

GeneProof®

PCR Kit

This manual is designed for the following kits:

Mycobacterium tuberculosis PCR kit, *Borrelia burgdorferi* PCR kit, *Chlamydia trachomatis* PCR kit, *Chlamydia pneumoniae* PCR kit, *Neisseria gonorrhoeae* PCR kit, *Cytomegalovirus* (CMV) PCR kit, *Epstein-Barr virus* (EBV) PCR kit, *Herpes Simplex virus* (HSV) PCR kit, *Varicella Zoster virus* (VZV) PCR kit, *Hepatitis B virus* (HBV) PCR kit and *Legionella pneumophila* PCR kit

CE
in vitro Diagnostics

Manuals for use with the following device

SLAN®
Real-Time PCR Detection System

VERSION K1-02-AJ

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GeneProof PCR kit

GeneProof PCR kits, designed for the detection and quantification of pathogen DNA, are based on the principle of amplifying specific target sequences of microorganisms and measuring the amplification product concentration growth in the course of the polymerase chain reaction by means of fluorescence-marked probes (the probe designated for pathogen detection is marked by the FAM or Cy5 fluorophore). The reaction mix includes an Internal Standard (IS) controlling the possible inhibition of the PCR reaction and the efficiency of DNA isolation process (the probe designed for IS detection is marked by the HEX fluorophore). The detection kit takes an advantage of the “hot start” technology, minimizing non-specific reactions and assuring maximum sensitivity and contains the uracil-DNA-glycosylase (UDG) controlling possible contamination of the PCR reaction by amplification products.

GeneProof PCR kits

- Use the “hot start” technology, minimizing non-specific reactions and assuring maximum sensitivity.
- Contain uracil-DNA-glycosylase (UDG), controlling possible contamination of the PCR reaction by amplification products.
- All PCR kits for pathogen DNA detection can be amplified by means of a universal amplification program.
- Are easy to use; the kits always contain one tube with MasterMix and one tube with Positive Control (or with an Internal Standard) or a set of calibration controls.
- Are designed for *in vitro* diagnostics (CE IVD certification).

ISIN and ISEX versions of the GeneProof PCR Kit

All GeneProof PCR kits include an Internal Standard providing for an effective monitoring of eventual inhibition of the PCR amplification and also of the isolation process efficiency. The Internal Standard is a precisely defined and quantified construct of a plasmid and insert, prepared by genetic engineering methods. **GeneProof develops and sells two basic versions of PCR kits with various compositions of the Internal Standard:**

PCR kit ISIN (Cat. No. *PCR kit/ISIN/*)

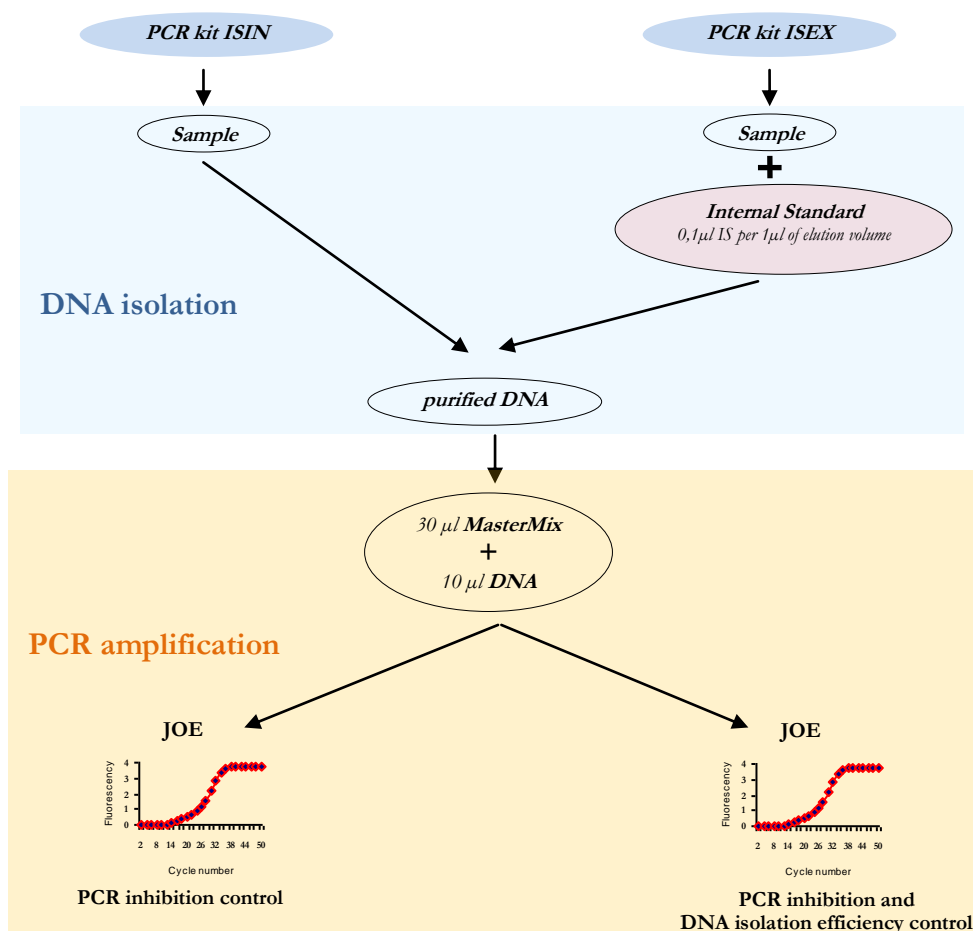
In this version of the PCR kit the Internal Standard is included in the MasterMix tube. This PCR kit version enables PCR inhibition control.

