

**LightCycler<sup>®</sup> 480**  
**LightCycler<sup>®</sup> 480 Instrument II**

**Designed for GeneProof diagnostic kits**  
**Microbiological DNA diagnostics**

**See [www.geneproof.com](http://www.geneproof.com) for the current list of available kits**



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## 1. Purpose

This device manual describes in detail the process of using GeneProof PCR kits for microbiological DNA diagnostics with the following devices: LightCycler® 480 and LightCycler® 480 Instrument II.

## 2. PCR Reaction Preparation

1. Add **30 µl of MasterMix** and **10 µl of DNA isolate** or **10 µl of Positive Control** into the tube in case of qualitative detection or **10 µl of calibrators** in case of quantitative detection. The final reaction mix volume should be **40 µl**.
2. Close the tubes, centrifuge shortly, insert into the device and start the PCR test.

## 3. Device Programming

When using the GeneProof PCR kits for the first time it is necessary to program the amplification profile and save it as a template. During subsequent uses of the GeneProof PCR kits start from chapter **4. PCR Amplification Start**. The software remembers the saved settings.

### 3.1. Software Start

1. Start **LightCycler® 480 Software**.
2. Click **New Experiment** in the main window.

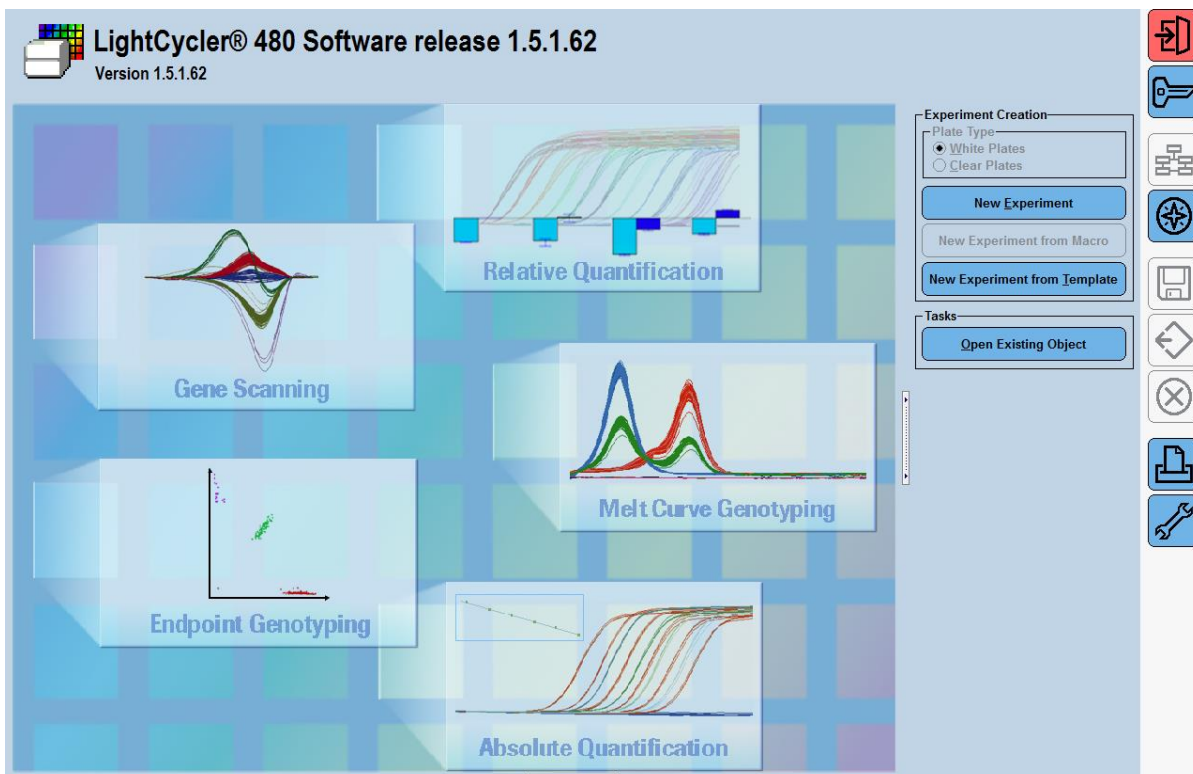


Fig. 3.1 New Experiment

3. Window for setting the experiment will be displayed.

## 3.2. Experiment Programming

1. Set **3 Color Hydrolysis Probe** in the **Detection Format** field. Enter **40 µl** in the **Reaction Volume** field.

The screenshot displays the GeneProof software interface for experiment programming. The 'Setup' section is active, showing the following settings:

- Detection Format:** 3 Color Hydrolysis Probe
- Block Size:** 96
- Plate ID:** (empty)
- Reaction Volume:** 40

Below the setup section is the 'Programs' table:

Program Name	Cycles	Analysis Mode
Program	1	None

The 'Temperature Targets' section shows the following parameters:

Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)
95	None	00:00:01	4.4	0	0	0	0

The 'Overview' graph shows the temperature profile over time. The y-axis is Temperature (°C) ranging from 30 to 100. The x-axis is Estimated Time (h:mm:ss) ranging from 0 to 25. The profile starts at 30°C at 0 minutes, ramps up to 95°C at 14 minutes, and then holds at 95°C until 25 minutes.

Fig. 3.2 Experiment Setting

2. Set the UDG decontamination step. Enter **UDG decontamination** in the **Program Name** field of the **Programs** window, enter **1** in the **Cycles** field and enter **None** in the **Analysis Mode** field. Enter **37** in the **Target (°C)** field in the **Temperature Targets** window, enter **None** in the **Acquisition Mode** field and enter **00:02:00** in the **Hold (hh:mm:ss)** field; leave other items without any changes.

3. Set the initial denaturation step. Click + to add a new step in the **Programs** window. Enter **Initial denaturation** in the **Program Name** field, enter **1** in the **Cycles** field and enter **None** in the **Analysis Mode** field. Enter **95** in the **Target (°C)** field in the **Temperature Targets** window, enter **None** in the **Acquisition Mode** field and enter **00:10:00** in the **Hold (hh:mm:ss)** field; leave other items without any changes.

4. Set the PCR program. Click + to add a new step in the **Programs** window. Enter **PCR** in the **Program Name** field, enter **45** in the **Cycles** field and enter **Quantification** in the **Analysis Mode** field.

- Enter **95** in the **Target (°C)** field in the **Temperature Targets** window, enter **None** in the **Acquisition Mode** field and enter **00:00:05** in the **Hold (hh:mm:ss)** field; leave other items without any changes.
- Click + in the **Temperature Targets** window to add an additional program step. Enter **60** in the **Target (°C)** field, enter **Single** in the **Acquisition Mode** field and enter **00:00:40** in the **Hold (hh:mm:ss)** field; leave other items without any changes. The program will automatically determine the optimum Ramp rate value.
- Click + in the **Temperature Targets** window to add an additional program step. Enter **72** in the **Target (°C)** field, enter **None** in the **Acquisition Mode** field and enter **00:00:20** in the **Hold (hh:mm:ss)** field; leave other items without any changes.

