

Temperature Stability and Applicability of ConviFlex™ DNAm Mix

INTRODUCTION

Temperature stability of PCR systems is an important issue, especially in regard to convenient transport and storage. ConviFlex™ DNAm Mix contains core components for DNA amplification which include *Taq* polymerase and dNTPs. Protective additives help stabilize 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 applicability of ConviFlex™ DNAm Mix to different PCR systems, as well as its stability at various ambient temperatures covering most transport-related, “stress”-storage, and in-use situations.

PROCEDURE

In the context of testing the applicability of our ConviFlex™ DNAm Mix to different PCR systems, we performed various assays using human and bacterial genomic target DNA. Both SYBR® Green and TaqMan® based qPCR techniques were tested, applied in two-step and three-step PCR programs on two different cyclers. Additionally, stress- and in-use stability testing were carried out, in which a dilution series of target DNA (50000 – 50 GU/PCR) was set up. For stress-testing, temperature stability and robustness of the lyophilized PCR mix was tested at room temperature, 4 °C, 37 °C, and 60 °C. Lyophilized PCR mix was incubated at an indicated temperature for 1, 3, or 7 days, and subsequently rehydrated and tested. Every PCR mix testing was done in duplicate. For in-use stability testing, functionality of PCR mix was tested after several freeze/thaw cycles. Two lyophilized PCR mixes were rehydrated and stored at either 4 °C or -20 °C for 7 days. Both PCR mixes were tested after 1, 2, 3, 4, and 7 days of the storage period. With every testing, performance was compared with that of a fresh PCR mix.

Results

1. Stress- and storage-testing

Functional testing was done comparing sensitivity, C_t -values and amplification curves (incl. fluorescence levels) after storage at various temperatures. Functionality of lyophilized ConviFlex™ DNAm Mix was positive for all temperatures and time points tested. No sensitivity loss was observed for any of the tested temperatures (shifts in C_t -values ≤ 1 and fluorescence intensity ≥ 70 % in reference to 4 °C, s. Table 1 and 2 and Figure 1).

Table 1. C_t values from FAM™ (sample) and HEX™ (internal control) channels for our ConviFlex™ DNAm Mix, after storage at room temperature, 37 °C or 60 °C for indicated time periods.

Sample	Storage at RT			Storage at 37 °C			Storage at 60 °C		
	1 day FAM™/ HEX™	3 days FAM™/ HEX™	7 days FAM™/ HEX™	1 day FAM™/ HEX™	3 days FAM™/ HEX™	7 days FAM™/ HEX™	1 day FAM™/ HEX™	3 days FAM™/ HEX™	7 days FAM™/ HEX™
NTC	No Ct / 31	No Ct / 31	No Ct / 31	No Ct / 31	No Ct / 31	No Ct / 31	No Ct / 31	No Ct / 31	No Ct / 31
50000 GU/PCR	22 / no Ct	22 / no Ct	21 / no Ct	21 / no Ct	22 / no Ct	22 / no Ct	22 / no Ct	21 / no Ct	21 / no Ct
5000 GU/PCR	25 / 31	25 / 30	25 / 31	25 / 31	25 / 31	25 / 30	25 / 31	25 / 31	25 / 31
500 GU/PCR	28 / 30	28 / 31	28 / 30	28 / 31	28 / 31	28 / 30	28 / 31	28 / 31	28 / 30
50 GU/PCR	32 / 31	31 / 31	32 / 31	32 / 31	32 / 31	32 / 31	32 / 31	32 / 31	32 / 31

Table 2. Assessment of storage-time (C_t / Sensitivity / Fluorescence level) for our ConviFlex™ DNAm Mix stored at various temperatures.

Temperature	Storage-time (C_t / Sensitivity / Fluorescence level)		
	1 d	3 d	7 d
Room temperature	+++	+++	+++
4 °C	+++	+++	+++
37 °C	+++	+++	+++
60 °C	+++	+++	+++