PCR kit ISEX (Cat. No. *PCR kit/ISEX/*)

In this PCR kit version the Internal Standard is included as an independent item within the package. This PCR kit enables both, PCR inhibition control and DNA isolation process efficiency control.

The Internal Standard should be added into the sample at the beginning of the isolation process as follows: 0.1 μ l of the Internal Standard per 1 μ l of elution volume:

Elution Volume	25 μ l	50 μ l	100 μ l	200 μ l
Internal Standard	2.5 μ l	5 μ l	10 μ l	20 μ l



User Manual

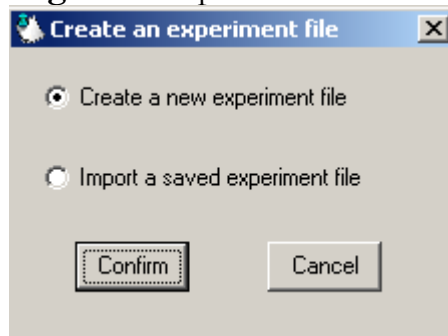
Reaction preparation

1. Add **30 µl** of the **MasterMix** and **10 µl** of the **DNA isolate** or **10 µl** of the **Positive Control** into a tube. The final reaction mix volume should be 40 µl.
2. Close the tubes, centrifuge them shortly and insert them into the device (*empty tubes must be inserted in the corners of the device block to assure correct adhesion of the heated lid*)

Device programming

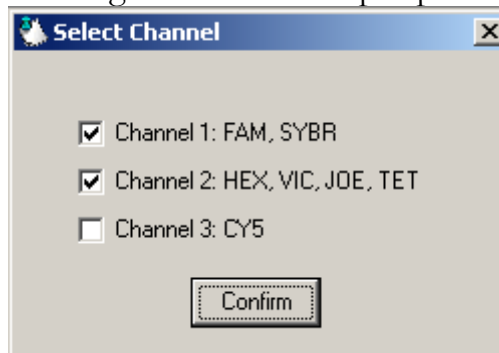
1. Start the software. Select **New** and then select **Create a new experiment file** in the **Create an experiment file** dialog. Click **Confirm** to continue (Fig. 1).

Fig. 1. PCR protocol creation.



