GeneProof
Molecular diagnostics for your routine

DEVICE MANUAL

Shanghai Hongshi Medical Technology

SLAN® Real-Time PCR System

Designed for GeneProof diagnostic kits

Microbiological DNA diagnostics

See www.geneproof.com for the current kits list

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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 device SLAN® Real-Time PCR System.

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. For next use click **Open** in the **Experiment Program** box and select the profile (see chapter **3.3 Amplification Profile Programming**).

3.1. Starting the Software

1. Start the software. Select **New** and in the **Create an experiment file** dialog choose **Create a new experiment file**. Click **Confirm** to continue.

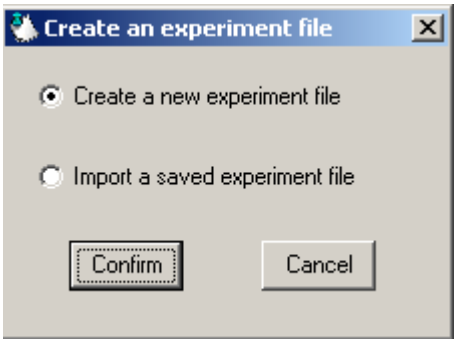


Fig. 3.1 Create New Experiment

2. In the **Select Channel** box check the following reading channels: **Channel 1: FAM, SYBR** and **Channel 2: HEX, VIC, JOE, TET** (for 3-channel detection kits select also **Channel 3: CY5**). Click **Confirm** to continue.

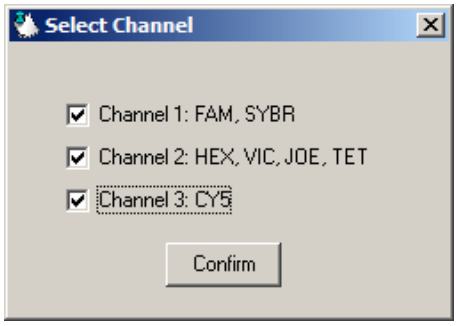


Fig. 3.2 Select Channels

3.2. Sample description

1. Select samples for detection in the **Module Edit** tab. Right-click and select **Template Editor(E)**. In the **Type** column in **channel 1** (for 3-channel kits also **channel 3**) select **Standard**, **Sample**, and/or **Negative**. In **channel 2** select **Control**. Select the type of the analyzed microorganism in the **Test Item** column.

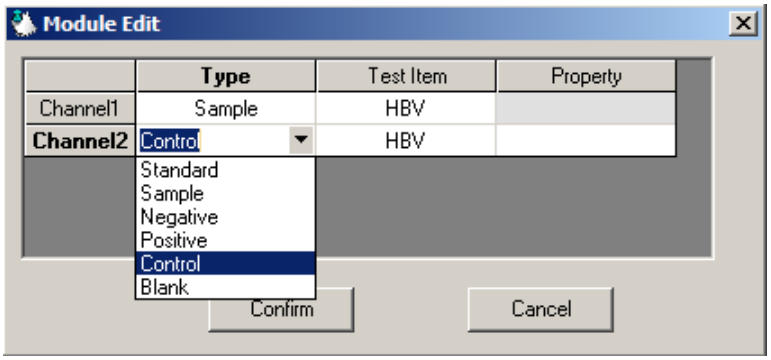


Fig. 3.3 Sample description

Use the **Set Fields** box to create and manage types. Select **Set Information Items(C)** in the main menu of the **Edit(E)** tab, near the **Information(P)** item, to open this box.

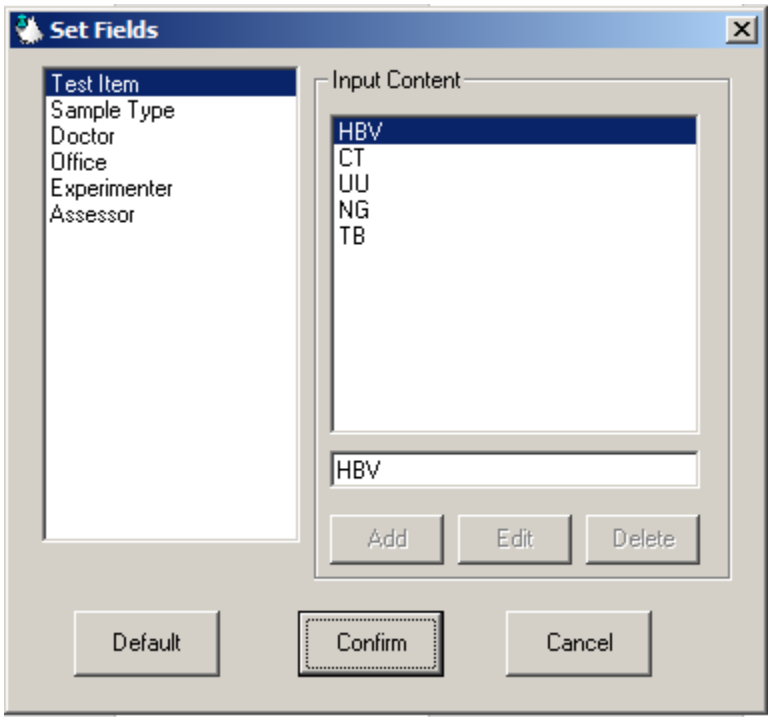


Fig. 3.4 Analyzed microorganism type management

2. Use the **Edit Patient Information** box to describe the samples specified in **Sample**. Select **Patient Information(X)** in the main menu of the **Edit(E)** tab, near the **Information(P)** item. In the **Name** column enter a description (e.g. the sample number), which will display next to the individual samples in the **Module Edit** tab. For additional information about patients and samples just open the **Edit Patient Information** box.