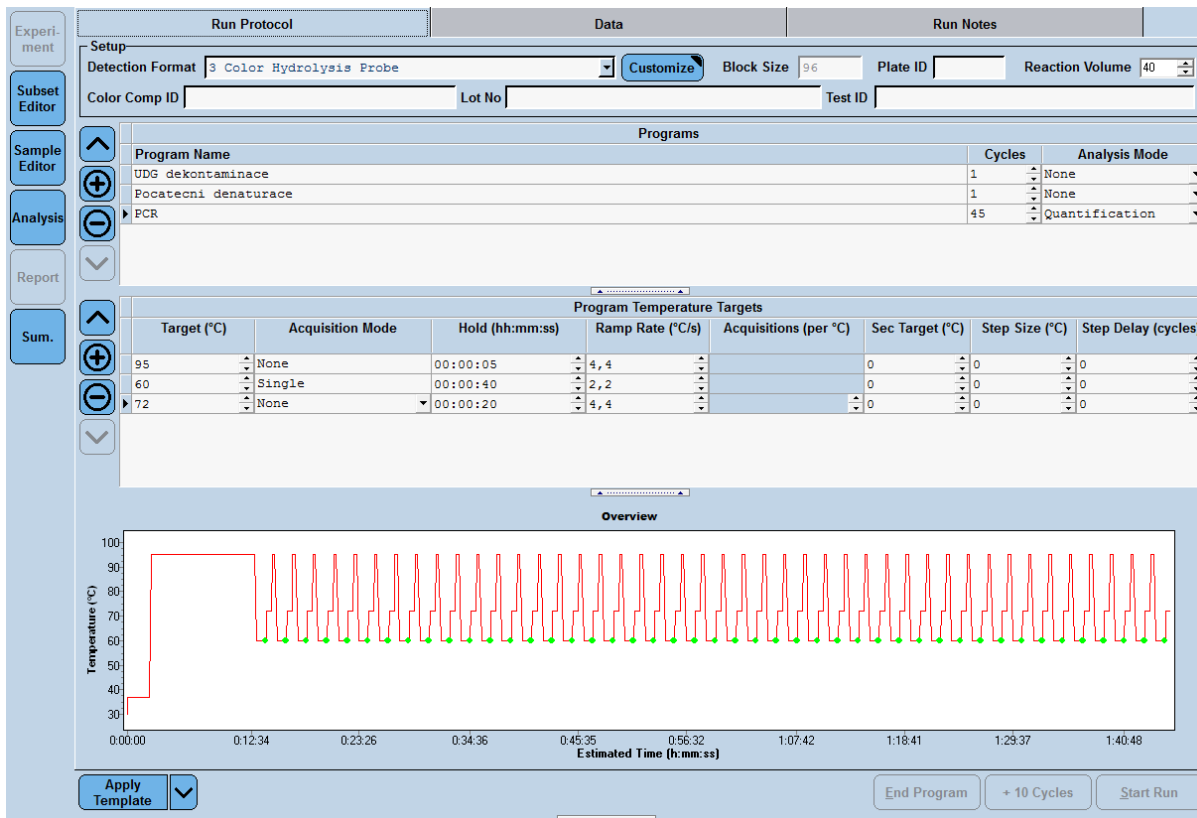


Fig. 3.3 Amplification Profile Setting

5. Use the arrow next to the **Apply Template** button in the lower left section of the screen to open a menu, select **Save As Template** and save it as **GeneProof DNA PCR** into the **Run Templates** folder.

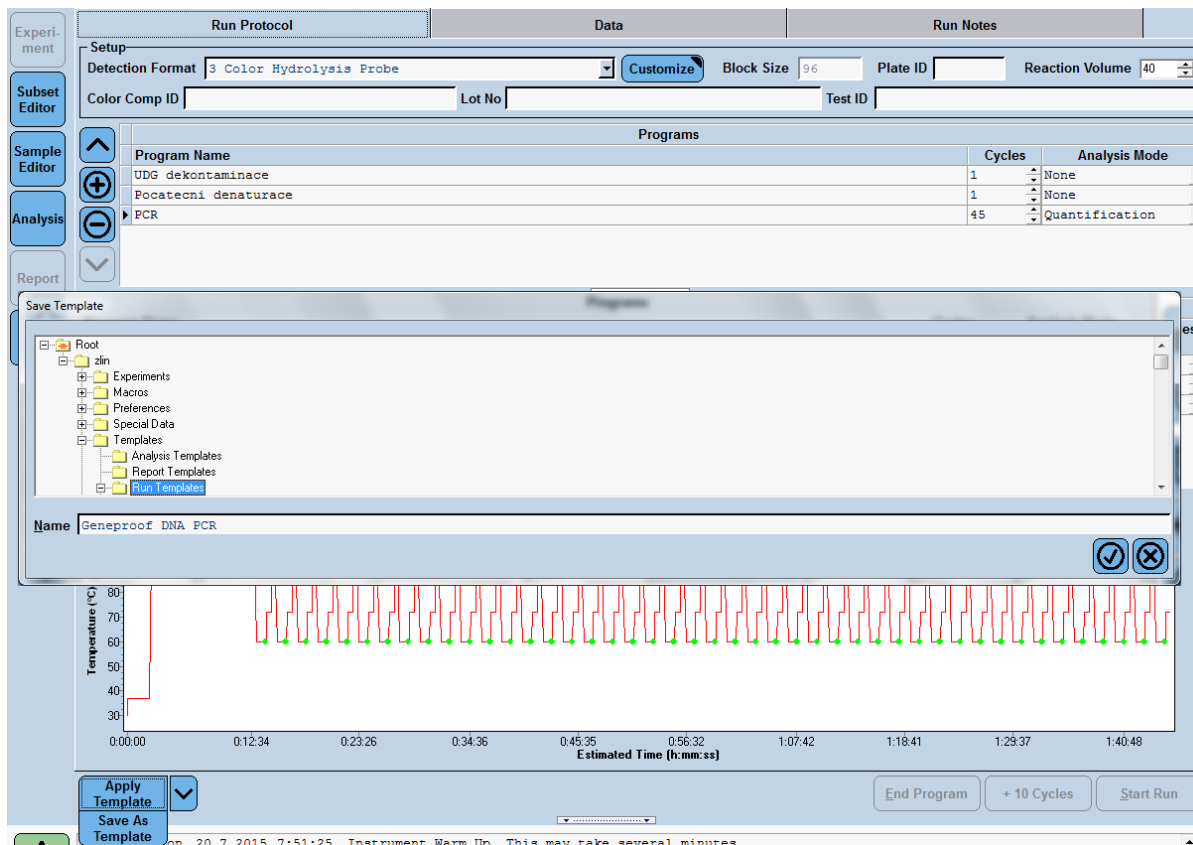


Fig. 3.4 Save Template

## 4. PCR Amplification Start

When using the GeneProof PCR kits for the first time it is necessary to program the amplification profile and save it as a template (see chapter 3. **Device Programming**). The software will remember the saved settings for subsequent GeneProof PCR kits uses.