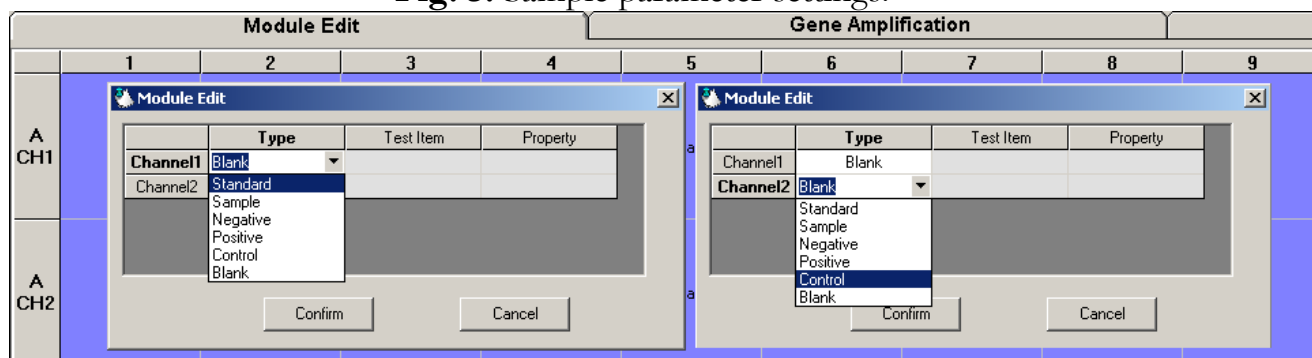
2. In the **Select Channel** box check the following reading channels: **Channel 1: FAM, SYBR** and **Channel 2: HEX, VIC, JOE, TET** Click **Confirm** to continue (Fig. 2).

Fig. 2. Setting of the device input parameters:



3. Select positions and a number of samples for detection in the **Module Edit** tab. Set the sample type (Standard, Sample and/or Negative) in **Channel1** for each position. In **Channel2** set a sample type as **Control** for each position. Channel 2 is used for Internal Standard detection (Fig. 3).

Fig. 3. Sample parameter settings.

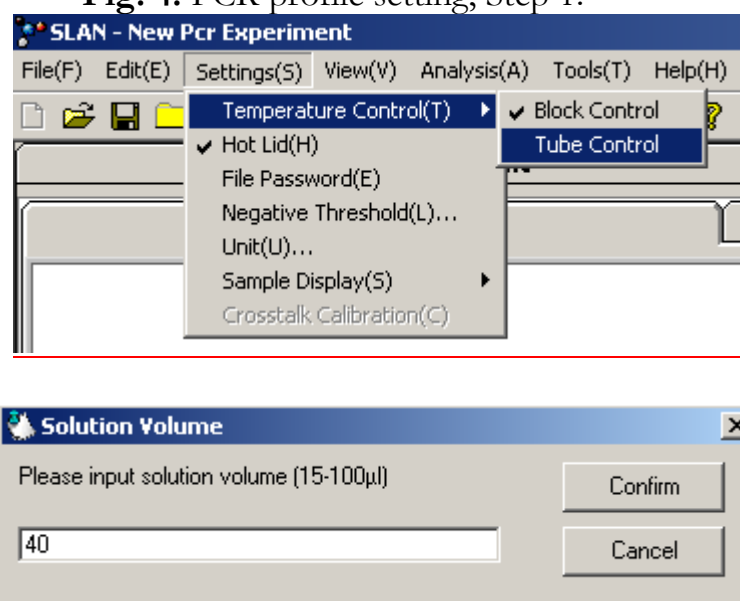


4. **PCR detection profile creation.**

Step 1:

Switch to the **Gene Amplification** tab and open **Settings(S)** in the upper menu. 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. 4.).

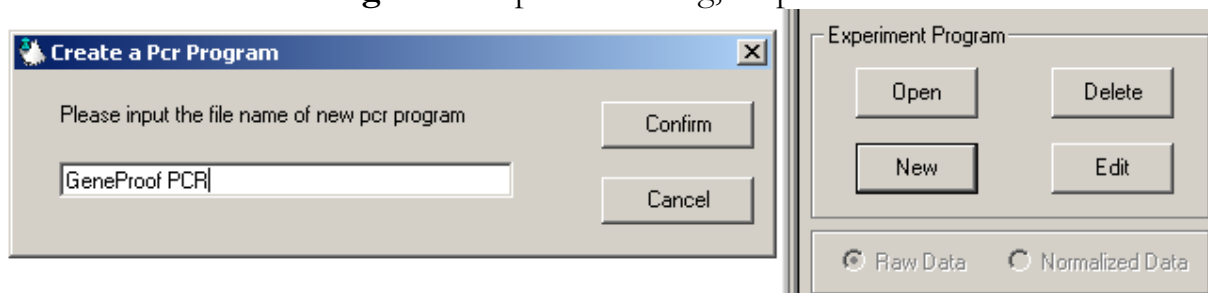
Fig. 4. PCR profile setting, Step 1.