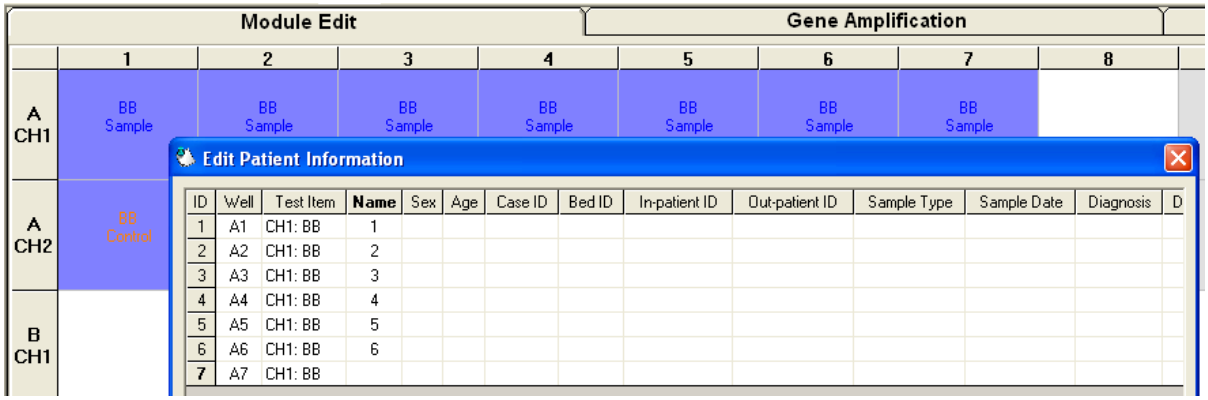


Fig. 3.5 Patient information

3.3. Amplification profile programming

1. Switch to the **Gene Amplification** tab and open **Settings(S)** in the main menu. **Hot Lid(H)** must be checked. Check **Tube Control** in the **Temperature Control(T)** menu item. **Solution Volume** box will open. Enter the reaction volume of **40 µl** here and then click **Confirm**.

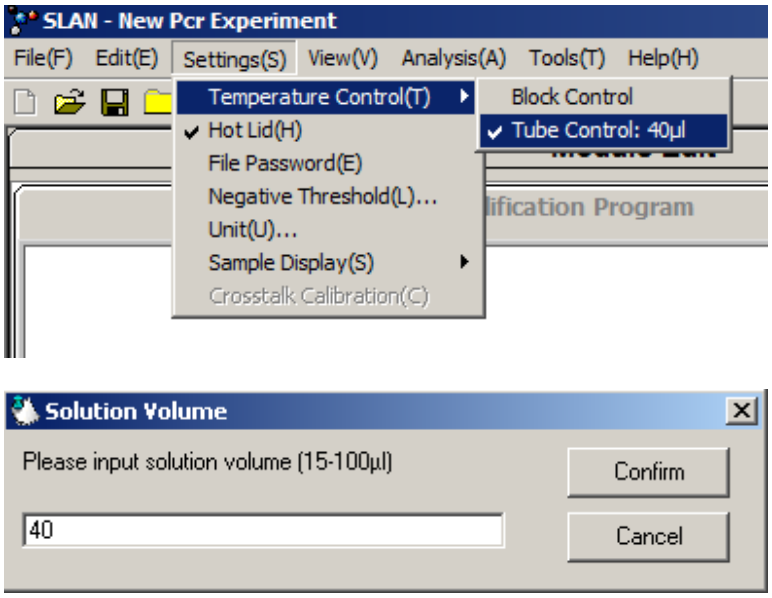


Fig. 3.6 Reaction volume

2. Click **New** in the **Experiment Program** box located in the right section of the program main panel. This will open the **Create a Pcr Program** dialog. Type in **GeneProof PCR DNA** as program name. Click **Confirm** to continue.

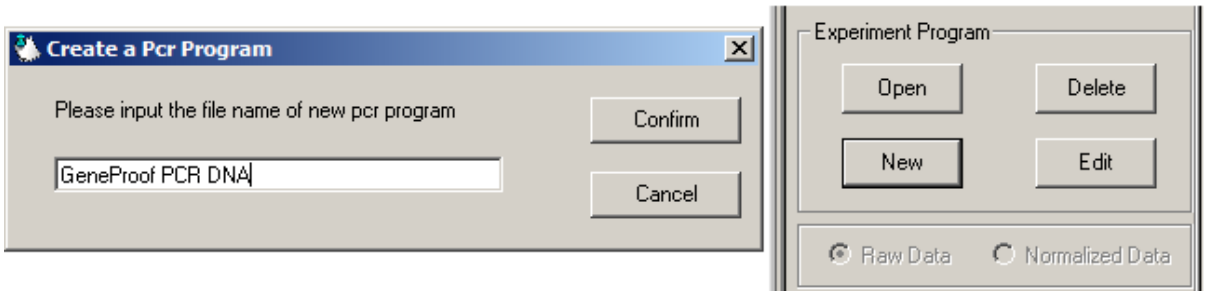


Fig. 3.7 New amplification profile creation

3. Program **Segment 1** of the PCR program:

Cycle times = 1

Step 1: Temperature = 37.0; Holding time = 02:00

Step 2: Temperature = 95.0; Holding time = 10:00

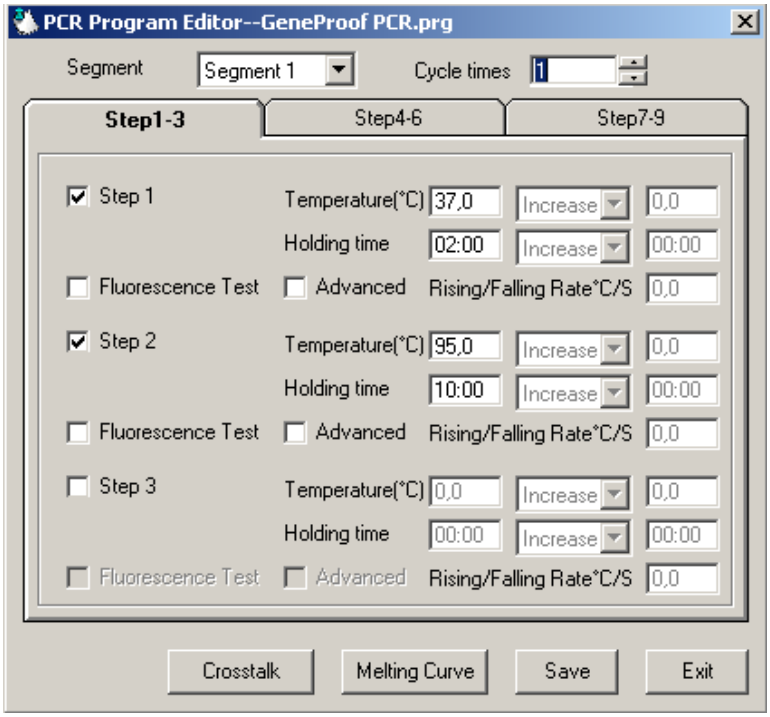


Fig. 3.8 Segment 1

4. Program **Segment 2** of the PCR:

Cycle times = 45

Step 1: Temperature = 95.0; Holding time = 00:05

Step 2: **Temperature = 60.0; Holding time = 00:40**; check the **Fluorescence Test**

