GeneProof
Molecular diagnostics for your routine

DEVICE MANUAL

Roche

LightCycler® 2.0

Designed for GeneProof diagnostic kits

Microbiological DNA diagnostics

See www.geneproof.com for the current list of available kits

CONTENTS

1. Purpose

This device manual describes in detail the process of using GeneProof PCR kits for Microbiological DNA diagnostics with the following device: LightCycler® 2.0.

2. PCR Reaction Preparation

1. Add **30 µl of MasterMix** and **10 µl of DNA isolate** or **10 µl of Positive Control** into a 100 µl capillary in case of qualitative detection or **10 µl of calibrators** in case of quantitative detection. The final reaction mix volume is **40 µl**.
2. 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® Software**.
2. Click New... to open the **Create New Object** box.
3. Select **LightCycler Experiment** and click **OK** to confirm.

Fig. 3.1 Create New Experiment

3.2. Basic Parameter Programming

1. In the **Run** box select **530** in the **Default Channel** field.
2. Enter **37** in the **Seek Temperature** field.
3. Enter **32** in the **Max. Seek Pos.** field.
4. Select **6 Ch.** in the **Instrument Type** field.
5. Select **100** in the **Capillary Size** field.

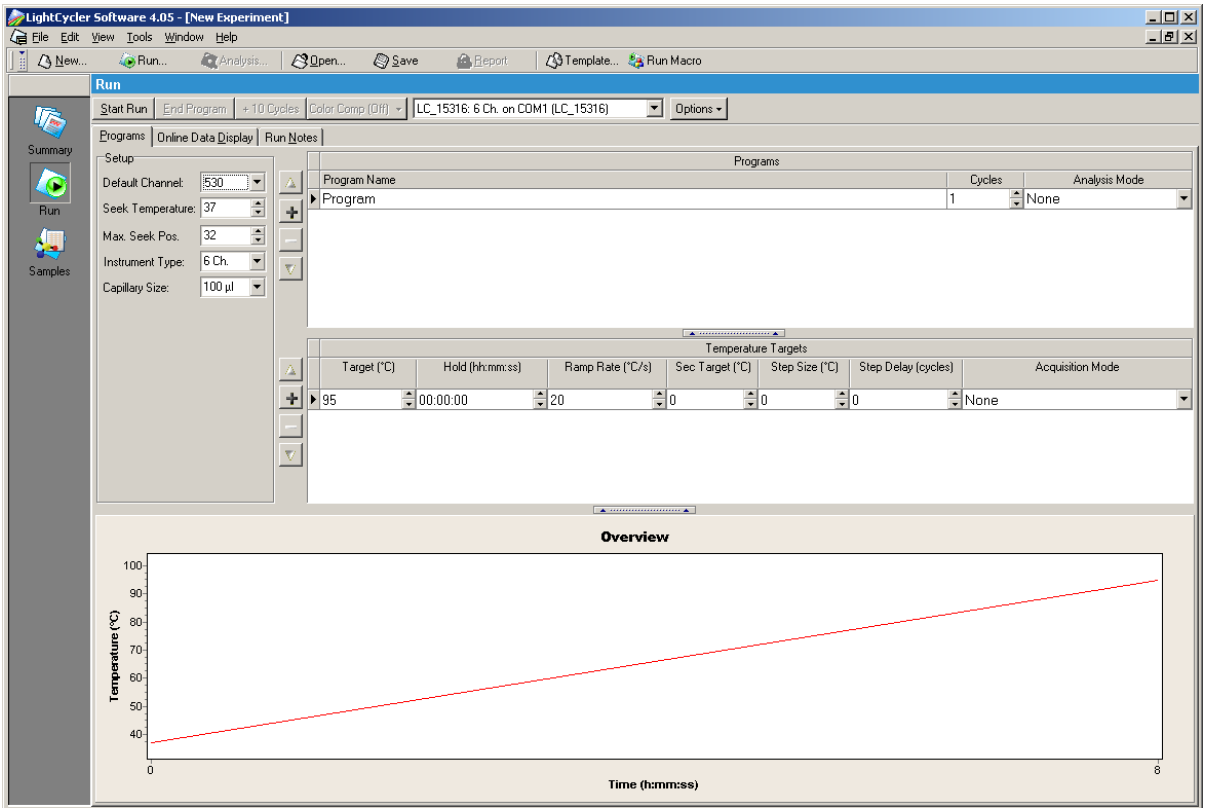

Fig. 3.2 Basic Parameters

3.3. Amplification Profile Programming

1. 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 **00:02:00** in the **Hold (hh:mm:ss)** field and enter **None** in the **Acquisition Mode** field; leave other items without any changes.

2. 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 **00:10:00** in the **Hold (hh:mm:ss)** field and enter **None** in the **Acquisition Mode** field; leave other items without any changes.

3. 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 **00:00:05** in the **Hold (hh:mm:ss)** field and enter **None** in the **Acquisition Mode** 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 **00:00:40** in the **Hold (hh:mm:ss)** field and enter **Single** in the **Acquisition Mode** field; leave other items without any changes.
- Click **+** in the **Temperature Targets** window to add an additional program step. Enter **72** in the **Target (°C)** field, enter **00:00:20** in the **Hold (hh:mm:ss)** field and enter **None** in the **Acquisition Mode** field; leave other items without any changes.

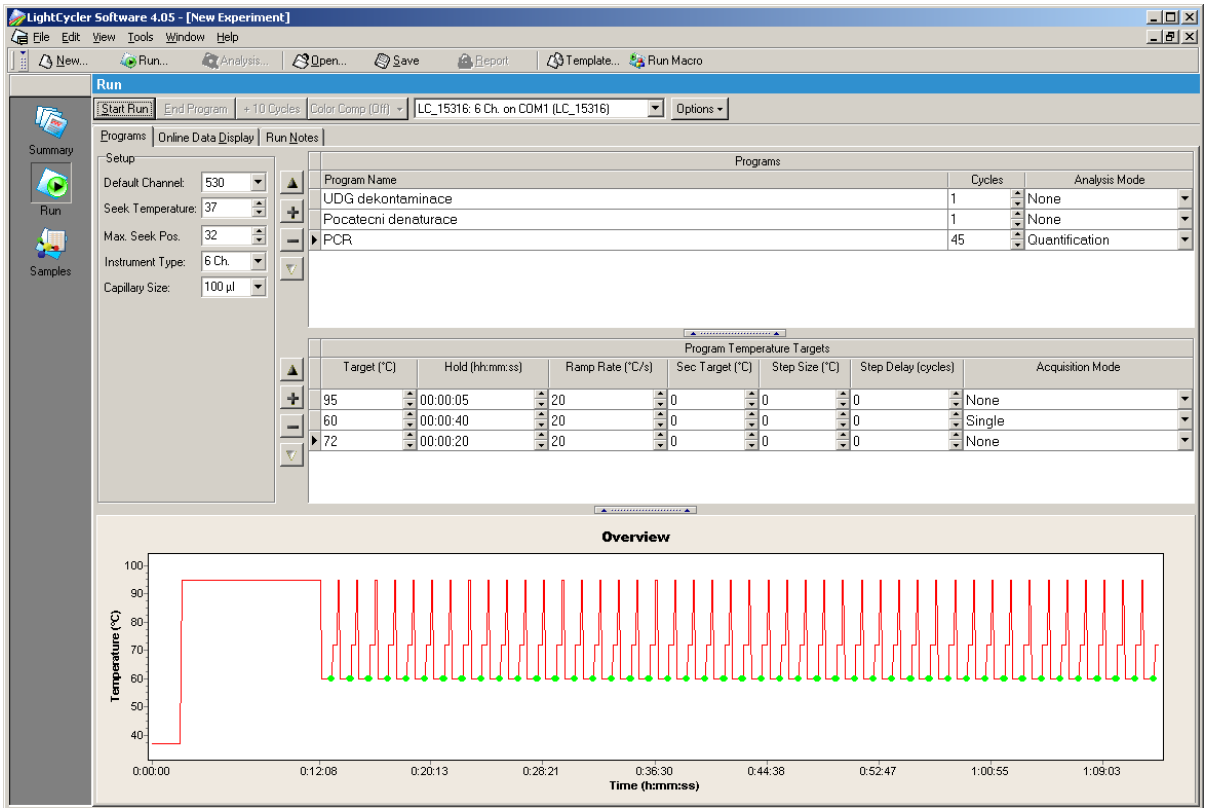

Fig. 3.3 Amplification Programming

3.4. Save Template

1. Select **Tools** in the upper menu bar and then select **Create Macro/Kit/Template….**
2. Check **Run Protocol Template** in the **Create Experiment Template** box and click **OK** to confirm.