Step 2:

Click **New** in the **Experiment Program** box. This will open the **Create a Pcr Program** dialog. Type in **GeneProof PCR** as program name. Click **Confirm** to continue (Fig. 5).

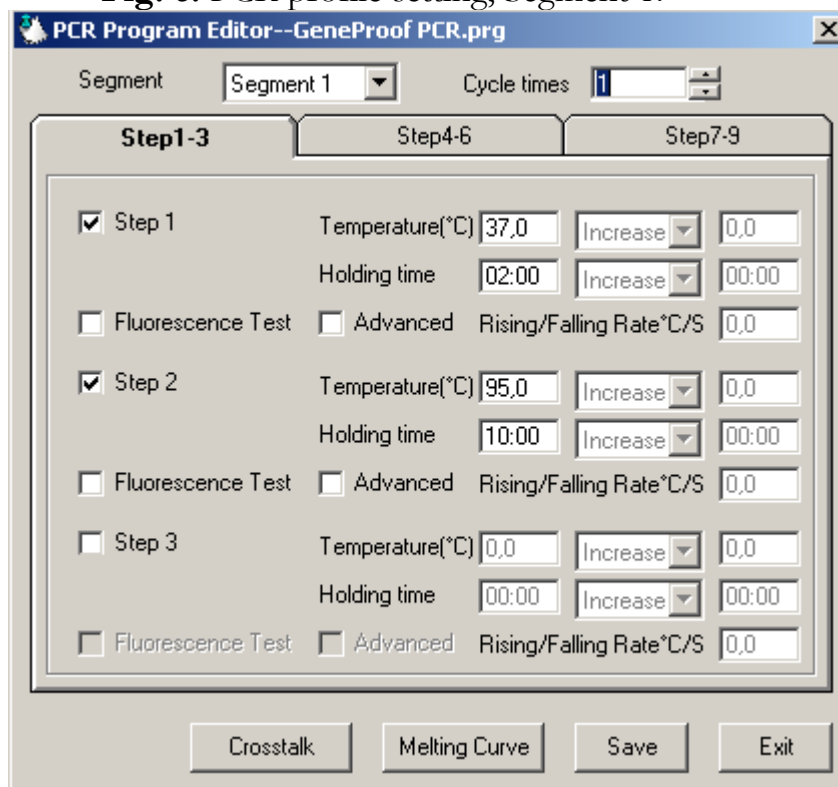
Fig. 5. PCR profile setting, Step 2.



Step 3:

Program **Segment 1** of the PCR program by setting the **Step 1** parameter (**Temperature 37.0** and **Holding time 02:00**) and by setting the **Step 2** parameter (**Temperature 95.0** and **Holding time 10:00**). Enter **1** into the field **Cycle times**. Leave other parameters without any changes and click **Save** (Fig. 6).

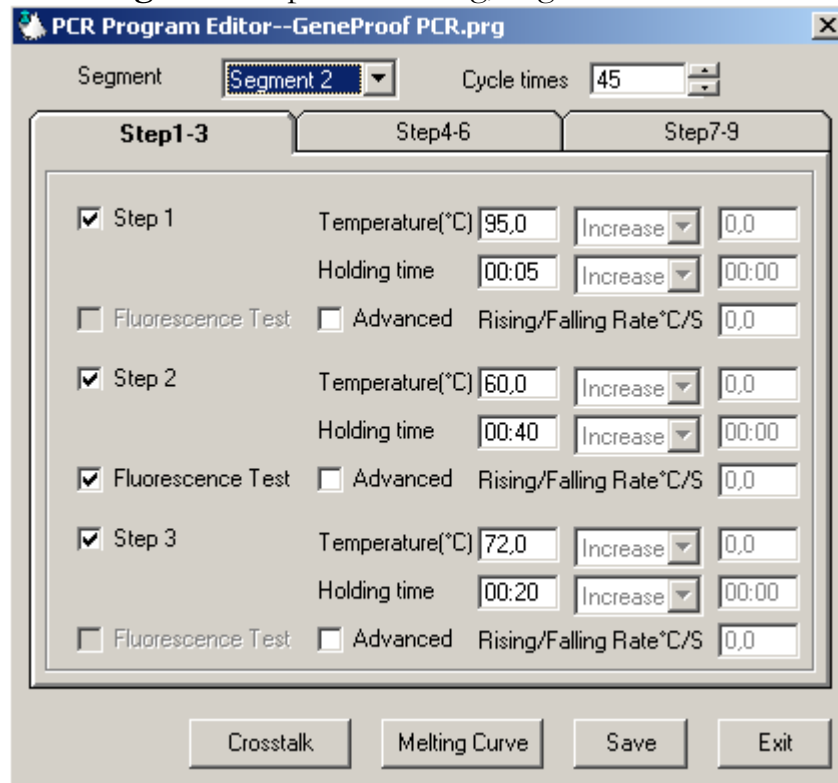
Fig. 6. PCR profile setting, Segment 1.