Step 3: Temperature = 72.0; Holding time = 00:20

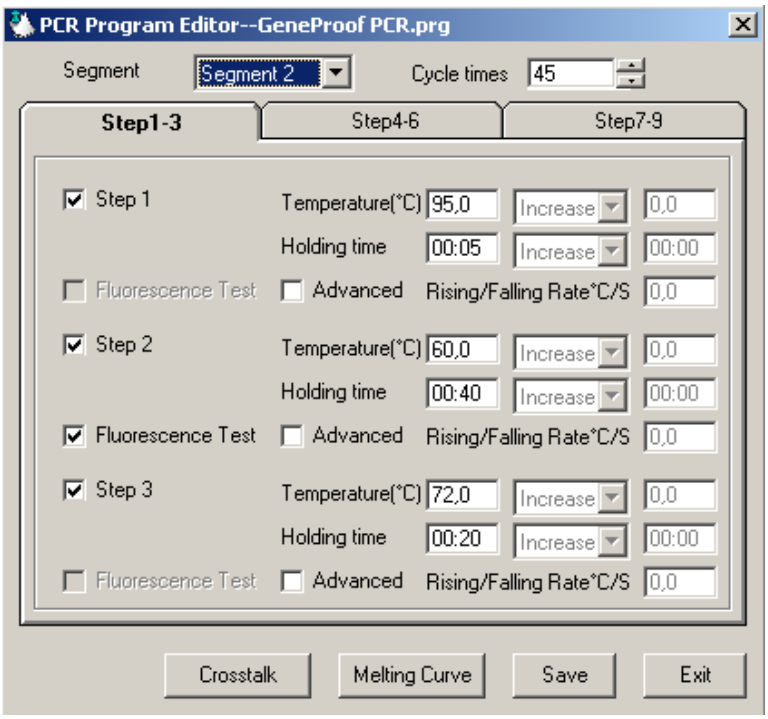


Fig. 3.9 Segment 2

5. Click **Save** and **Exit**.

3.4. Starting the experiment

1. Click **Open** to open the newly created PCR program in the **Experiment Program** box of the **Gene Amplification** tab. Select **GeneProof PCR DNA** in the menu and click **Open**. The created PCR program will open, graphic representation of the PCR profile will display and the **Start** button will activate.