### 4.1. Open Saved Template

1. Start LightCycler® 480 Software.
2. Select **New Experiment from Template** in the initial screen. Select **GeneProof DNA PCR** in the menu.

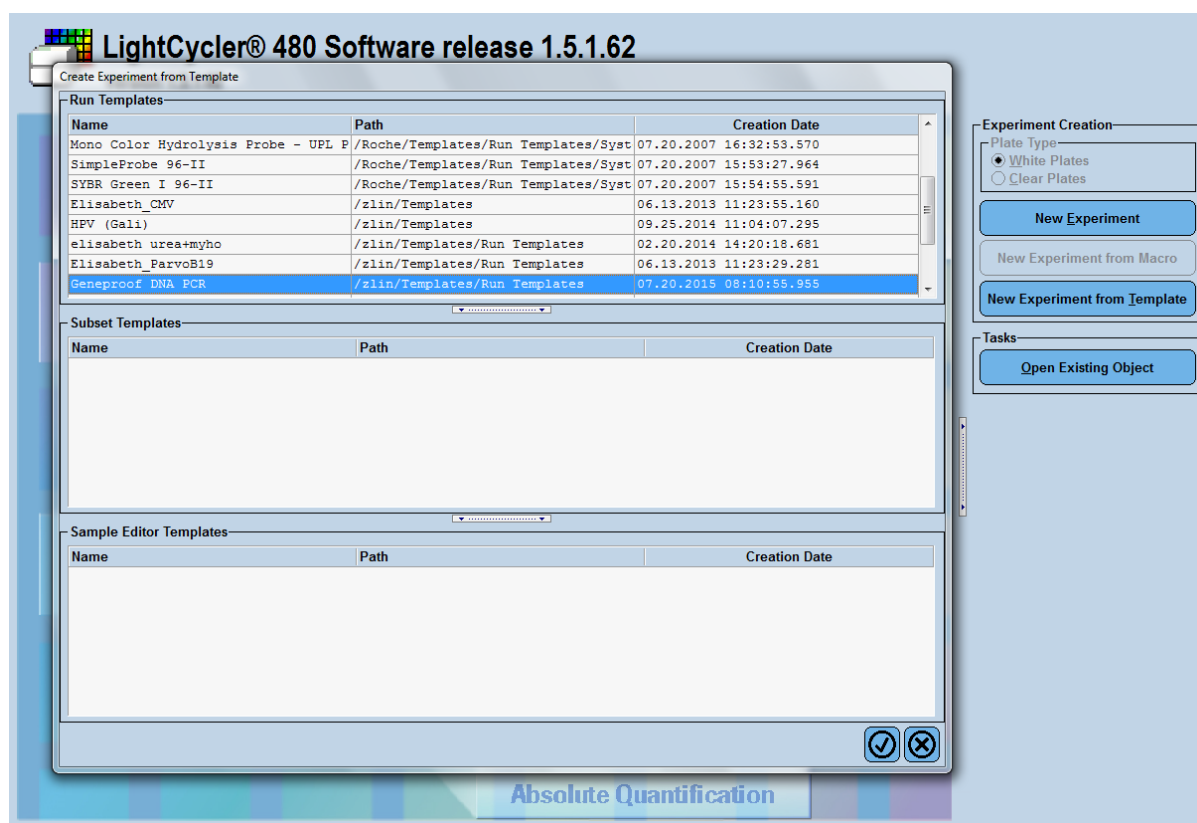


Fig. 4.1 Open Template

## 4.2. Creating Subsets and Sample Editing

Separate samples into groups according to the examined pathogens to make orientation in the sample layout on the plate easier.

1. Select **Subset editor** in the left bar.
2. Use **+** in the lower section of the **Subsets** window to add a new subset and assign a name to this subset.
3. Ctrl-click to select appropriate samples in the plate schema and click **Apply** in the lower section to confirm.

