification curve of the PC (FAM channel): Amplification of the 1x-10x frozen and thawed samples. The relative fluorescence units were plotted against the number of cycles; 1x-10x PC (green), reference PC (blue) and NC (red).

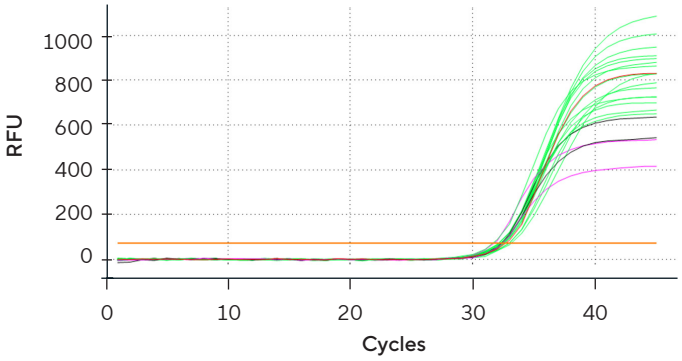

Figure 2: Amplification curve of the IC (ROX channel) Amplification of the 1x-10x frozen and thawed samples. The relative fluorescence units were plotted against the number of cycles; reference IC (red), 1x-8x IC (green), 9x IC (black) and 10x IC (pink).

Testing kit reagent stability after rehydration and subsequent storage for 12 months at ≤ -18°C:
All samples of the *Mycoplasma fermentans* dilution series (Table 1) were amplified with the freshly rehydrated reference kit and the test kit in which the rehydrated components were stored at ≤-18 °C for 12 months. The test kit shows no changes in the Ct values in the FAM channel (Figure 3) when compared to the freshly rehydrated reference kit. After long-time storage a slight decrease in fluorescence intensity compared to the reference kit was observed.

Sample (genome copies/ml)	Test sample (Ct)	Reference sample (Ct)
100 000	18	18
10 000	21	21
1 000	25	25
100	28	28
10	31	31

Table 1: Dilution series of *Mycoplasma fermentans* in genome copies/reaction and the corresponding Ct values in the FAM channel of the sample and the reference value.

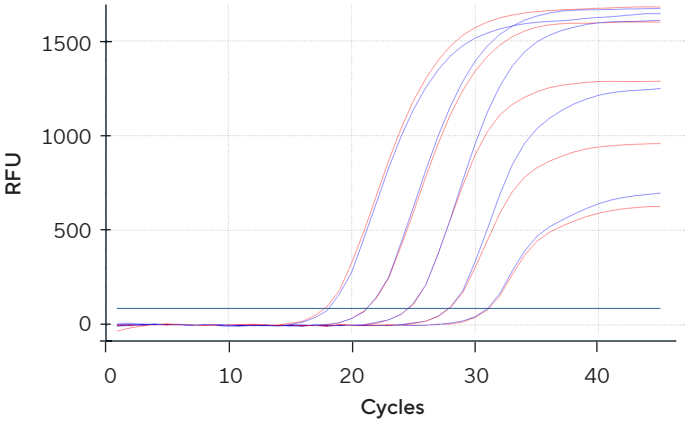

Figure 3: Amplification curve (FAM channel) of the dilution series 100000-10 genome copies per reaction of *Mycoplasma fermentans*. The relative fluorescence units were plotted against the number of cycles; the sample (red) and the reference values (blue).

The IC and PC stored for 12 months at ≤ -18 °C showed no loss of function compared to the freshly dissolved IC and PC. The Ct value (Table 2) and the fluorescence intensity (Figures 4 & 5) showed only minimal deviations from the reference kit values.

	Test sample		Reference sample	
Sample type	CT (FAM)	Ct (ROX)	Ct (FAM)	Ct (ROX)
PC	23	31	25	33
PC	23	32	24	32
IC	N/A	31	N/A	32
IC	N/A	31	N/A	32

Table 2: The Ct values of the tested IC and PC of the sample after 12 months of storage at ≤ -18 °C and the reference value in the FAM channel and ROX channel.

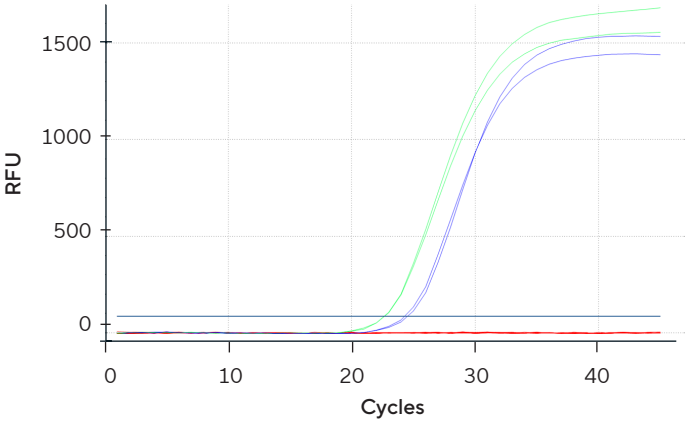

Figure 4: PC amplification curve (FAM channel): of the rehydrated and frozen PC after 12 months of storage at ≤ -18 °C. The relative fluorescence units were plotted against the number of cycles; PC (green), reference value (blue) and NC (red).

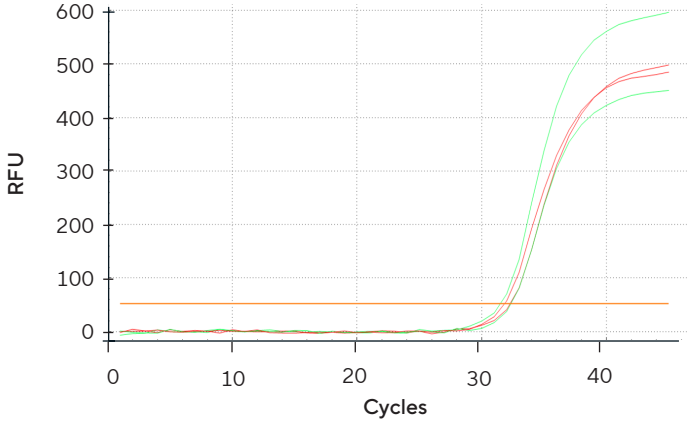

Figure 5: Amplification curve of the IC (ROX channel): of the rehydrated and frozen IC after 12 months of storage at ≤ -18 °C. The relative fluorescence units were plotted against the number of cycles; reference IC (red) and sample IC (green).

Conclusion

The study revealed no major influence on the functionality of the IC and PC even after up to ten repeated freezing and thawing cycles. However, the IC showed a slight loss of flourescence intensity after the 9th thawing cycle.

Dissolved kit components which were stored for 12 months at -18°C generated similar Ct values and flourescence signal intensity for measured *Mycoplasma fermentas* samples when compared to a freshly rehydrated reference kit. While the flourescence intensity decreases slightly after 12 months, the sensitivity of the product remains unchanged (Ct values).

Neither long-term storage of rehydrated kit components for 12 months nor repeated freezing and thawing of the dissolved components reduced the performance. Nevertheless, we recommend avoiding repeated freezing cycles.

It is recommended that freshly dissolved components such as IC, PC and Mycoplasma Mix are divided into aliquots after initial use to minimize potential stress of repeated freezing and thawing cycles. Furthermore, we recommend to aliquot each rehydrated kit component (IC, PC, Mycoplasma Mix) in nuclease-free and DNA low-binding tubes for sufficient storage.

Trademarks

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