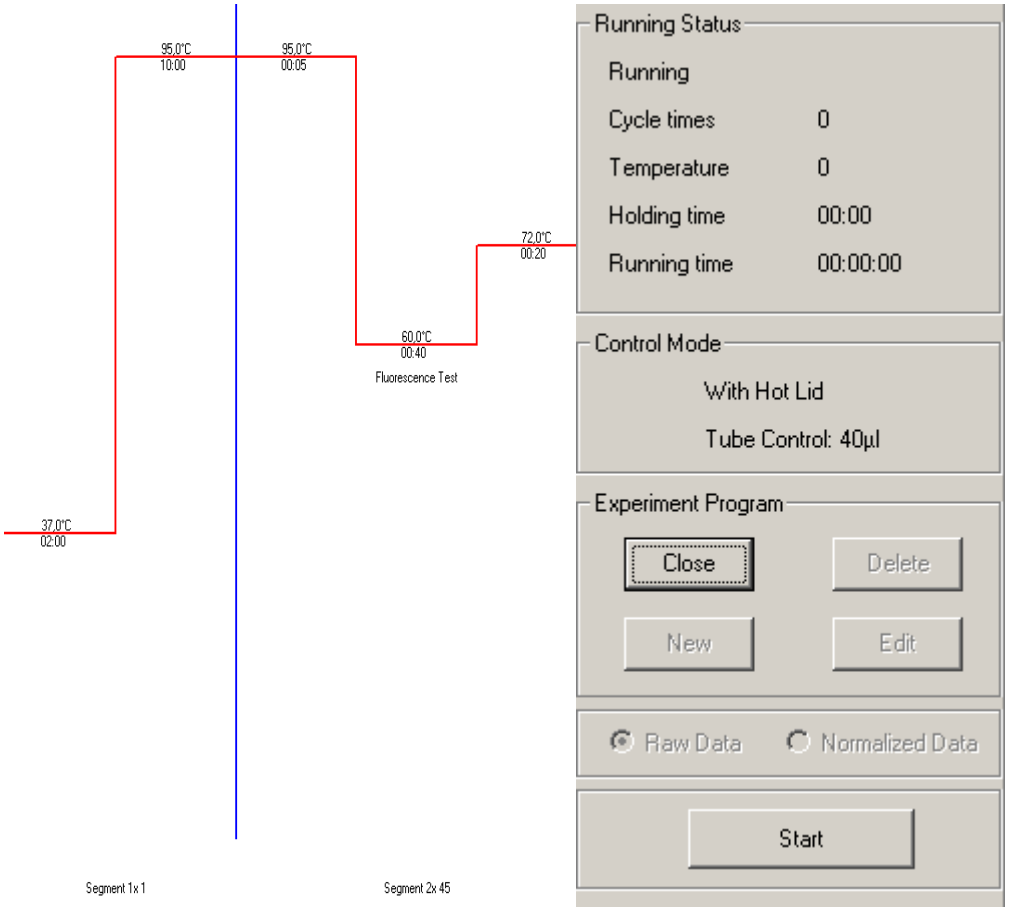


Fig. 3.10 Opening of the created PCR program

2. Click **File(F)** and **Save as(A)** to save the created experiment under a designation used by your laboratory.

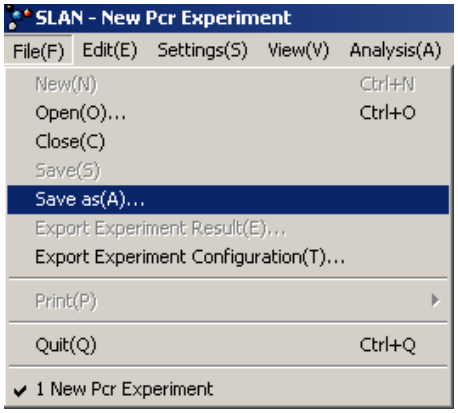


Fig. 3.11 Save experiment

3. Click **Start** and confirm in the **Hint** box to start the experiment.

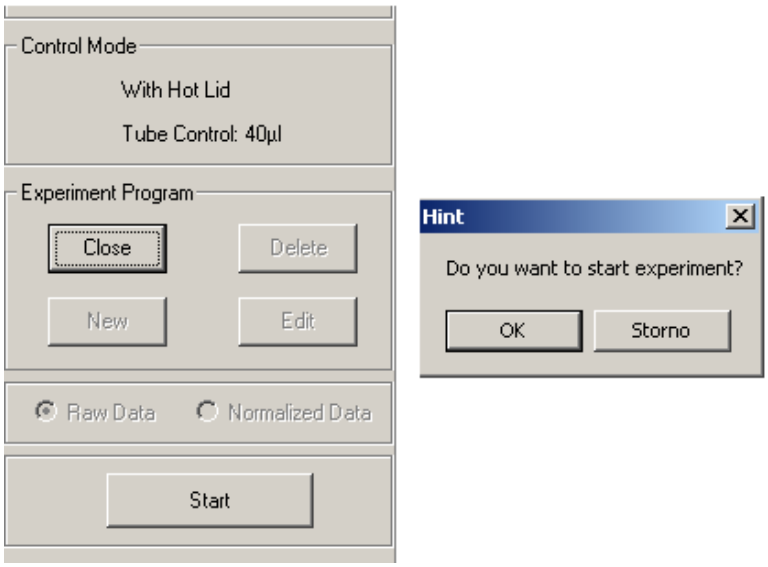


Fig. 3.12 Start experiment

After the experiment has finished, it is necessary to click the button in the upper bar to open the **Hot Lid** and provide access to the tubes inside the device.

4. Result qualitative analysis and detection evaluation

PCR detection results evaluation must be **always** performed qualitatively first; if you use the PCR kit for quantitative assessment, continue to quantify positive samples in the second step.

1. Select values for the detection qualitative analysis in the **Module Edit** box. **Positive Control** and **Negative Control** are the minimum required samples for a valid assessment of a PCR reaction.

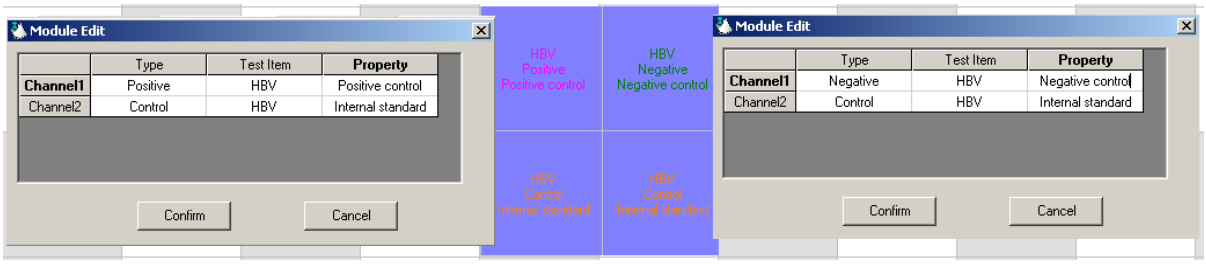


Fig. 4.1 Sample description during qualitative detection

2. Switch to the **Test Results** box and select **Amplification Curve** tab. Select **Settings(S)** and **Negative Threshold(L)**. Enter **1** into the **Quantitative Threshold** column and **45** into the **Qualitative Threshold** column. Click **Confirm**to continue.

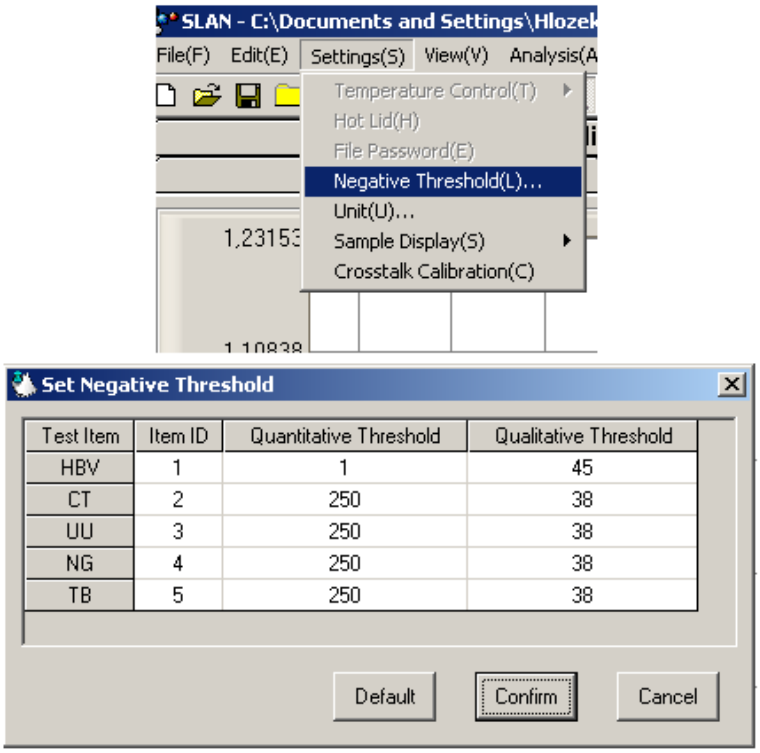


Fig. 4.2 Negative threshold settings for qualitative detection

3. Select **Parameter Setup(S)** in the main menu of the **Analysis(A)** tab. In both channels **Channel 1 and Channel 2** (for 3-channel detection kits also **Channel 3**) for qualitative detection analysis select **Analysis Type Qualitative** and set the **Manual Threshold** above the reaction basal noise. Select **Manual Optimization** and use **Digital Filter**. Click **Apply** and **Confirm** to apply the settings for both detection channels.