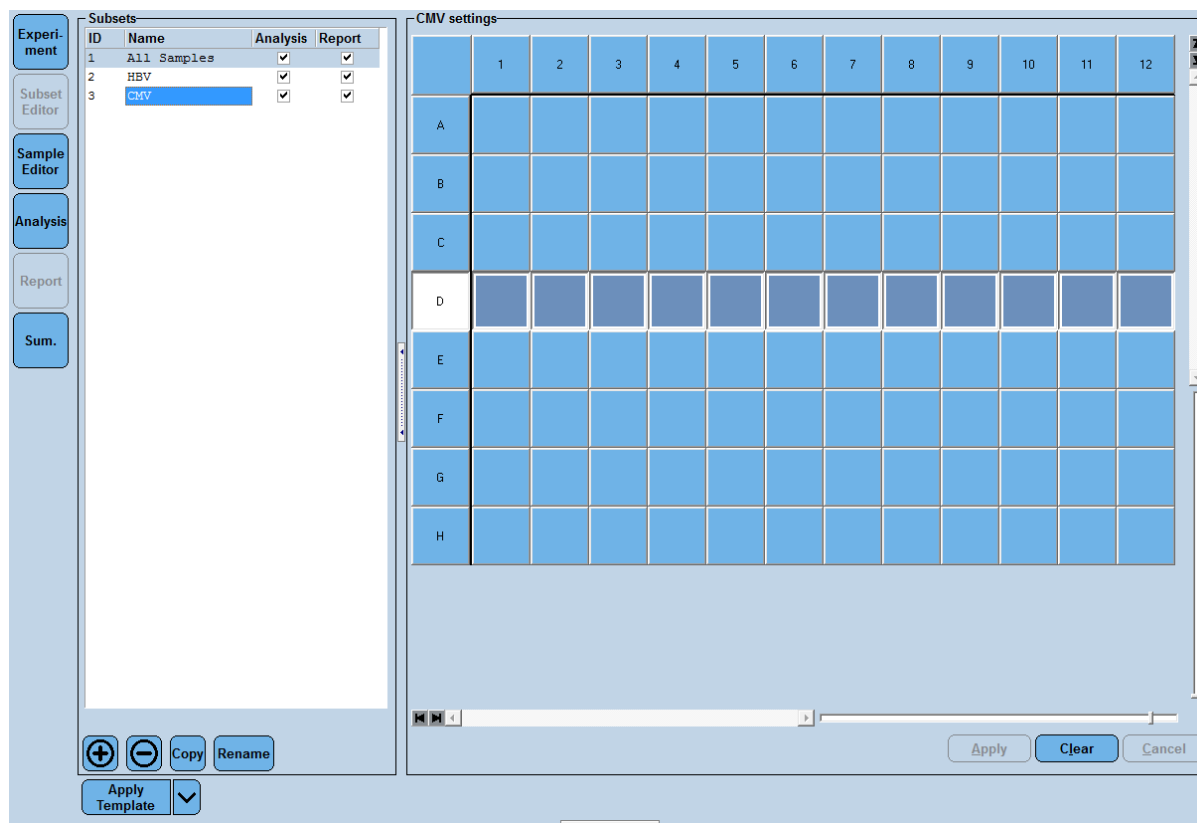


Fig. 4.2 Creating Subsets



4. Select **Sample editor** in the left bar to assign names to the individual samples.
5. Select the appropriate subset in the drop-down menu of the **Step 2: Select Samples** window.
6. Enter the sample name in the **Sample Name** column of the sample table.

The screenshot displays the GeneProof software interface for sample description. The interface is organized into several sections:

- Left Sidebar:** Contains navigation buttons for Experiment, Subset Editor, Sample Editor, Analysis, Report, and Sum.
- Top Panel:**
  - Step 1: Select Workflow:** Includes radio buttons for Abs Quant, Rel Quant, Scanning, Color Comp, Tm, Melt Geno, and Endpt Geno.
  - Select Filter Combinations:** Features checkboxes for 465-510, 533-580, and 618-660.
  - Abs Quant:** Includes a Units input field.
- Step 2: Select Samples:**
  - Subset:** A dropdown menu currently set to 'CMV'.
  - Grid:** A 12x8 grid (A-H, 1-12) for selecting sample positions.
  - Color Legend:** A grid of colored squares corresponding to sample positions 1-12, PK, and NK.
- Table:** A table with the following data:

Pos	Color	Repl Of	Sample Name
D1	Blue		1
D2	Red		2
D3	Green		3
D4	Magenta		4
D5	Grey		5
D6	Yellow		6
D7	Cyan		7
D8	Black		8
D9	Dark Green		9
D10	Orange		10
D11	Purple		PK
D12	Light Green		NK
- Step 3: Edit Properties:** Includes a Sample Name input field and a Make Replicates dropdown menu.
- Bottom Panel:** Contains buttons for Apply Template, Configure Properties, Toggle View (Table), Reset All, Import, and Export.

Fig. 4.3 Sample Description

For quantitative analysis only

7. Check **Abs. Quant.** in the **Step 1: Select Workflow** window. A table with samples and other parameters displays on the right.

8. Select **Standard** in the **Quantification Sample type** column of the positions containing calibrators for channel **FAM 465-510** (in case of **3-channel** detection also for the **Cy5 618-660** channel) and enter the calibrator concentration into the **Concentration** field.

The screenshot displays the GeneProof software interface. On the left, there are navigation buttons: Experiment, Subset Editor, Sample Editor, Analysis, Report, and Sum. The main window is divided into three sections:

- Step 1: Select Workflow:** Includes radio buttons for 'Abs Quant' (selected), 'Rel Quant', 'Scanning', 'Color Comp', 'Tm', 'Melt Geno', and 'Endpt Geno'. It also has checkboxes for filter combinations: 465-510, 533-580, and 618-660.
- Step 2: Select Samples:** Features a grid for sample selection (rows A-H, columns 1-12) and a 'Subset' dropdown set to 'CMV'. Below the grid is a 'Quantification Sample Type' dropdown menu.
- Table:** A table with columns: Pos, Filter combination, Color, Repl Of, Sample Name, Quantification Sample Type, and Concentration. The table lists 22 samples (D1 to D11) with various filter combinations and concentrations. A dropdown menu is open for sample D4, showing options: 'Standard', 'Positive Control/Calibrator', and 'Negative Control'.
- Step 3: Edit Abs Quant Properties:** Includes fields for 'Sample Name', 'Sample Type' (radio buttons for 'Unknown', 'Positive Control/Calibrator', 'Standard'), and 'Concentration'. There is an 'Auto Std Curve' button and a 'Make Replicates' dropdown.

At the bottom, there are buttons for 'Apply Template', 'Configure Properties', 'Toggle View (Table)', 'Reset All', 'Import', and 'Export'.

Fig. 4.4 Calibrators Settings

## 4.3. Start Experiment


It is necessary to save the experiment to the required location using the  (floppy disk) button on the right bar before starting the device.