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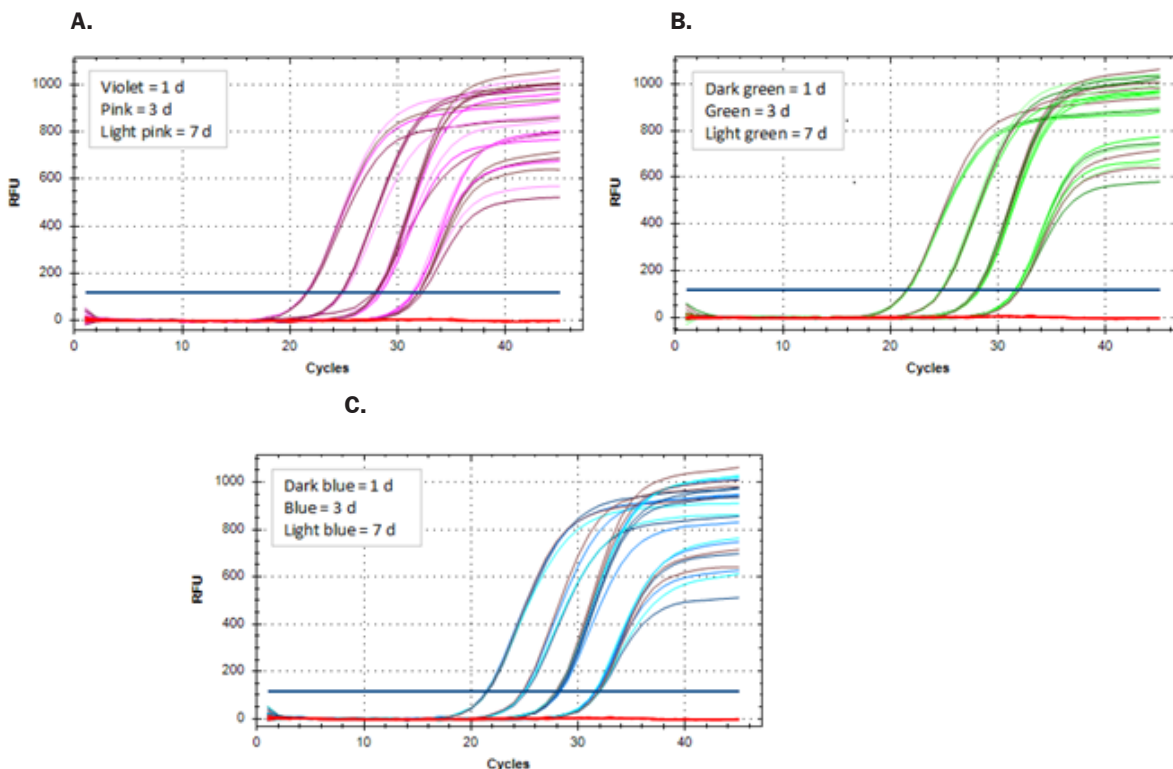


Figure 1. DNA amplification curves of qPCR using ConviFlex™ DNamp Mix after incubation at A. room temperature, B. 37 °C, and C. 60 °C, in reference to 4 °C (brown curves).

2. In-Use stability testing

Functional testing was done comparing sensitivity, C_t -values and amplification curves (incl. fluorescence levels) after storage at 4 °C, or at -20 °C with several freeze/thaw cycles. ConviFlex™ DNamp Mix was stable even after several freeze/thaw cycles, whereat no sensitivity loss was observed and fluorescence intensity was relatively constant (shifts in C_t -values ≤ 1 and fluorescence intensity $\geq 70\%$ in reference to a fresh PCR mix, s. Table 3 and 4 and Figure 2).

Table 3. C_t values from FAM™ (sample) and HEX™ (internal control) channels for our ConviFlex™ DNamp Mix, after storage at 4 °C or -20 °C for indicated time periods, in comparison with a fresh PCR mix.

Sample	Storage at RT			Storage at 37 °C			Storage at 60 °C		
	Fresh mix	4 °C	-20 °C	Fresh mix	4 °C	-20 °C	Fresh mix	4 °C	-20 °C
	FAM™/ HEX™	FAM™/ HEX™	FAM™/ HEX™	FAM™/ HEX™	FAM™/ HEX™	FAM™/ HEX™	FAM™/ HEX™	FAM™/ HEX™	FAM™/ HEX™
NTC	No Ct / 32	No Ct / 32	No Ct / 32	No Ct / 32	No Ct / 32	No Ct / 32	No Ct / 32	No Ct / 31	No Ct / 32
50000 GU/PCR	21 / no Ct	21 / no Ct	21 / no Ct	20 / no Ct	20 / no Ct	20 / no Ct	21 / no Ct	21 / 37	21 / no Ct
5000 GU/PCR	24 / 32	24 / 32	24 / 32	23 / 33	23 / 33	24 / 33	25 / 32	24 / 31	25 / 32
500 GU/PCR	27 / 31	27 / 31	27 / 31	27 / 32	27 / 32	27 / 32	28 / 31	28 / 31	28 / 31
50 GU/PCR	30 / 32	30 / 31	30 / 31	30 / 32	30 / 32	30 / 32	32 / 31	33 / 31	33 / 31

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Table 4. Assessment of storage-time (Ct / Sensitivity / Fluorescence level) for our ConviFlex™ DNAmix stored at various temperatures.