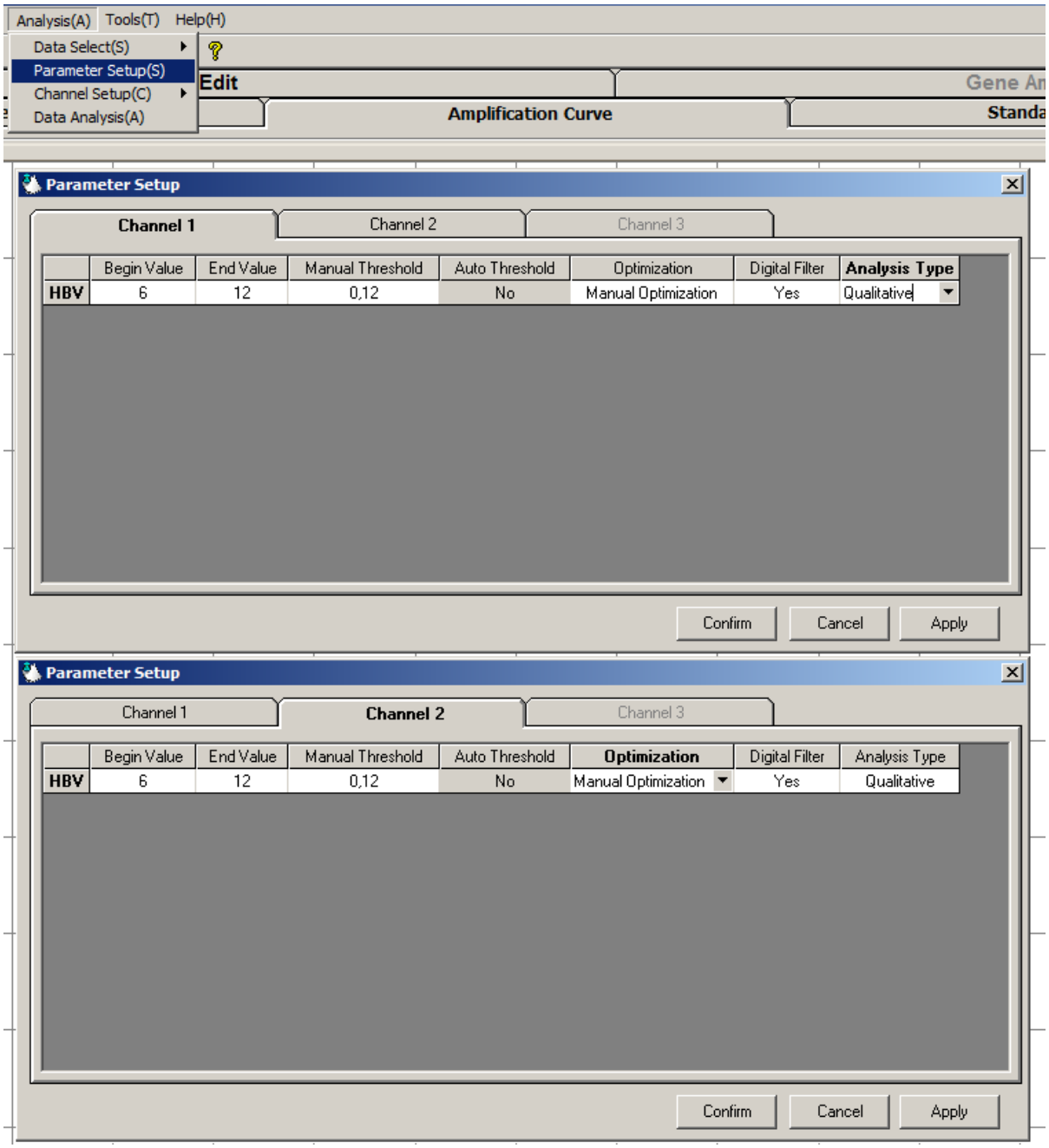


Fig. 4.3 Parameter Setup Settings

4. Select **Data Analysis(A)** in the main menu of the **Analysis(A)** tab and click **Confirm** to confirm the results evaluation for the selected detection channels. Use **Channel Setup(C)** in the **Analysis(A)** tab to select the channels.