Fig. 4.5 Save Experiment

1. Select **Experiment** in the left bar.
2. In the lower right corner click **Start Run** to start the experiment.

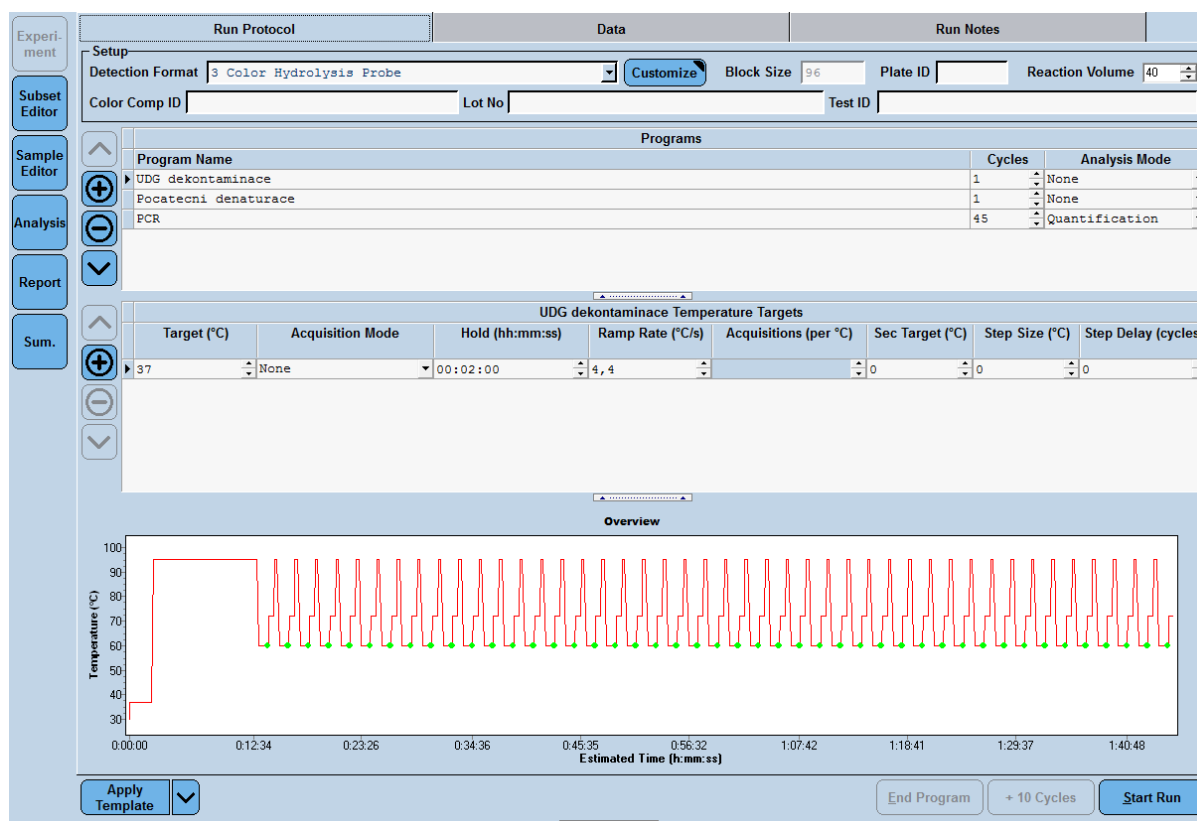


Fig. 4.6 Start Experiment

You can watch the experiment progress on the **Data** tab.

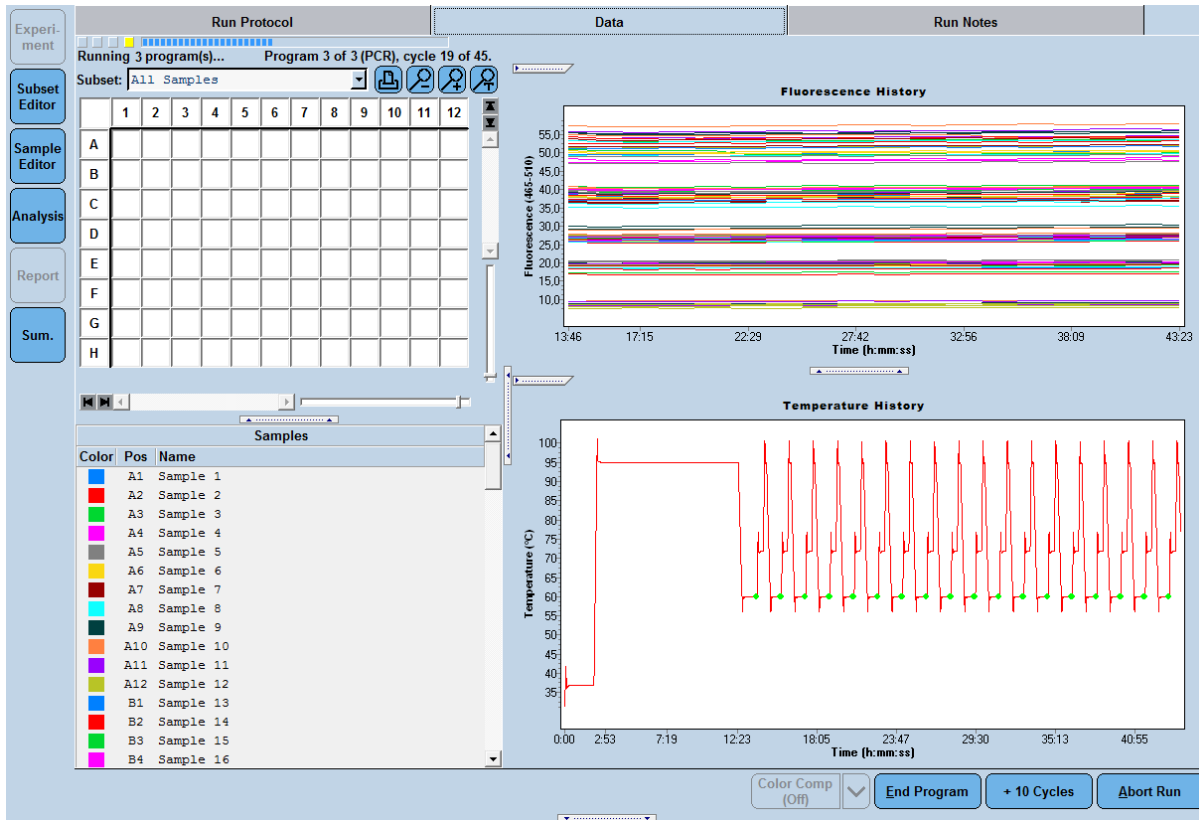


Fig. 4.7 Experiment Progress

## 5. Result Analysis and Detection Evaluation

PCR detection end is announced by the **Run complete...** message in the upper left corner of the software window.

### 5.1. Detection Analysis of the Studied Microorganism

1. Use the **Analysis** button in the left bar to display the analysis menu.
2. Click **Abs Quant/Fit Points** in the **Create New Analysis** window.

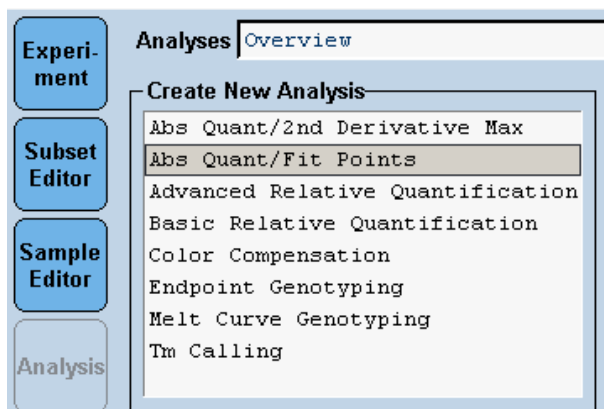


Fig. 5.1 Analysis Selection

3. Select a subset for analysis in the **Subset** row of the newly opened window. You can assign a name to the new analysis in the **Name** row.