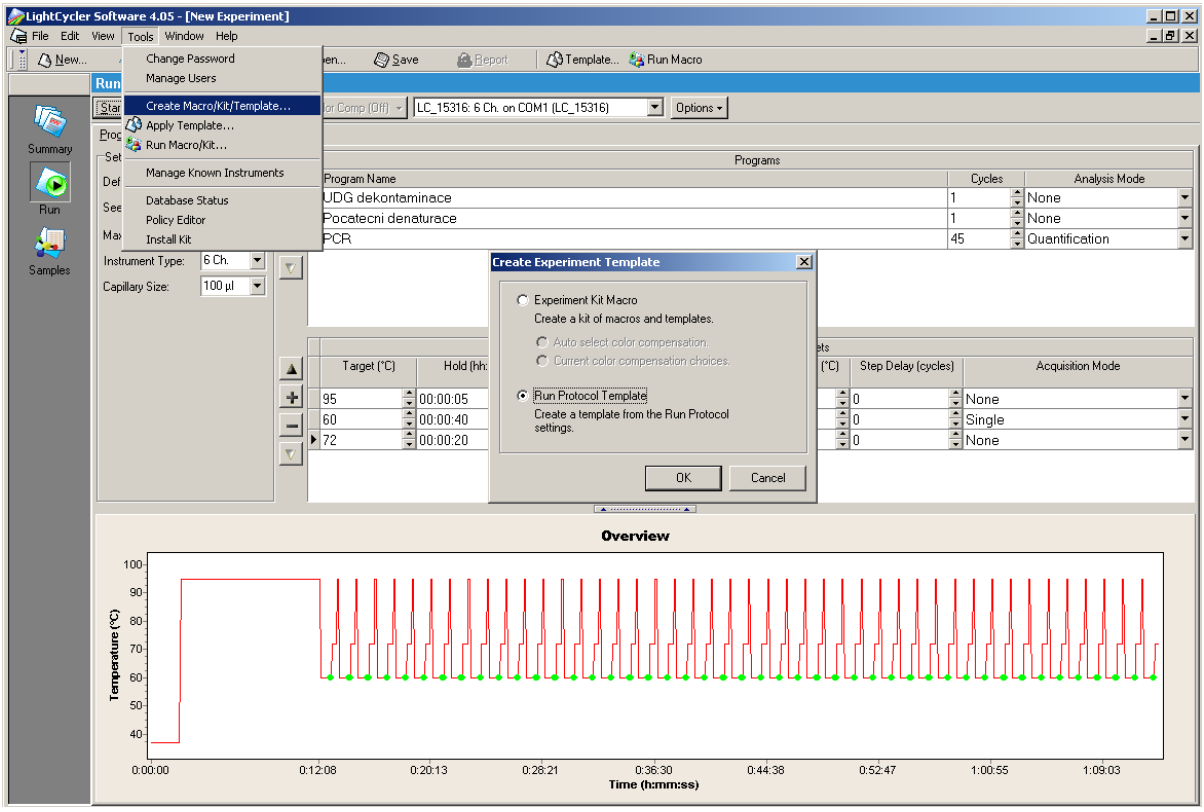

Fig. 3.4 Template Selection

3. Save the template into the **Run Templates** folder with the following name: **GeneProof DNA PCR**.

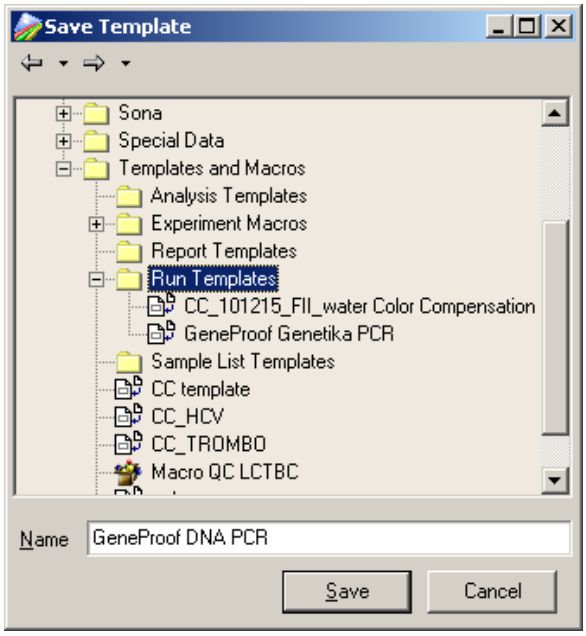

Fig. 3.5 Save Template

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**). It is not necessary to program the amplification profile again for subsequent GeneProof PCR kit uses.

4.1. Open Saved Template

1. Start **LightCycler® Software**.
2. Click New... to open the **Create New Object** box.
3. Select **LightCycler Experiment** and click **OK** to confirm.

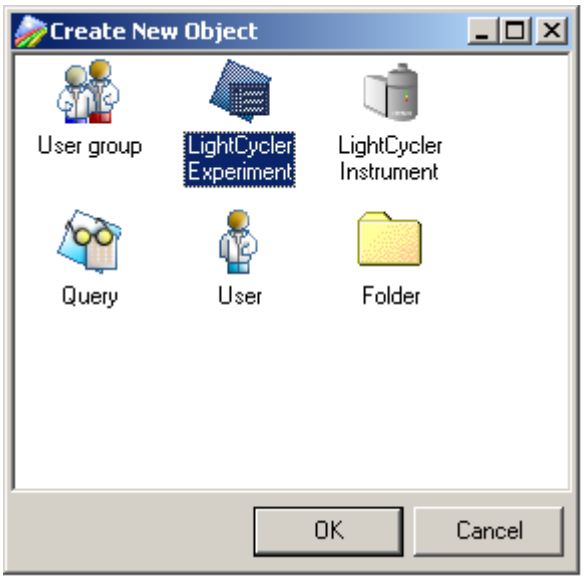

Fig. 4.1 Create New Experiment

4. Click the Template... button to open the **Apply Template** box.
5. Select **GeneProof DNA PCR** in the **Run Templates** folder and click **Open**.

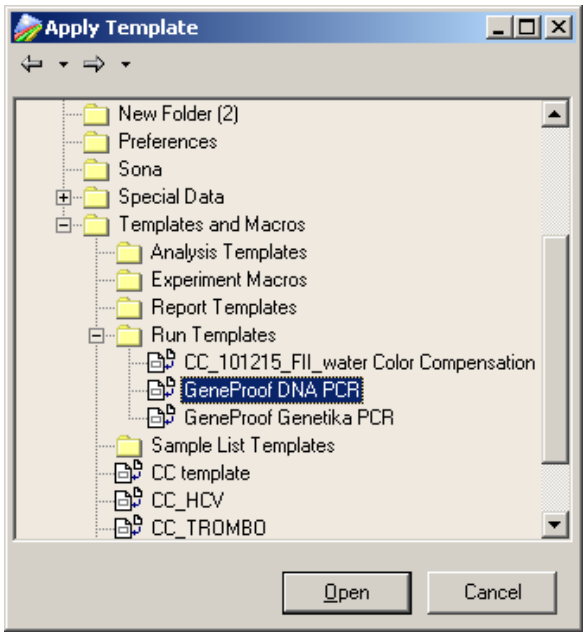

Fig. 4.2 Open Template

4.2. Edit Sample

1. Switch to **Samples** in the left side of the main window.
2. In the **Sample data** box enter the **number of samples under examination** in the **Sample Count** field.
3. In the **Sample Name** column you can assign names to the samples and in the **Sample Note** column you can add notes.