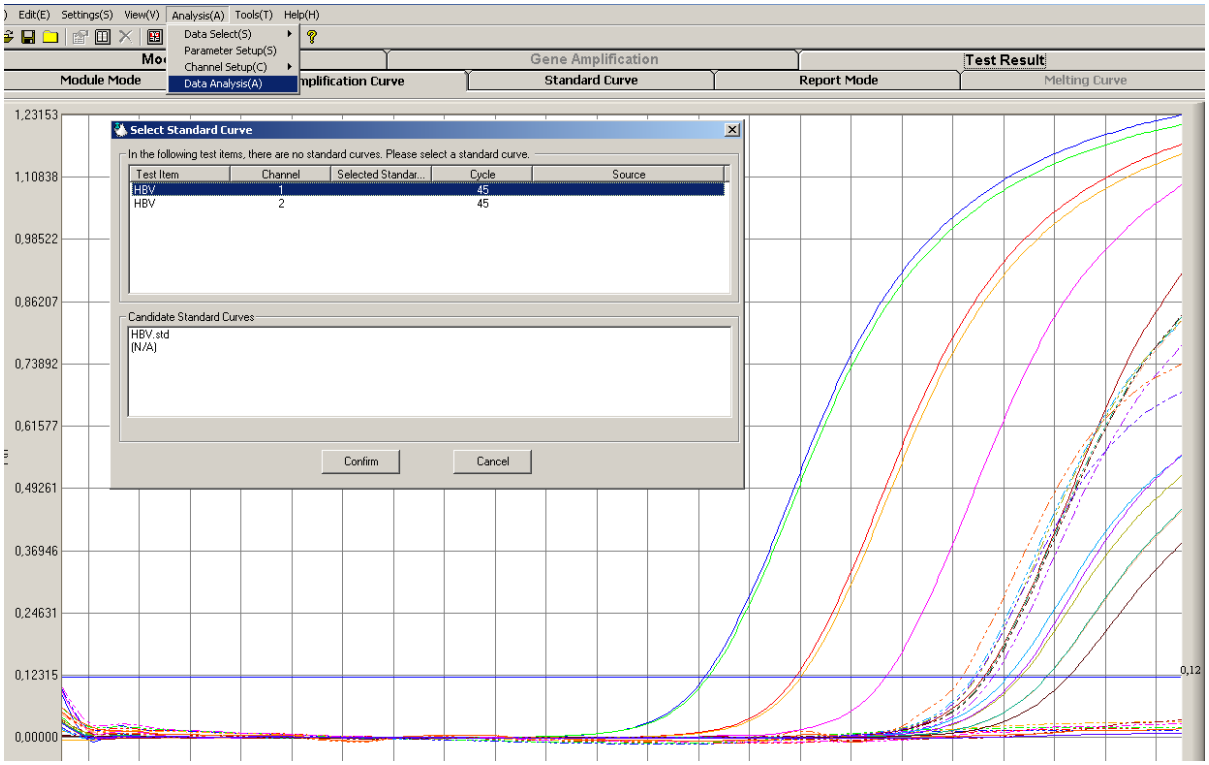
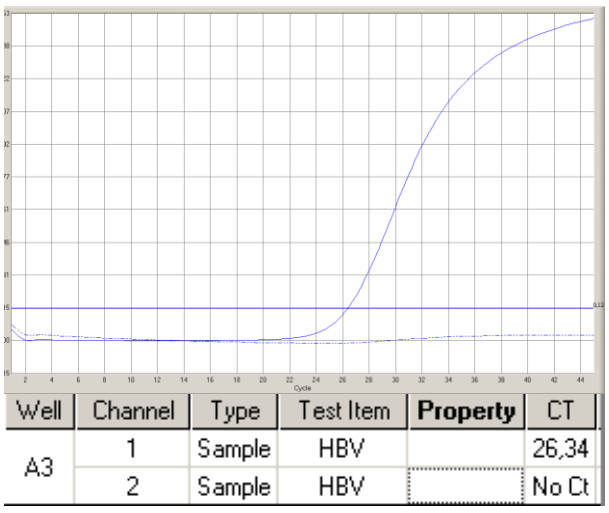


Fig. 4.4 Starting the qualitative detection results analysis

- **Positive sample amplification**

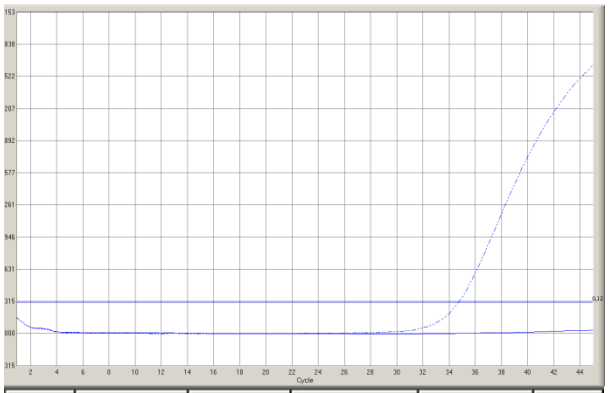
  In the graphics window for **Channel 1** (for 3-channel detection kits also **Channel 3**) you can see the rising curve intersecting the manually preset threshold; the **Ct** numeric value is assigned to this curve in the evaluation table (**Report Mode** tab).



Well	Channel	Type	Test Item	Property	CT
A3	1	Sample	HBV		26,34
	2	Sample	HBV		No Ct

Fig. 4.5 Positive sample

- **Internal Standard Amplification**
  In the graphic display for **Channel 2** you can see an ascending curve intersecting the manually set threshold. A numeric value **Ct** is assigned to this curve in the assessment table (**Report Mode** tab).



Well	Channel	Type	Test Item	Property	CT
B3	1	Negative	HBV	TE	No Ct
	2	Control	HBV		34,69

Fig. 4.6 Internal standard

Perform evaluation according to the leaflet of the used GeneProof PCR kit.

5. Result quantitative analysis and detection evaluation

1. Select values for the detection quantitative analysis in the **Module Edit** box. **Calibration Control** and **Negative Control** are the minimum required samples for a valid quantitative assessment of a PCR reaction. Select **Standard** in the **Type** column for each calibration control in the reaction (**Channel 1**, possibly **Channel 3**) and then select a **concentration** value in relevant units/µl in the **Property** column. Select **Negative** in the **Type** column for the negative control (**Channel 1**, possibly **Channel 3**) and then select a type of the negative sample in the **Property** column (sterile water, TE buffer. etc.). **Channel 2** is reserved for the Internal Standard amplification detection. Click **Confirm** to confirm the selected parameters.

