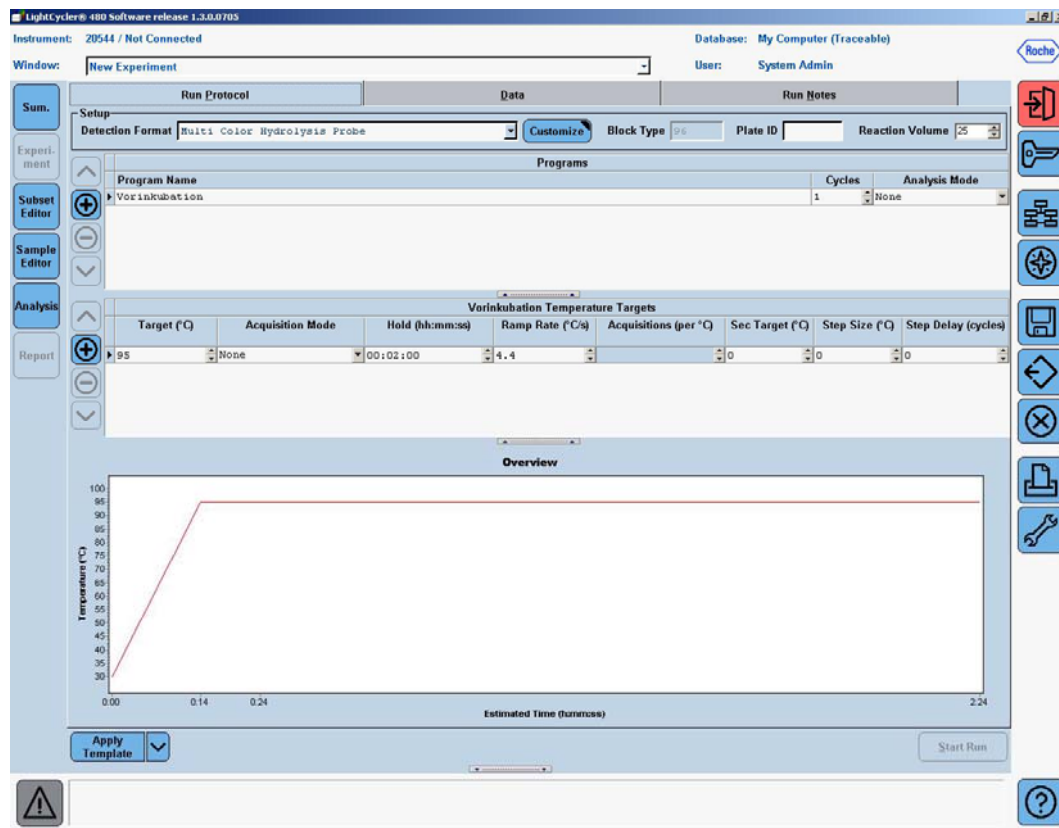


## Experimental Protocol and Evaluation for LightCycler® 480 with Minerva Biolabs real-time PCR kits

### 1. Experimental Protocol

#### Program 1: Pre-incubation

Cycles	1
Analysis Mode	None
<b>Temperature Targets [°C]</b>	<b>Segment 1</b>
Target temperature [°C]	95
Hold [min]	2:00
Ramp rate [°C/s]	4.4
Secondary target temperature [°C]	0
Step size [°C]	0.0
Step delay [Cycles]	0
Acquisition mode	None



#### Program 2: Amplification

Cycles	45			
Analysis Mode	Quantification			
<b>Temperature Targets [°C]</b>	<b>Segment 1</b>	<b>Segment 2</b>	<b>Segment 3</b>	<b>Segment 4</b>
Target temperature [°C]	95	55	60	72
Hold [s]	1	5	7	5
Ramp rate [°C/s]	4.4	2.2	4.4	4.4
Secondary target temperature [°C]	0	0	0	0
Step size [°C]	0.0	0.0	0.0	0.0
Step delay [Cycles]	0	0	0	0
Acquisition mode	None	None	Single	None



Please check the correct settings for the filter combination of LightCycler®480. For Target you must choose the filter FAM (483-533) and for the Internal Control-Target the filter ROX (558-610).