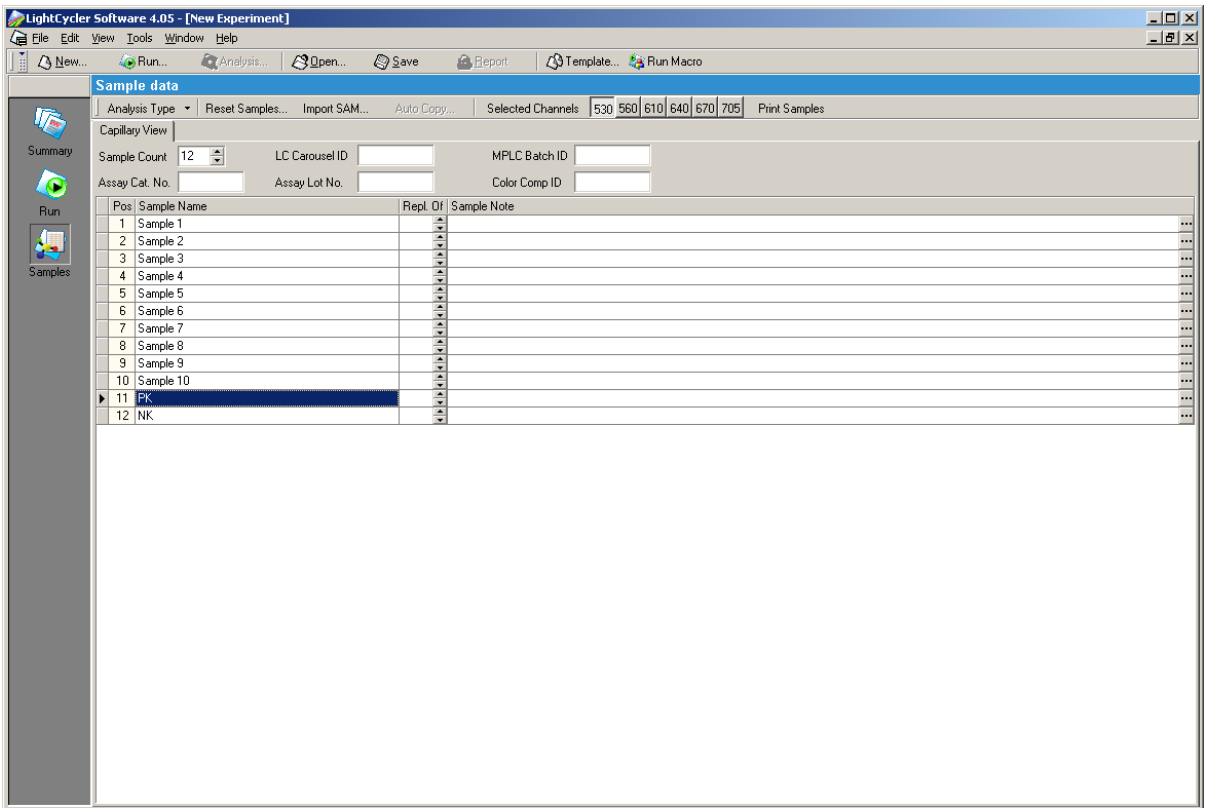

Fig. 4.3 Sample Description

For quantitative analysis only

4. Click **Analysis Type** and select **Absolute Quantification**.

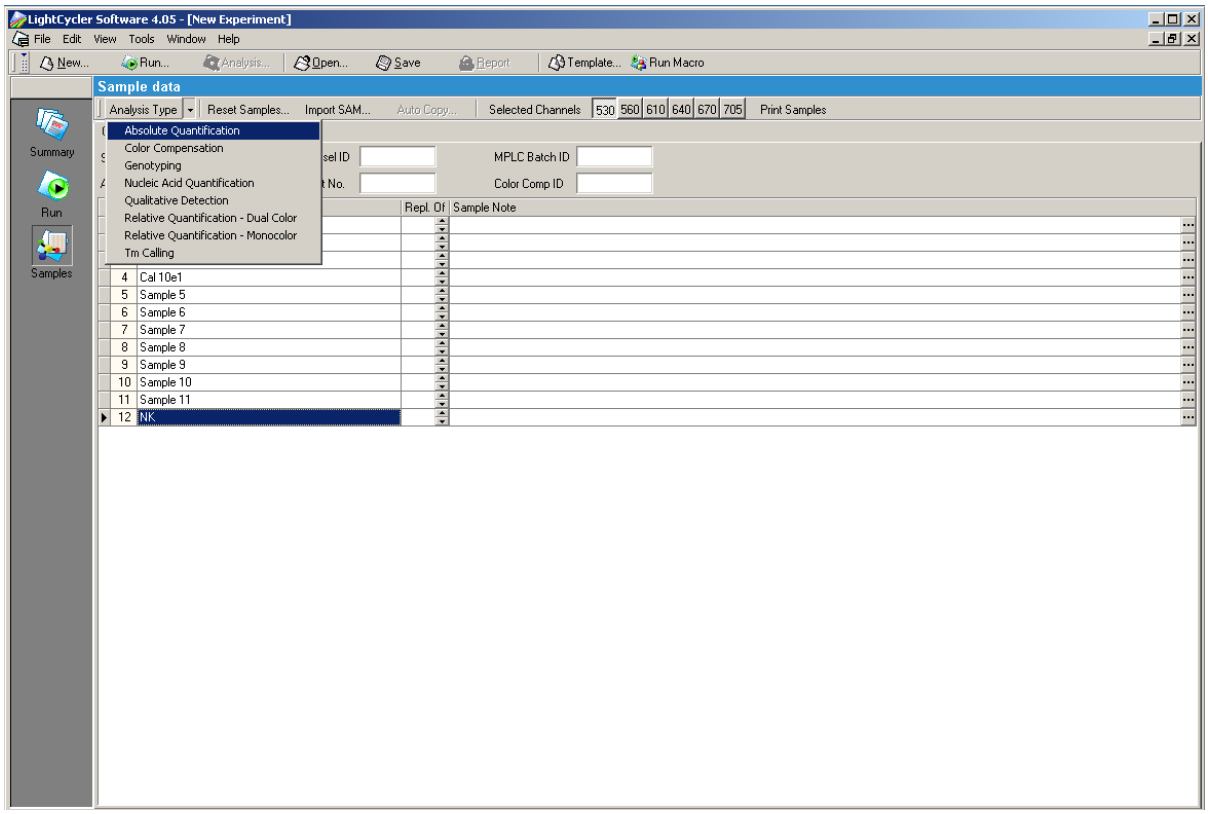

Fig. 4.4 Edit Samples for Quantitative Analysis

5. Select **Standard** in the **Sample type** column of the positions containing calibrators and enter the calibrator concentration into the **Concentration** field.

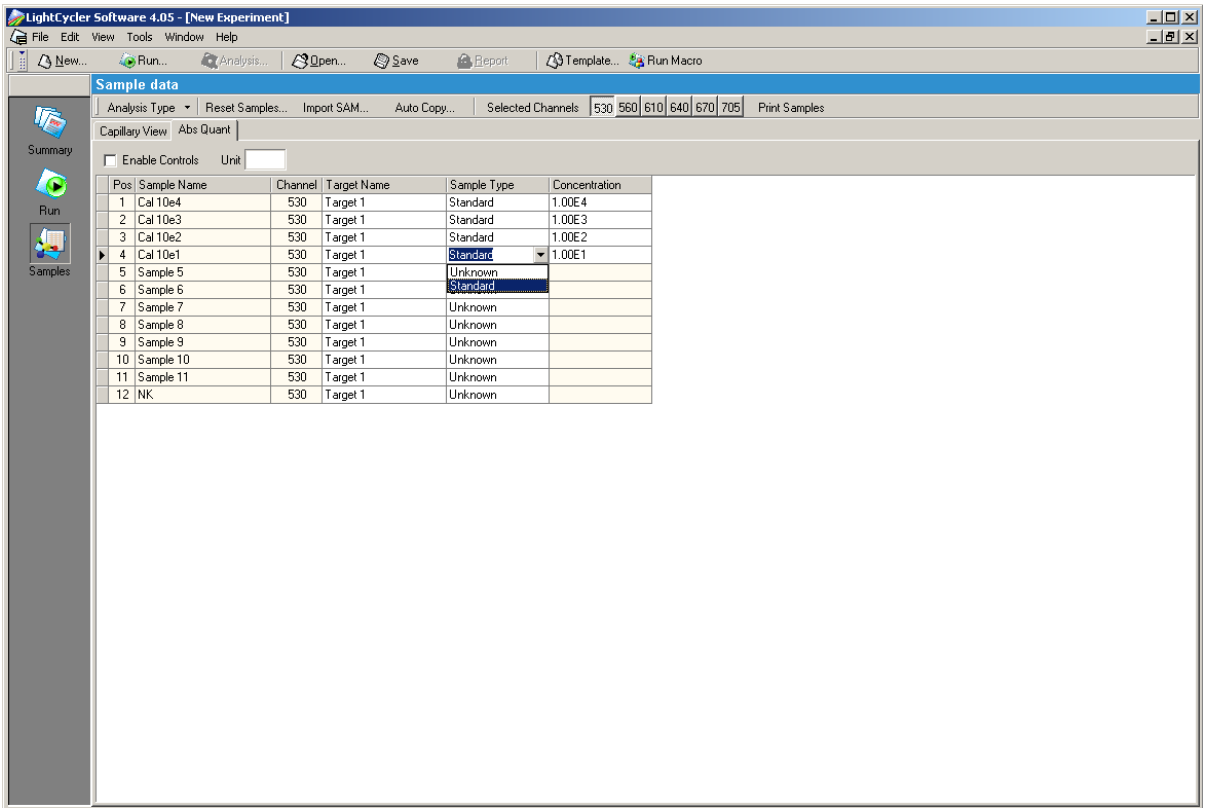

Fig. 4.5 Calibrators Setting

4.3. Start Experiment

Save the experiment before starting the device.