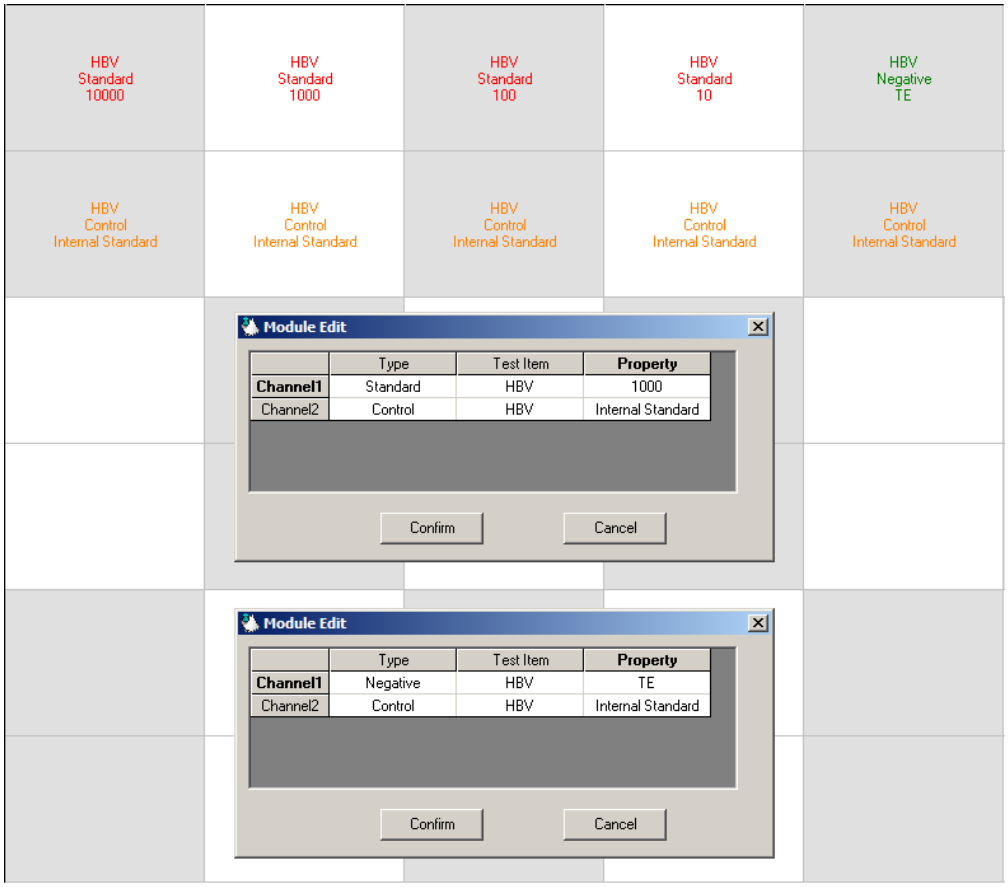


Fig. 5.1 Sample description during quantitative detection

2. Switch to the **Test Results** box and select **Amplification Curve** tab. Select **Settings(S)** and **Negative Threshold(L)**. Enter **1** into the **Quantitative Threshold** column and **45** into the **Qualitative Threshold** column. Click **Confirm**to continue.

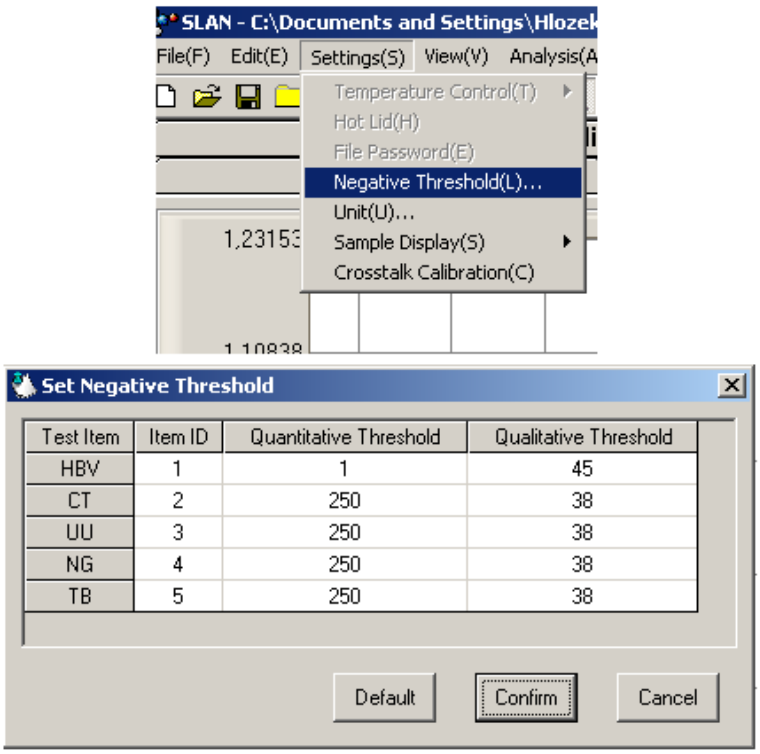


Fig. 5.2 Negative threshold settings for quantitative detection

3. Select **Parameter Setup(S)** in the main menu of the **Analysis(A)** tab. In both channels **Channel 1 and Channel 2** (for 3-channel detection kits also **Channel 3**) for quantitative detection analysis select **Analysis Type Quantitative** and set the **Manual Threshold** above the reaction basal noise. Select **Manual Optimization** and use **Digital Filter**. Click **Apply** and **Confirm** to apply the settings for both detection channels.