Step 4:

Program **Segment 2** of the PCR program by setting parameter **Step 1** (**Temperature 95.0** and **Holding time 00:05**), parameter **Step 2** (**Temperature 60.0**, **Holding time 00:40**, type in **Fluorescent Test**) and parameter **Step 3** (**Temperature 72.0**, **Holding time 00:20**). Enter **45** into the field **Cycle times**. Leave other parameters without any changes and click **Save** (Fig. 7).

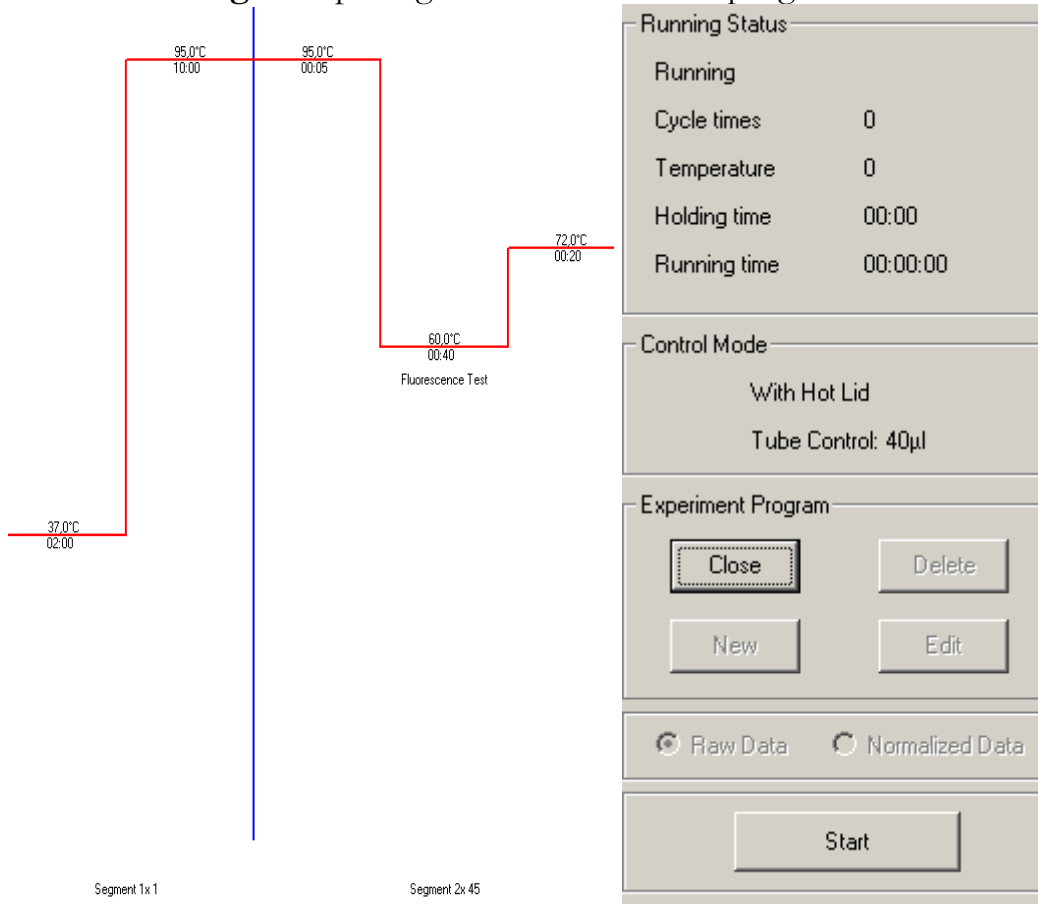
Fig. 7. PCR profile setting, Segment 2.