1. Click the Save button to open the **Save LightCycler Experiment** box.
2. Assign a name to the experiment and click **Save** to save it into the **Experiments** folder.

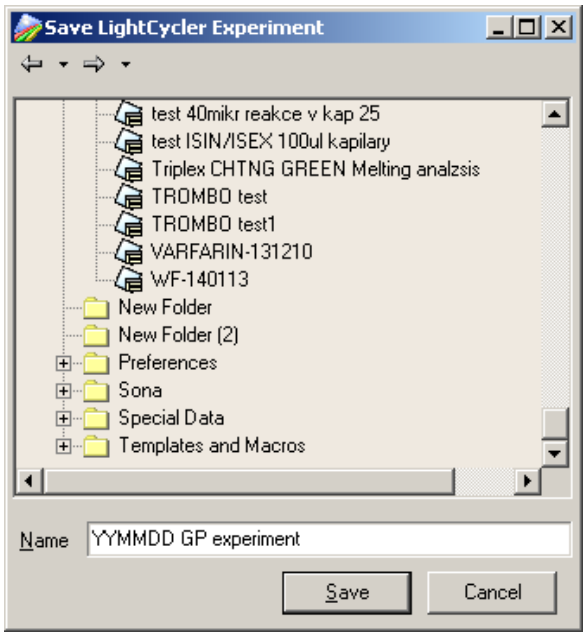

Fig. 4.6 Save Experiment

3. Switch back to **Run** in the left side of the main window.
4. Click **Start Run** in the **Run** box to start the experiment.

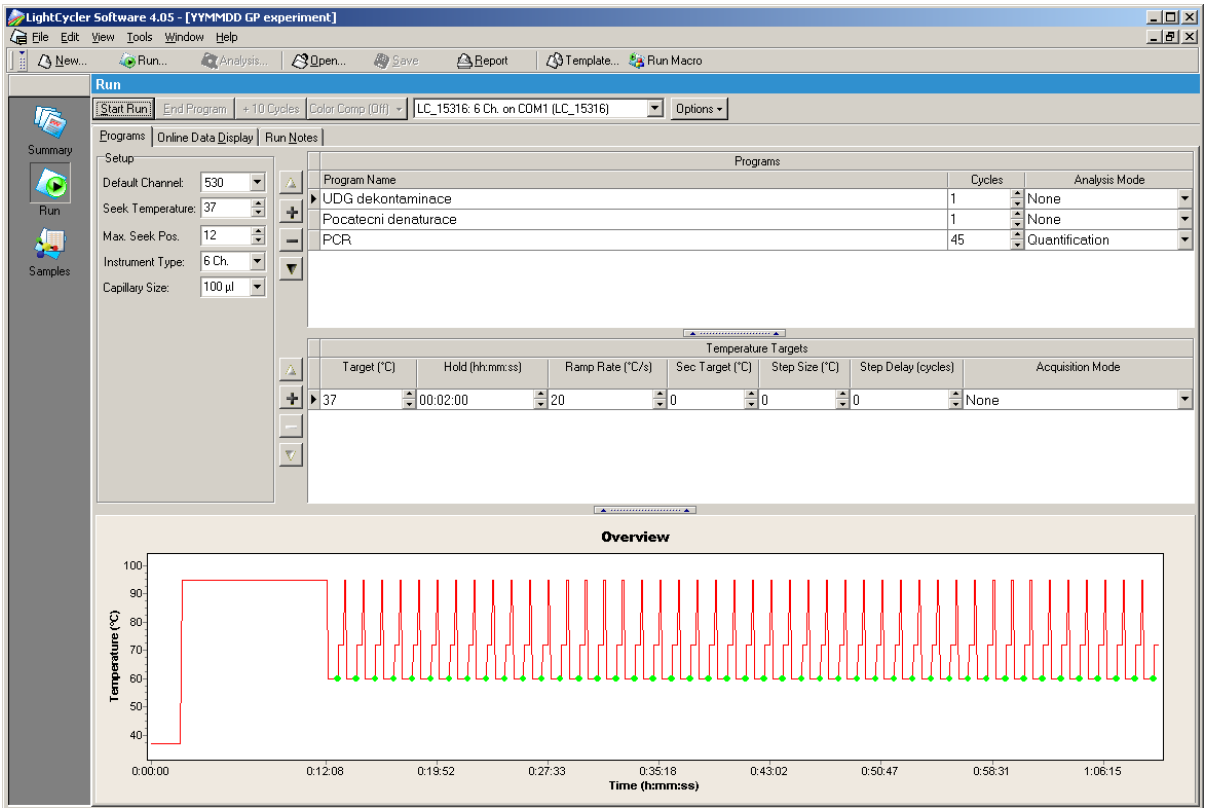

Fig. 4.7 Start Experiment

5. Result Analysis and Detection Evaluation

Run Complete will display in the upper part of the **Run** box after the experiment has been processed.

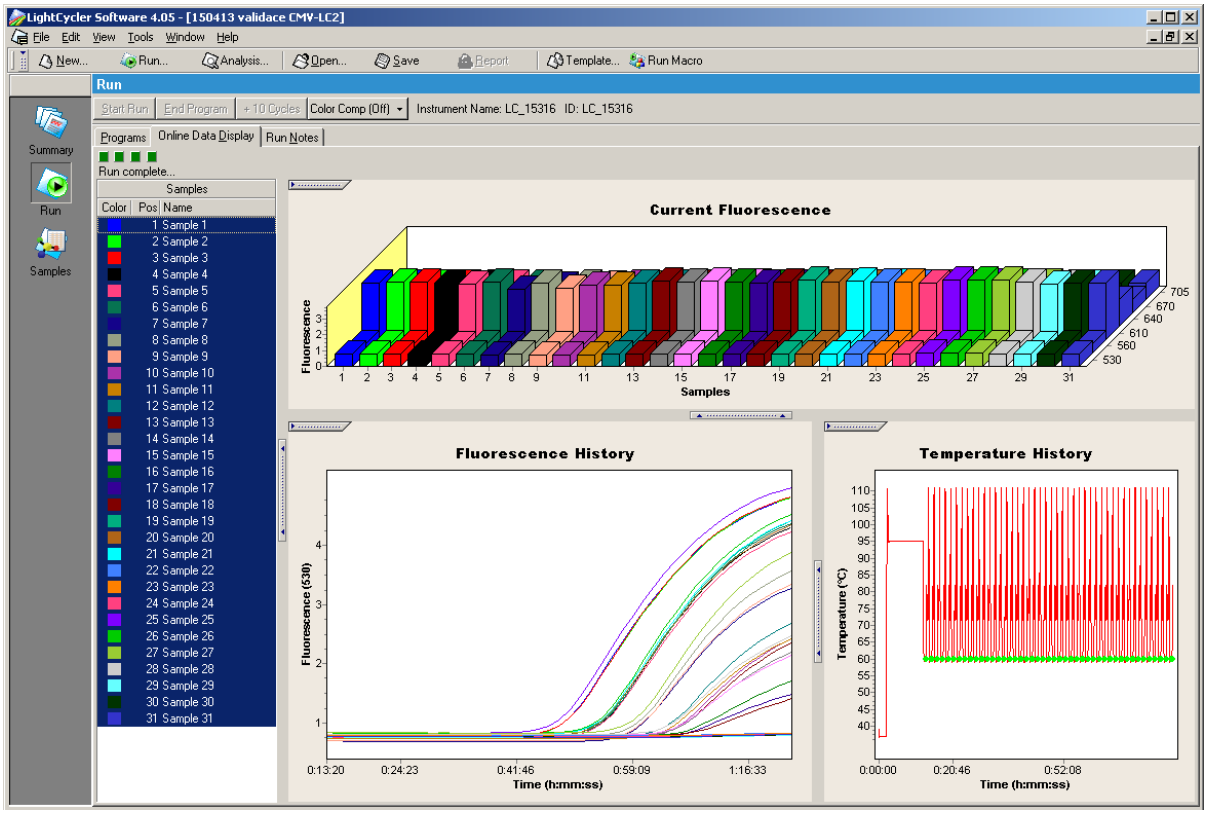

Fig. 5.1 Finished Experiment

5.1. Detection Analysis of the Studied Microorganism

1. Click Analysis... to open the **Create New Analysis** box.
2. Check **Absolute Quantification** and click **OK** to confirm.

