

## Experimental Protocol and Evaluation for Cepheid Smart Cycler® with Minerva Biolabs real-time PCR kits

### 1. Experimental Protocol

#### Phase 1:

Stopp

temp	sec	optic
95	180	off

#### Phase 2:

Repeat 40

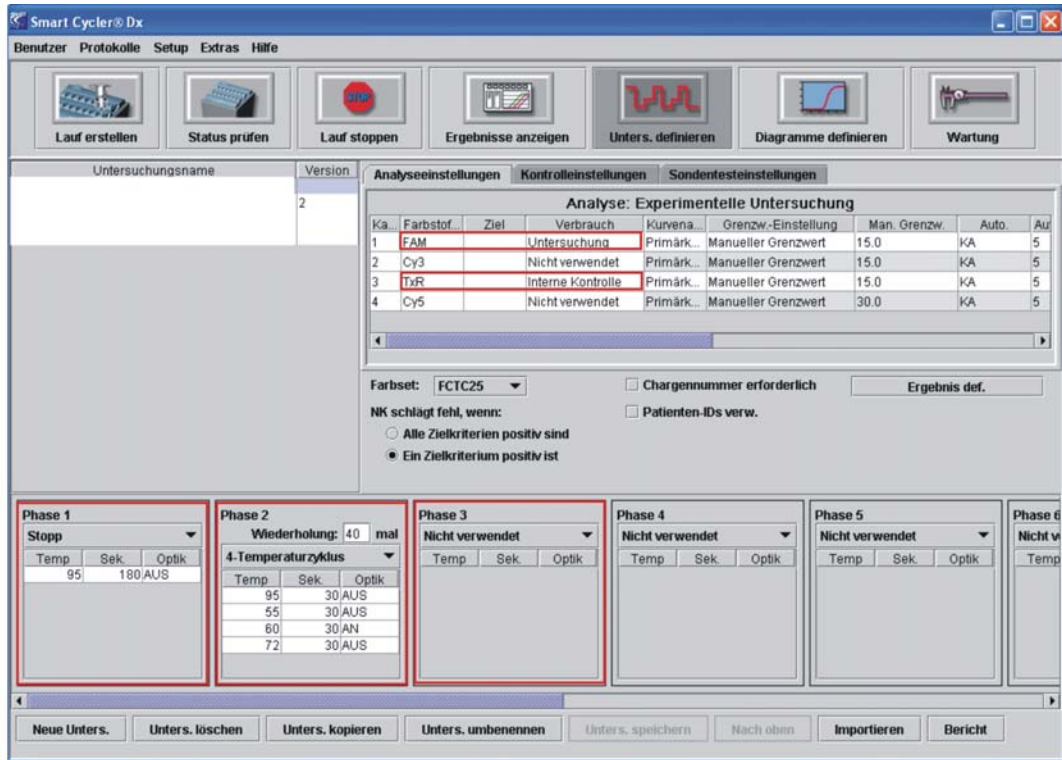
4-temperaturecycle

temp	sec	optic
95	30	off
55	30	off
60	30	on
72	30	off

#### Phase 3:

Stopp

temp	sec	optic
40	30	off



The screenshot shows the 'Smart Cycler Dx' software interface. The main window displays 'Analyse: Experimentelle Untersuchung' with the following table:

Ka	Farbstof	Ziel	Verbrauch	Kurvena	Grenzw-Einstellung	Man. Grenzw.	Auto.	Au
1	FAM	Untersuchung		Primärk...	Manueller Grenzwert	15.0	KA	5
2	Cy3	Nicht verwendet		Primärk...	Manueller Grenzwert	15.0	KA	5
3	TxR	Interne Kontrolle		Primärk...	Manueller Grenzwert	15.0	KA	5
4	Cy5	Nicht verwendet		Primärk...	Manueller Grenzwert	30.0	KA	5

Below the table, the 'Farbset' is set to 'FCTC25'. The 'NK schlägt fehl, wenn:' section has 'Ein Zielkriterium positiv ist' selected. The bottom of the interface shows phase settings for Phase 1 (Stopp), Phase 2 (4-Temperaturzyklus, Wiederholung: 40 mal), Phase 3 (Nicht verwendet), Phase 4 (Nicht verwendet), Phase 5 (Nicht verwendet), and Phase 6 (Nicht verwendet).

Please check the correct settings for the filter combination of Cepheid Smart Cycler®. For the target you must choose the filter FAM (483-533) and for the Internal Control-Target the filter TxR (558-610).

## 2. Evaluation and interpretation of the results

The analysis of the obtained data is divided into two parts:

- Quantitative analysis of DNA in fluorescence channel FAM-Signals (483-533)
- Quantitative analysis of Internal Control DNA in fluorescence channel TxR-Signals (558-610).

The amplification curves were obtained by performing the described procedure with a dilution series of a Quantification Standard and the Cepheid Smart Cycler<sup>®</sup> instrument. The fluorescence values versus cycle number are displayed. In the same run the amplification of the Internal Control DNA was shown in channel TxR (558-610).