The screenshot shows the LightCycler 480 software interface. A dialog box titled "Detection Formats" is open, showing the "Multi Color Hydrolysis Probe" detection format. The "Integration Time Mode" is set to "Dynamic". The "Filter Combination" table is as follows:

Active	Filter Combination
<input type="checkbox"/>	Cyan 500 (450-500)
<input checked="" type="checkbox"/>	FAM (483-533)
<input type="checkbox"/>	Hex (523-568)
<input checked="" type="checkbox"/>	Red 610 (558-610)
<input type="checkbox"/>	Cy 5 (615-670)

Program 3: Cooling

Cycles	1
Analysis mode	None
<b>Temperature Targets [°C]</b>	<b>Segment 1</b>
Target temperature [°C]	40
Hold [s]	30
Ramp rate [°C/s]	2.2
Secondary target temperature [°C]	0
Step size [°C]	0.0
Step delay [Cycles]	0
Acquisition mode	None

The screenshot shows the LightCycler 480 software interface with the "Cooling" program selected. The "Kühlung Temperature Targets" table is as follows:

Target [°C]	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate [°C/s]	Acquisitions (per °C)	Sec Target [°C]	Step Size [°C]	Step Delay (cycles)
40	None	00:00:30	2.2	0	0	0	0

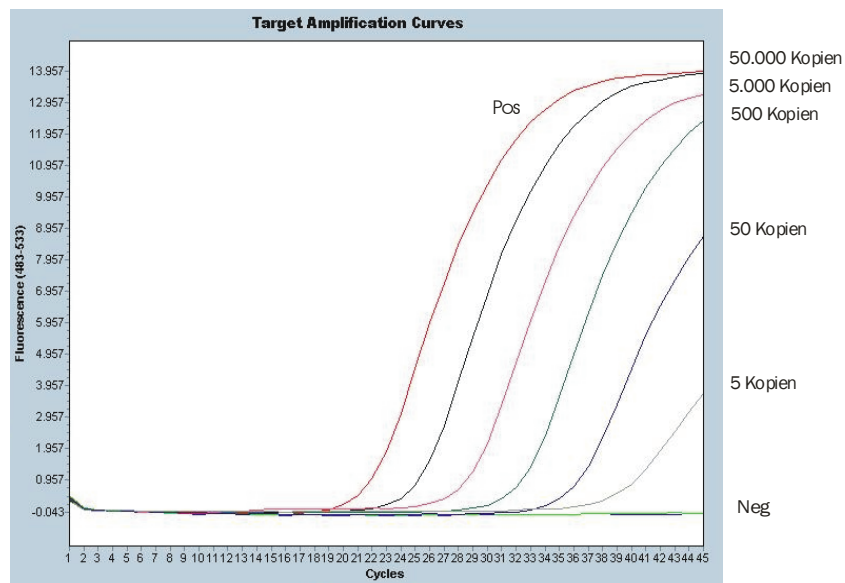
## 2. Evaluation and interpretation of the results

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

- quantitative analysis of DNA in fluorescence channel FAM-specific signal
- qualitative analysis of Internal Control DNA in fluorescence channel ROX-specific signal

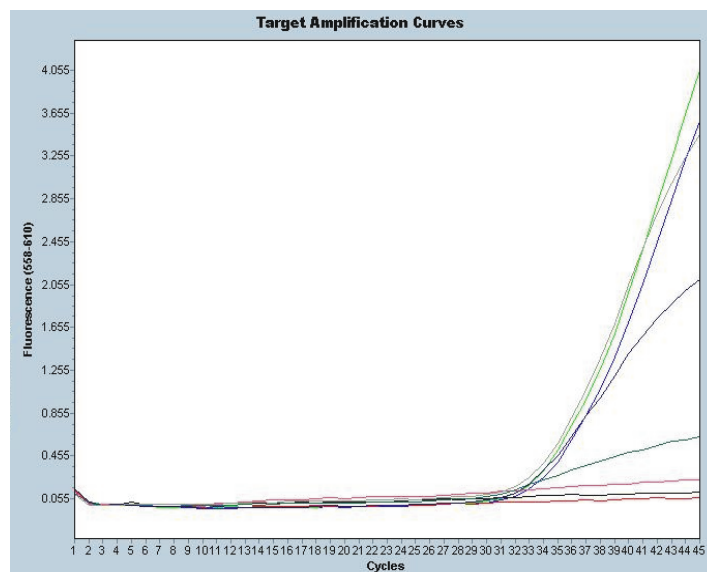
The following amplification curves were obtained by performing the described procedure with a dilution series of a Quantification Standard and the LightCycler® 480 instrument. The fluorescence values versus cycle number are displayed. In the same run the amplification of Internal Control DNA was shown in channel ROX (filter comb. 558-610).

### Amplifikation curves of the FAM Target – „filter combination 483-533“



Amplified dilution series of approx.  $5 \times 10^4$ ,  $5 \times 10^3$ ,  $5 \times 10^2$ , 50 and 5 genome equivalents of a Quantification Standard as starting template.

### Amplifikation curves of the ROX Internal Control-Target – „filter combination 558-610“



Amplified Internal Control DNA and a dilution series of a Quantification Standard as starting template.