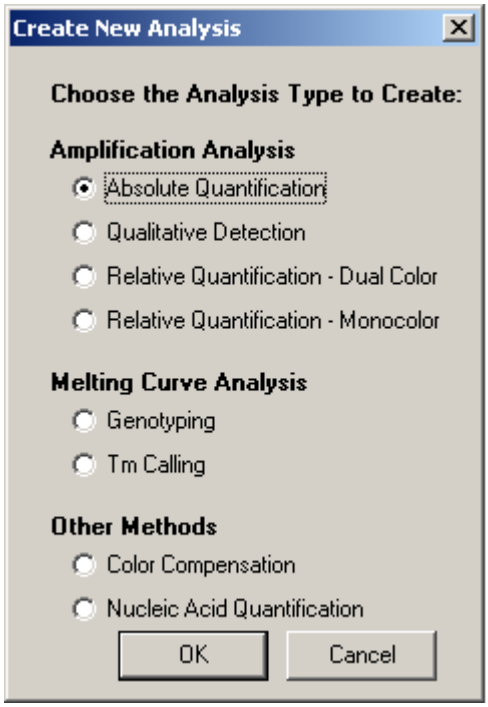

Fig. 5.2 New Analysis

3. Click **Channel** and select **530** in the **Absolute Quantification** box.

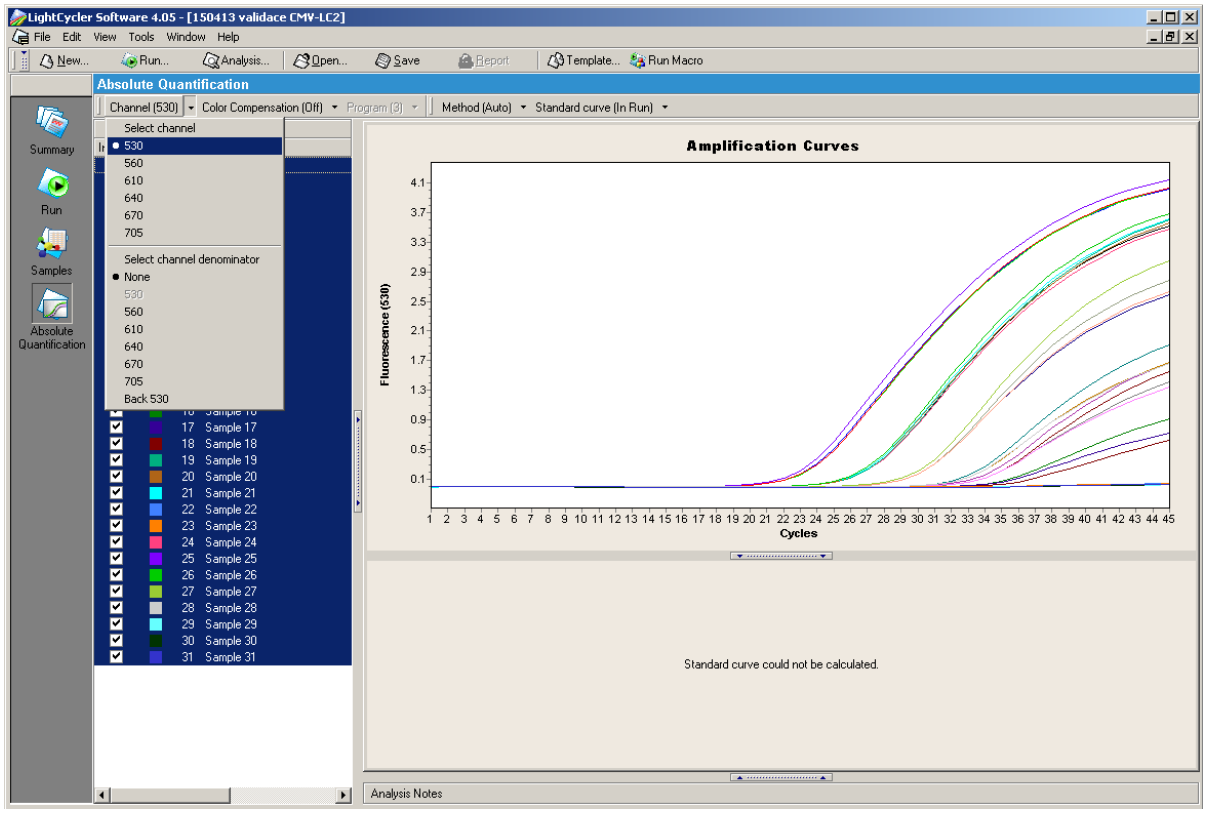

Fig. 5.3 FAM Channel Selection

4. Click **Color Compensation**; in the newly opened **Select Object** box select the appropriate color-compensation file and click **OK** to confirm.

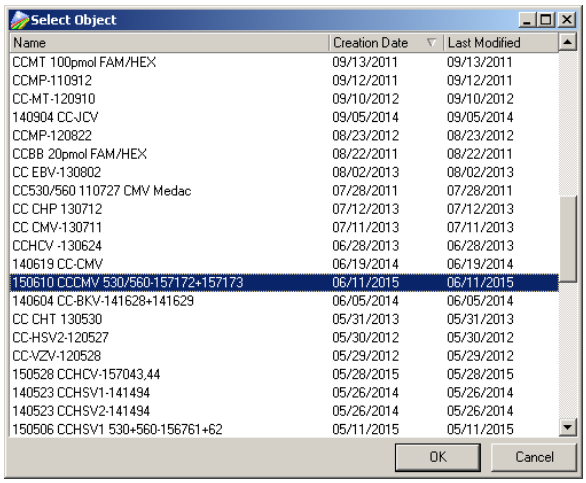

Fig. 5.4 Color-Compensation File Selection

5. Check **530** and **560** in the **Color Compensation Channels** box and click OK to confirm.

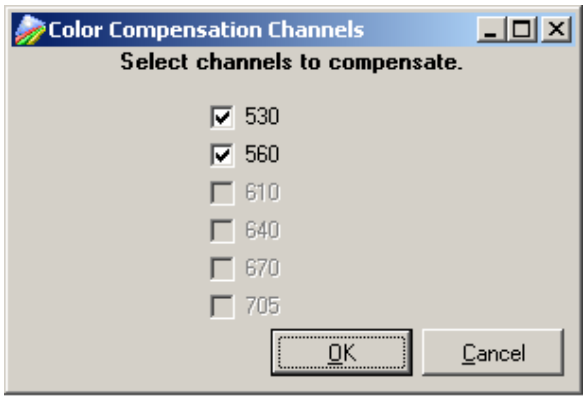

Fig. 5.5 Color-Compensation Channels Selection

The color-compensation will modify the curves.