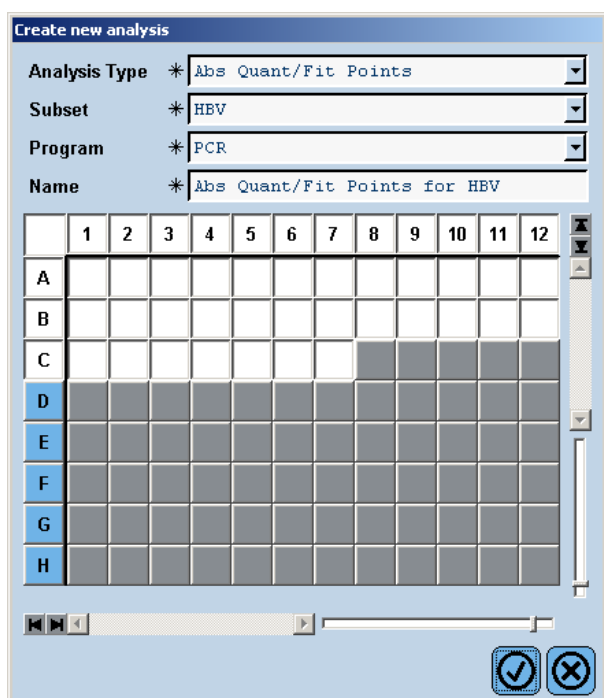


Fig. 5.2 Subset Selection

4. An analysis window is displayed. Select the appropriate color-compensation for FAM and HEX channels. Click **Color Comp** to open the **In Database** menu.

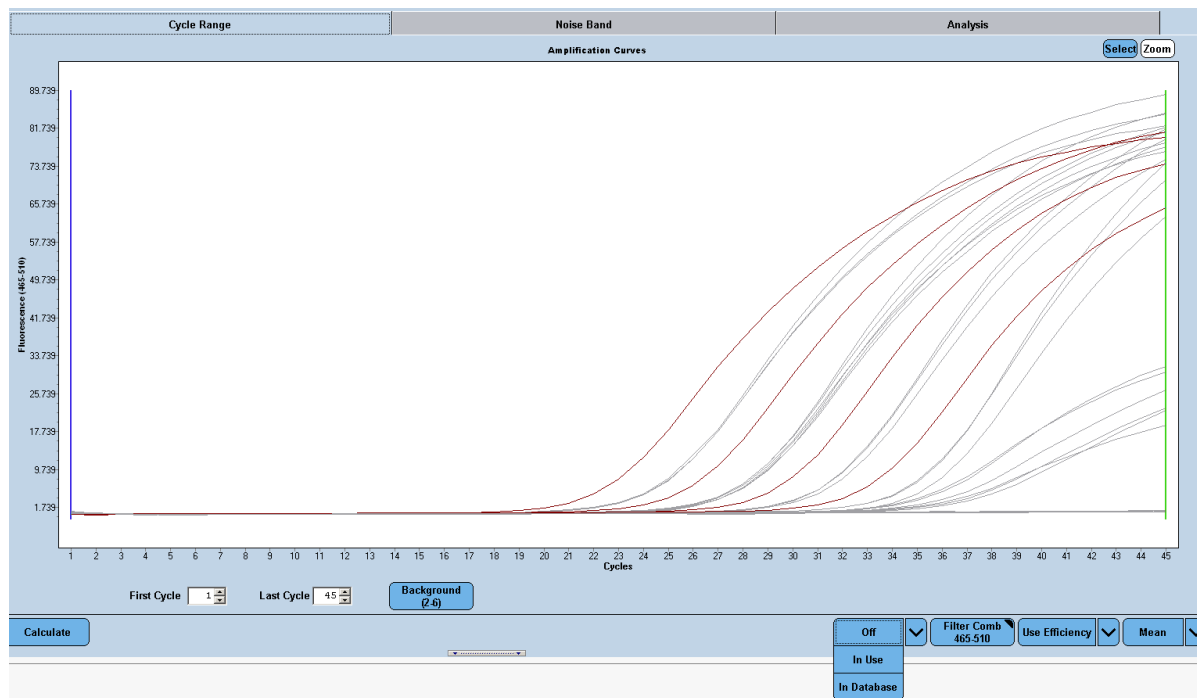


Fig. 5.3 Color-compensation

5. Select **Universal CC FAM (510) - VIC (580)** color-compensation and confirm.

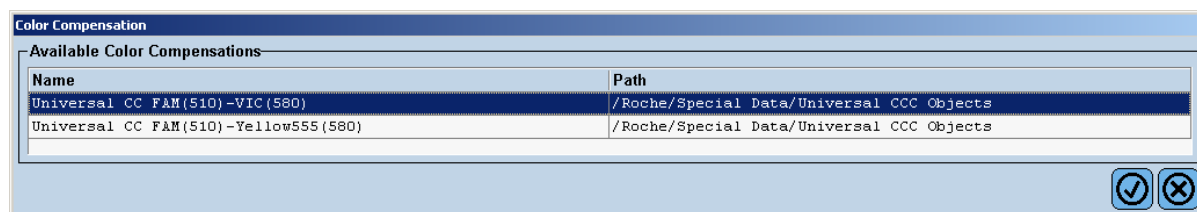


Fig. 5.4 Color-Compensation Selection

6. Check FAM and HEX channels in the **Color Compensation Channels** window and confirm.



Fig. 5.5 Color-Compensation Channels Selection

7. Click **Filter Comb** in the lower row to open the channel menu for analysis. Select the **FAM** channel. (When using a 3-channel detection kit repeat the above-described procedure also for the **Cy5** channel)

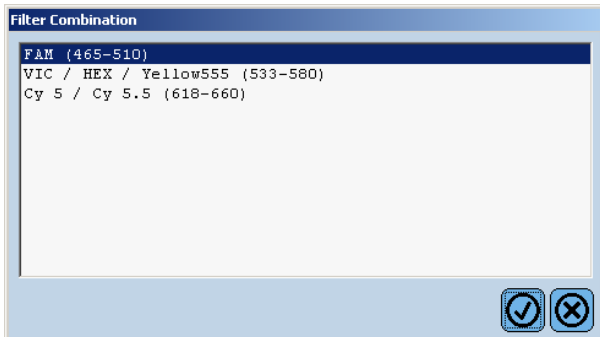


Fig. 5.6 Analysis Channel Selection

8. Switch to the **Noise Band** tab. Set the Noise Band button to **NoiseBand (Fluorescent)**. Use the mouse cursor to move the red line above the base noise of the reaction.