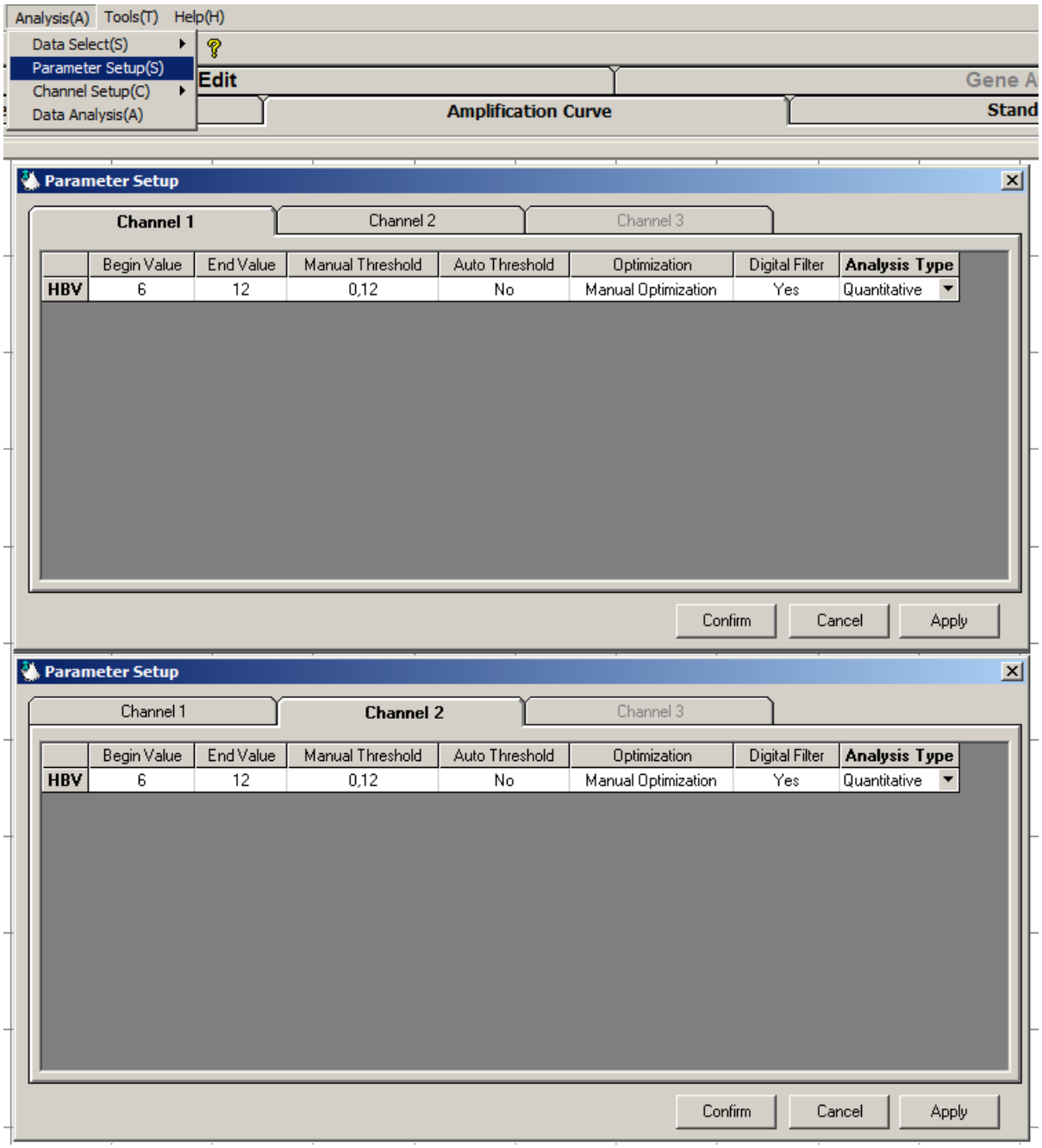


Fig. 5.3 Parameter Setup settings

4. Select **Data Analysis(A)** in the main menu of the **Analysis(A)** tab and click **Confirm** to confirm the results evaluation for the selected detection channels. Use **Channel Setup(C)** in the **Analysis(A)** tab to select the channels.

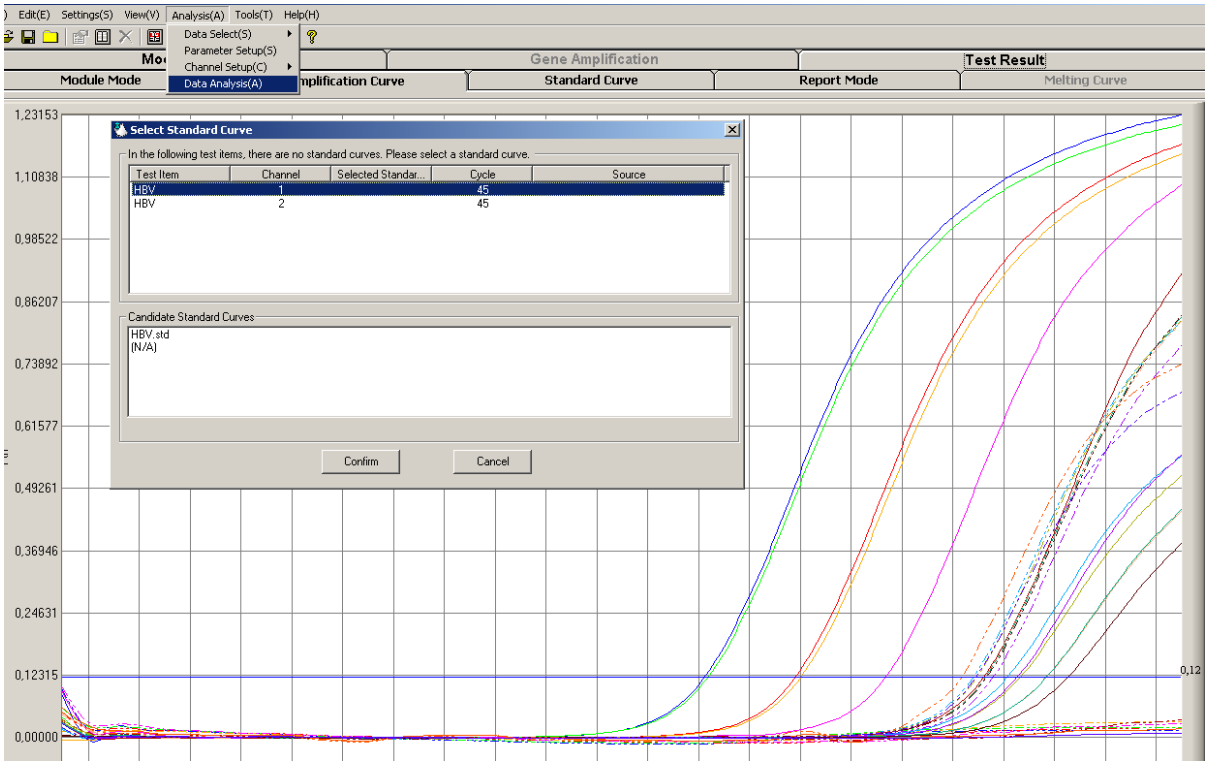


Fig. 5.4 Starting the quantitative detection results analysis

5. Verify the correlation coefficient of the calibration series (**R-Value**) in the **Standard Curve** tab. The correlation coefficient must be |0.9899| or higher. After reading the measured values click the **Report Mode** tab to display the numerical result of the unknown sample detection in the **Test Result** column.

Slope:	-3,55652
Intercept:	37,64050
R-Value:	-0,99991

Well	Channel	Type	Test Item	Property	CT	Test Result	Average	CV
A1	1	Sample	HBV		26,75	1,155E+03		10,54
	2	Control	HBV	Internal standard	No Ct	<1	8,247E-01	
A2	1	Sample	HBV		30,32	1,143E+02		10,54
	2	Control	HBV	Internal standard	No Ct	<1	8,247E-01	

Fig. 5.5 Assessment of the calibration curve quality and of the detection quantitative result

Perform evaluation, including the virus concentration calculation in copies/ml according to the leaflet of the used GeneProof PCR kit.

6. Customer Service

We appreciate all our customers and besides high-quality products we provided above-standard customer service including the following:

- Provision of free demonstration PCR kits
- 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. Contacts

Support and customer care

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Orders

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