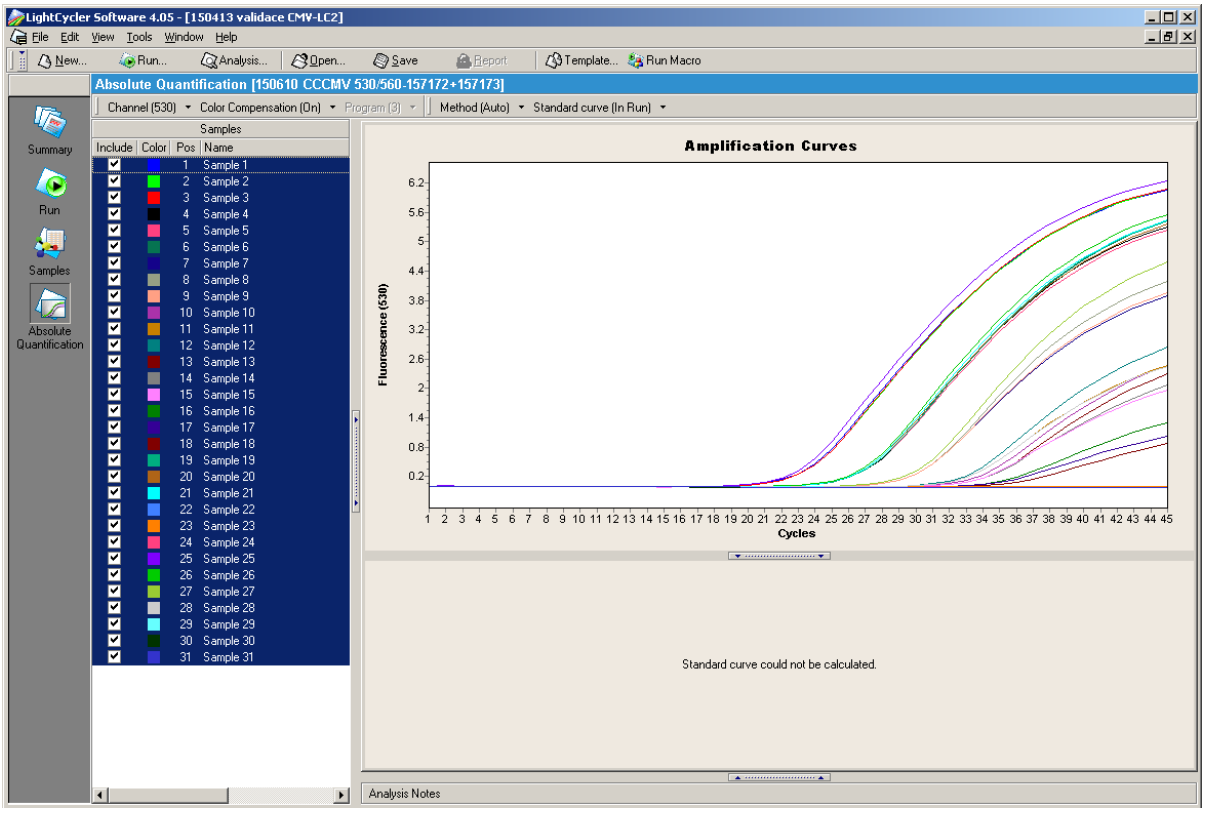

Fig. 5.6 Curves After Color-Compensation

6. Click **Method** and select **Fit Points**.

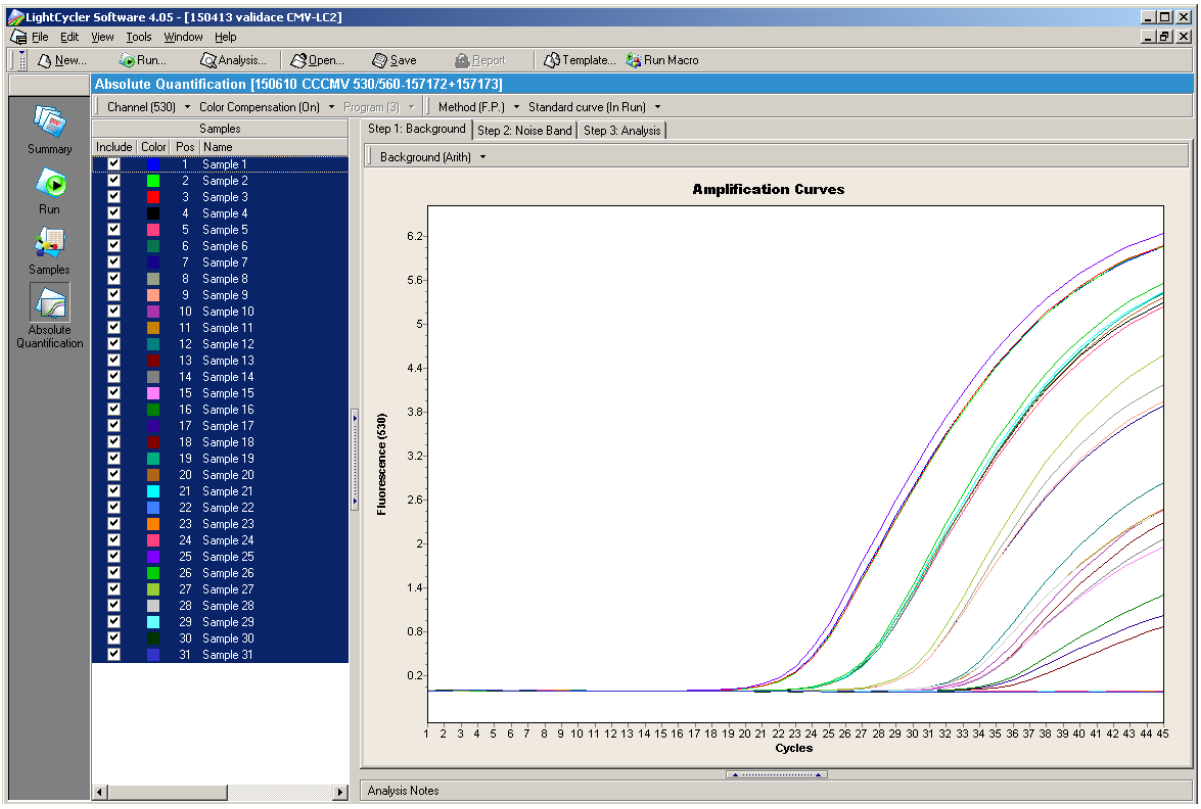

Fig. 5.7 Fit Points Method

7. Switch to the **Step2: Noise Band** tab and move the line above the reaction basal noise

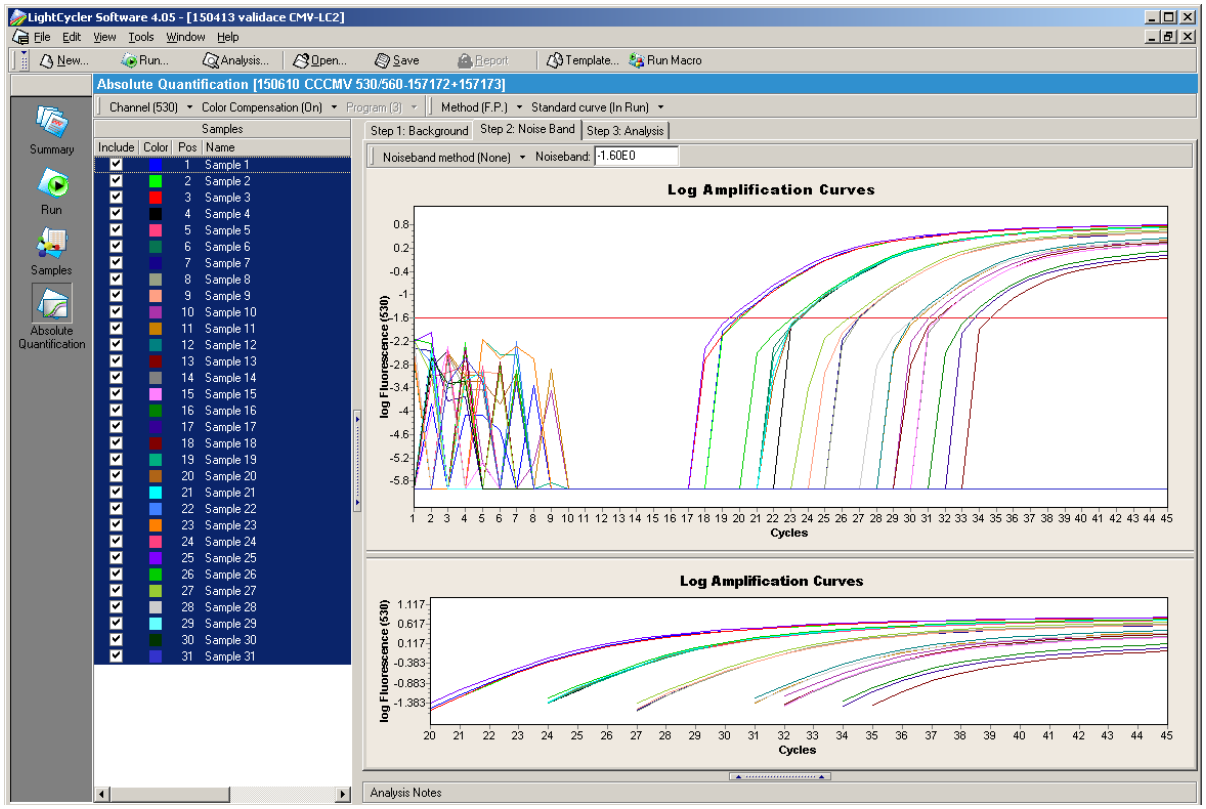

Fig. 5.8 Noise Band Setting

8. Switch to the **Step 3: Analysis** and move the **Threshold** line to intersect all the curves.