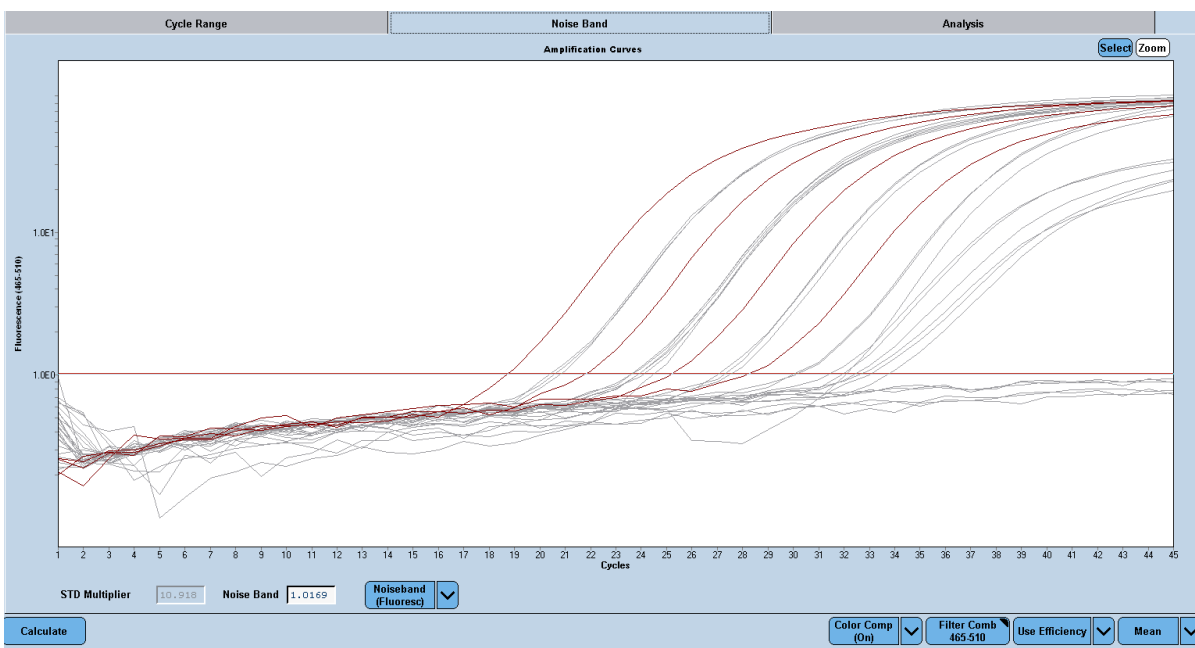


Fig. 5.7 Noise Band Setting

9. Switch to the **Analysis** tab. Use the mouse cursor to move the Threshold red line above the beginning of the curves to intersect all the curves. Click **Calculate** in the lower row.

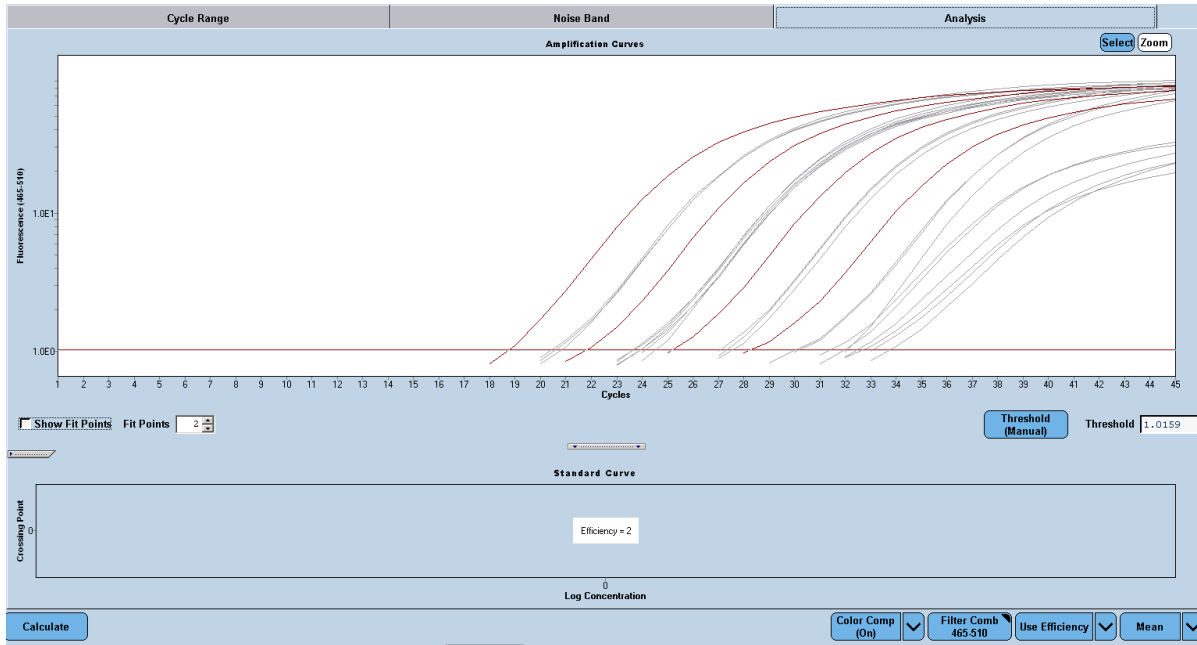



Fig. 5.8 Threshold Setting

10. Samples evaluated as positive will turn red, negative samples will turn green.

Click the separator  between the sample table and the chart to display an extended preview of the plate with positions of positive and negative samples and Cp values in a table.

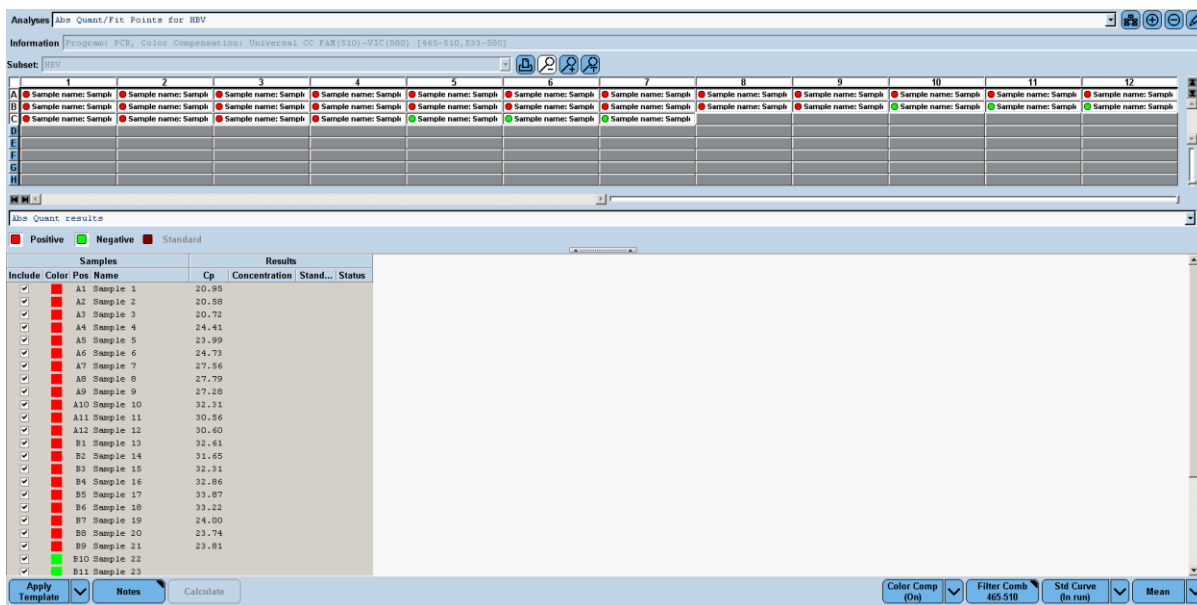


Fig. 5.9 Extended Preview



For quantitative evaluation only

Switch the **Use Efficiency** button in the lower row to **Std Curve (In run)**. Click **Calculate** to calculate a standard curve and concentrations of unknown samples.