Temperature	Storage-time (C _t / Sensitivity / Fluorescence level)				
	1 d	2 d	3 d	4 d	7 d
fresh	+++	+++	+++	+++	+++
4 °C	+++	+++	+++	+++	+++
-20 °C	+++	+++	+++	+++	+++

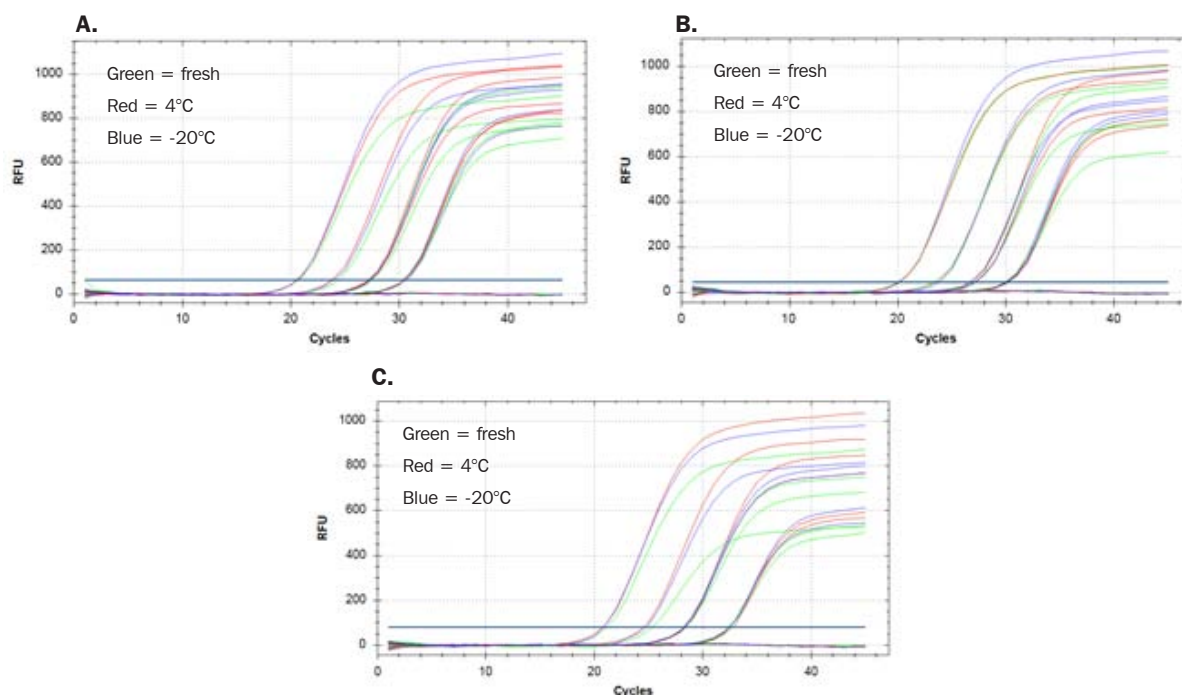


Figure 2. Amplification curves of qPCR using ConviFlex™ DNAmix after rehydration and incubation at 4 °C (red curves) or -20 °C (blue curves) for **A.** 1 day (1 thaw/freeze cycle), **B.** 4 days (4 thaw/freeze cycles), and **C.** 7 days (5 thaw/freeze cycles), in comparison with fresh ConviFlex™ DNAmix (green curves).

3. Applicability

ConviFlex™ DNAmix was tested using several different assays and showed a broad range compatibility and excellent characteristics with regard to applicability and convenience.

ConviFlex™ DNAmix showed functional compatibility when tested in PCR amplification of bacterial and human genomic DNA, in conventional and qPCR, in both TaqMan®- and SYBR® Green based methods, and on two different qPCR cyclers from different manufacturers, Rotor-Gene® 6000 (Corbett) and CFX96 Touch™ (Bio-Rad), applying both two-step and three-step PCR programs.

CONCLUSIONS

Temperature stability testing of our freeze-dried ConviFlex™ DNAmix showed it is highly stable at room temperature, 37 °C or even 60 °C for up to 7 days, and therefore requires regular shipping at ambient temperatures without the need for dry ice or cool packs. Results also showed that multiple freeze/dried cycles of the rehydrated PCR mix do not reduce its stability and functionality. We therefore suggest storage temperatures ≤ RT for ConviFlex™ DNAmix in the freeze-dried condition. For long-term storage, however, lyophilized ConviFlex™ DNAmix should ideally be stored at 2 – 8 °C. After reconstitution, we recommend storage at ≤ -18 °C.

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This Technical Note also applies to the SwabUp™ DNAm Mix, a component of the SwabUp™ Lab Monitoring Plus kit (Cat. No. 182-0010/-0050).

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Number: TN14.03EN
Date of Release 20.06.2019
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