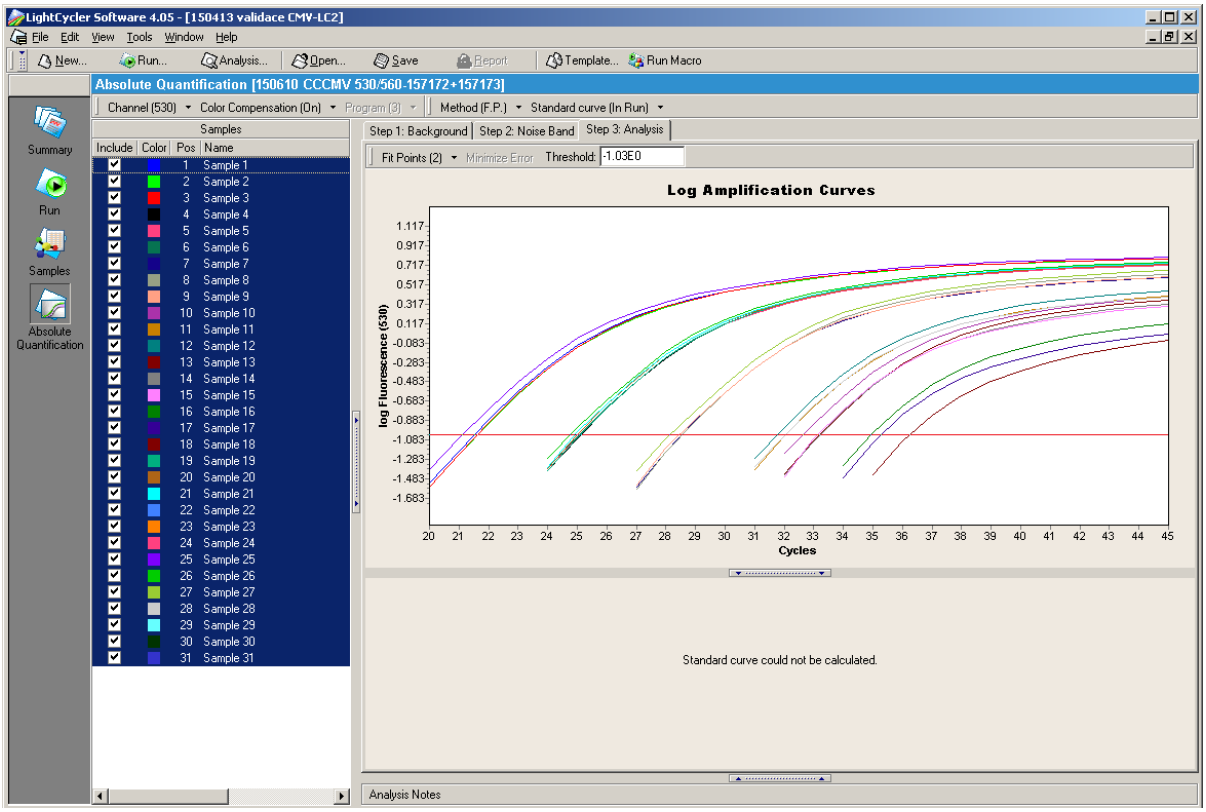

Fig. 5.9 Threshold Setting

9. Click the separator between the sample table and the chart to display the results. Samples with **CP** values displayed are positive.

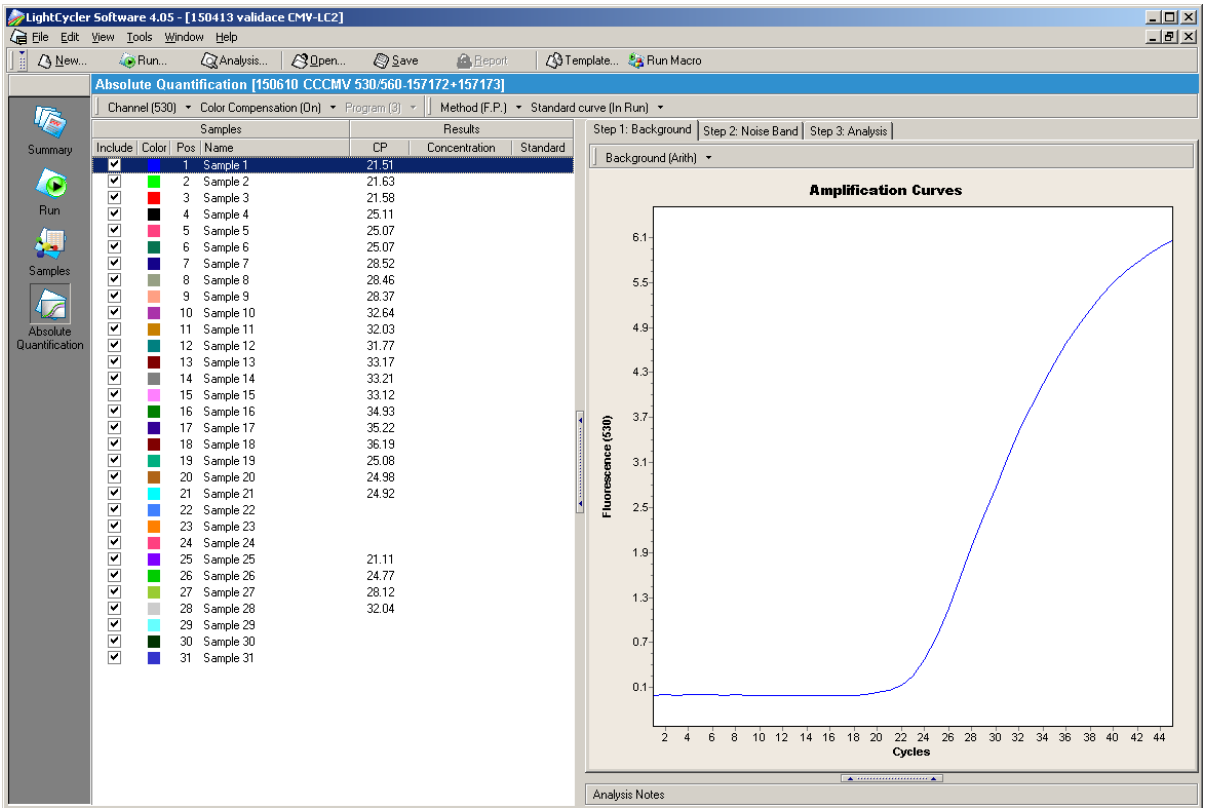

Fig. 5.10 Assessment

For quantitative analysis only

A standard curve is calculated in case of a quantitative examination.