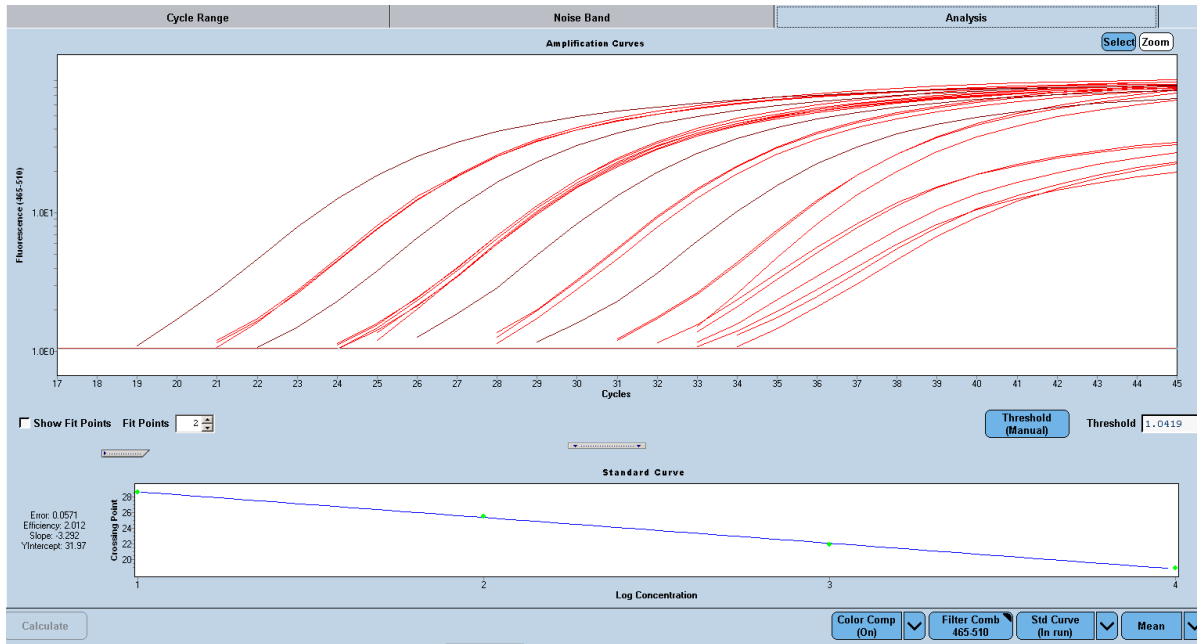



Fig. 5.10 Standard Curve

Click the separator  between the sample table and the chart to display Cp values and sample concentrations.

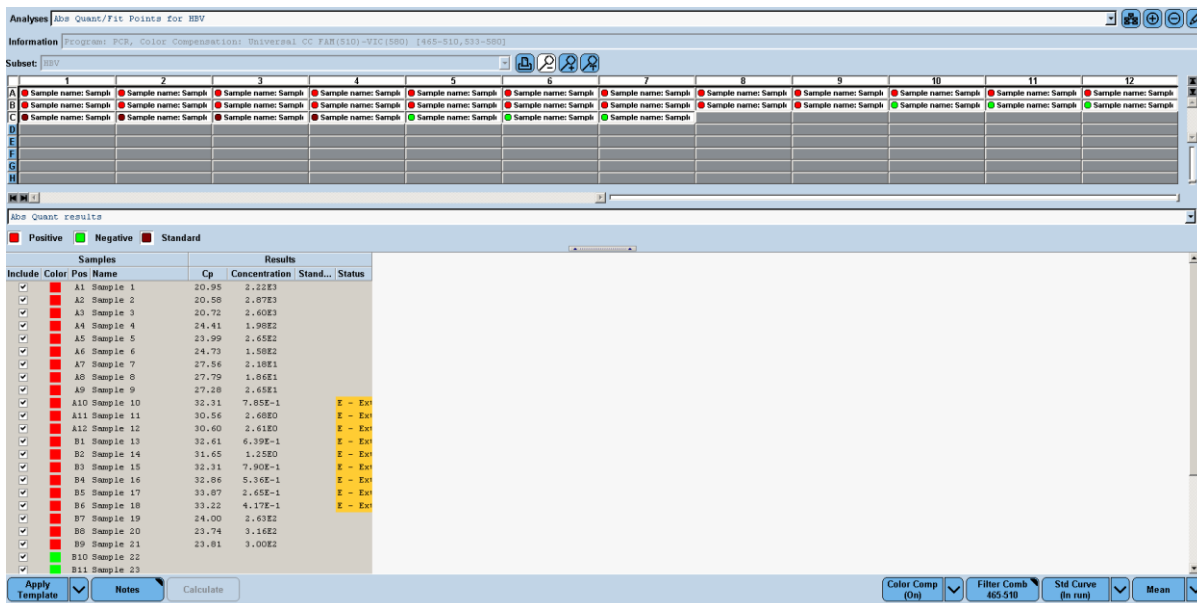



Fig. 5.11 Resulting Concentrations

Perform evaluation, including the pathogen concentration calculation in copies/ml (IU/ml) according to the package insert of the used GeneProof PCR kit.

## 5.2. Internal Standard Detection Analysis

1. Click **Filter Comb** in the lower row to open the channel menu for analysis. Select the **VIC/HEX/Yellow555** channel.
2. Move the red line above the base noise of the reaction in the **Noise Band** tab.
3. Move the red Threshold line above the beginning of the curves in the **Analysis** tab. Click **Calculate** in the lower row.

Perform evaluation according to the package insert of the used GeneProof PCR kit.

Click  (floppy disk) to save the evaluated detection. Use the **Report** button in the left bar to print the final protocol.



## 6. Customer Service

We appreciate all our customers and besides high-quality products we provide superior customer service including the following:

- Provision of free PCR kit samples, including demonstration in the customer's laboratory and personnel training
- Express deliveries
- Quick solution of problems related to the supplied products – service guaranteed within 24 hours from the time of announcement
- Consultations concerning technological and clinical interpretations

To assure the quickest possible solution of any problem we always require the GeneProof PCR Kit users to provide the following information:

- Kit name
- Problem definition
- Kit lot - specified on the kit package
- Used device
- File with the examination log from the used device

## 7. Contact Information

### Support and customer care

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