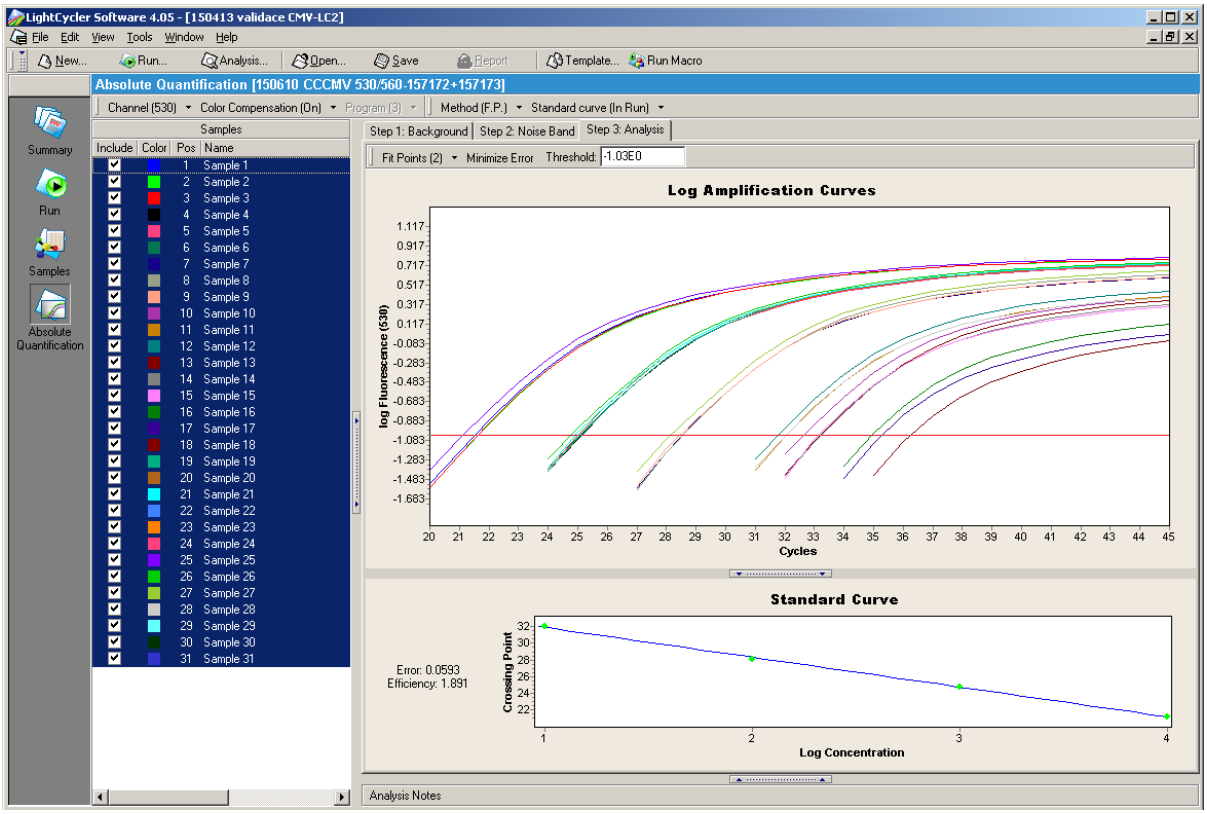

Fig. 5.11 Standard Curve

Click the separator between the sample table and the chart to display **CP** values and the resulting sample **concentrations**.

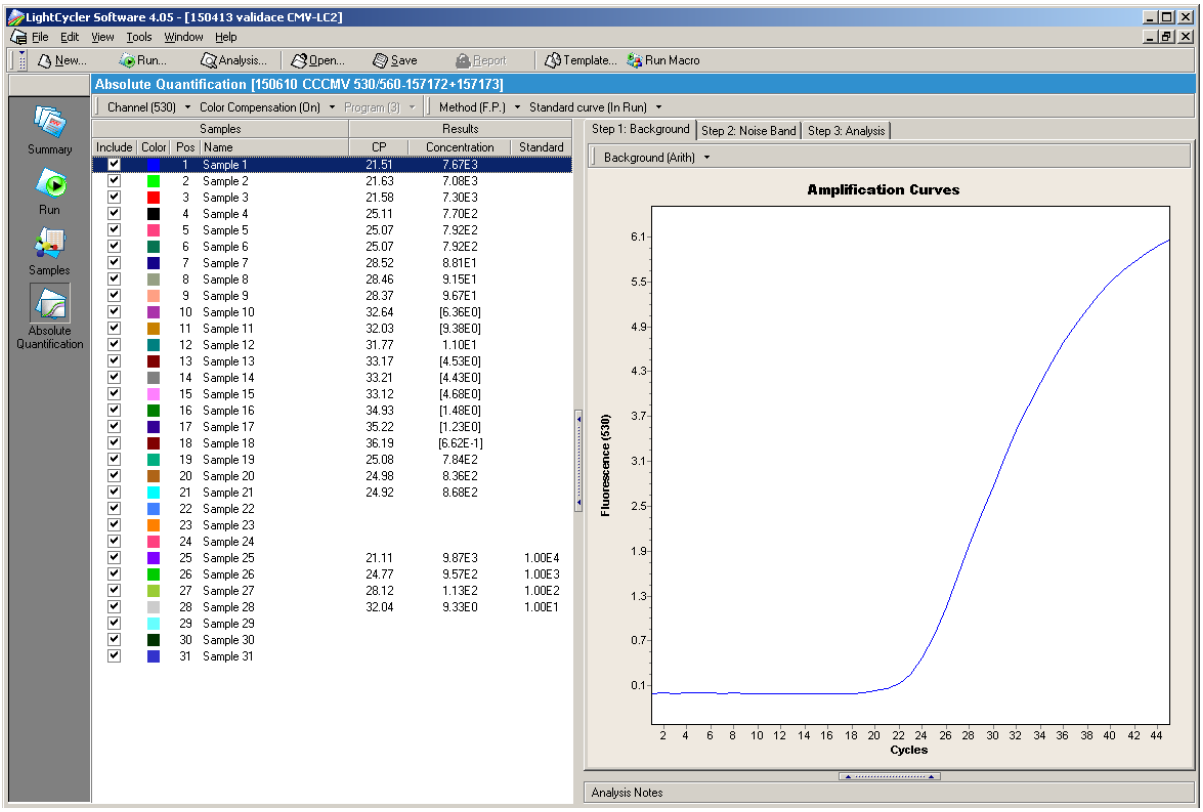

Fig. 5.12 Resulting Concentrations

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 **Channel** and select **560** in the **Absolute Quantification** box.

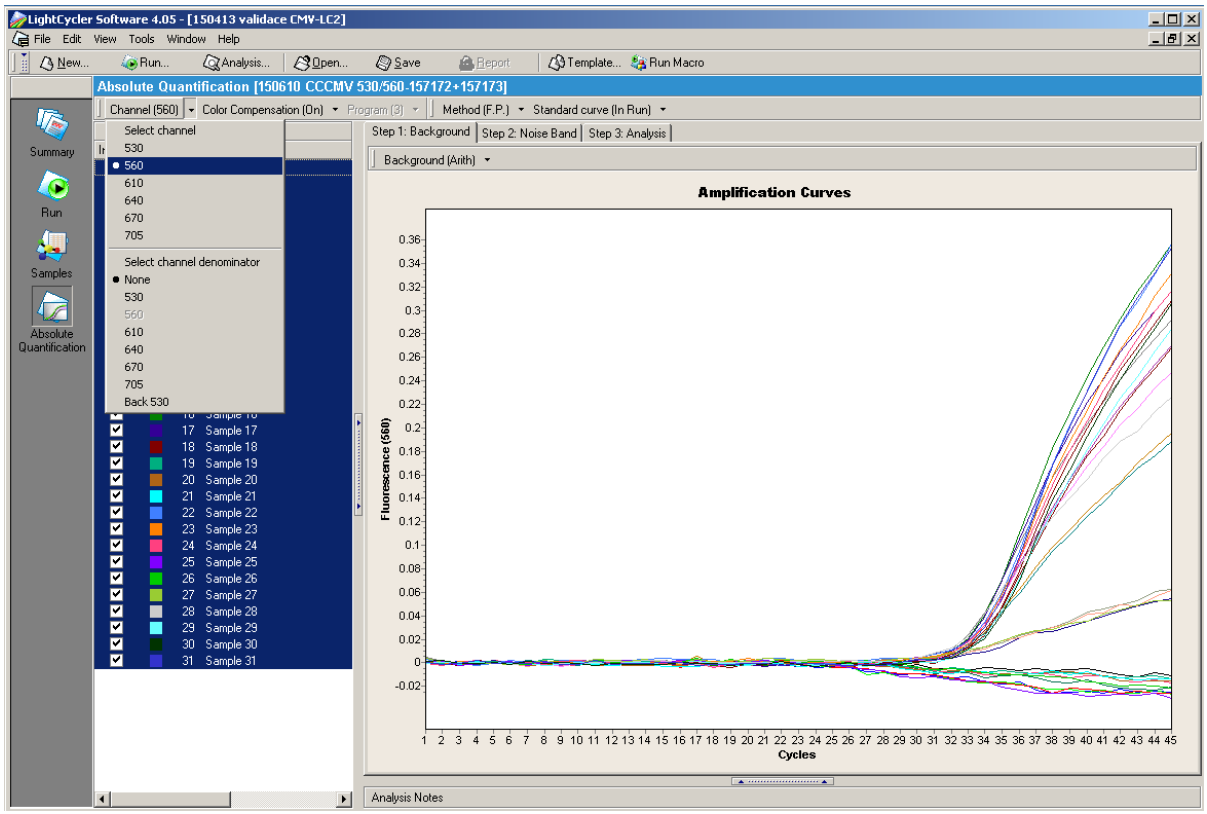

Fig. 5.13 HEX Channel Selection

2. In the **Step2: Noise Band** tab move the line above the reaction basal noise.
3. In the **Step 3: Analysis** tab move the **Threshold** line to intersect all the curves.

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

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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Orders

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