Step 5:

Click **Open** to open the newly created PCR program. Select **GeneProof PCR** and click **Open**. The created PCR program will open, graphic representation of the PCR profile will display and the **Start** button will be enabled (Fig. 8).

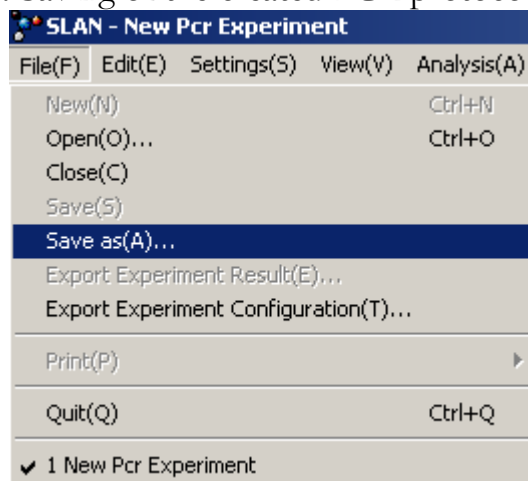
Fig. 8. Opening of the created PCR program.



Step 6:

Click **File(F)** and **Save as(A)** to save the created protocol under a designation used by your laboratory (Fig. 9).

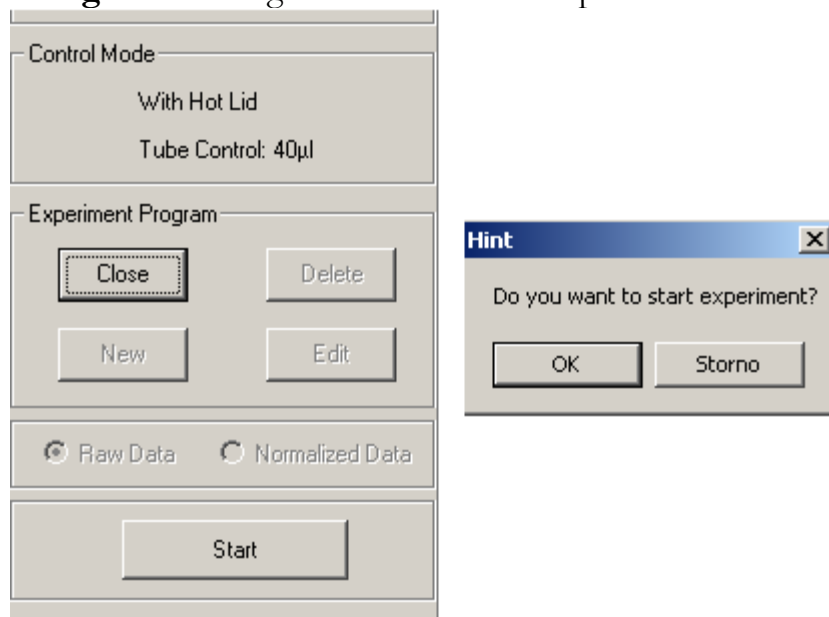
Fig. 9. Saving of the created PCR protocol.



Step 7:

Click **Start** and then **OK** in the **Hint** box to start the PCR program (Fig. 10).

Fig. 10. Starting of the created PCR protocol.

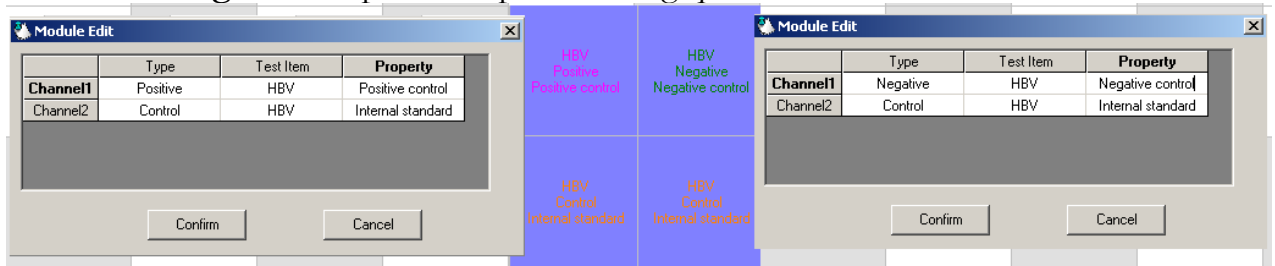


Detection evaluation

Qualitative analysis of the detection results

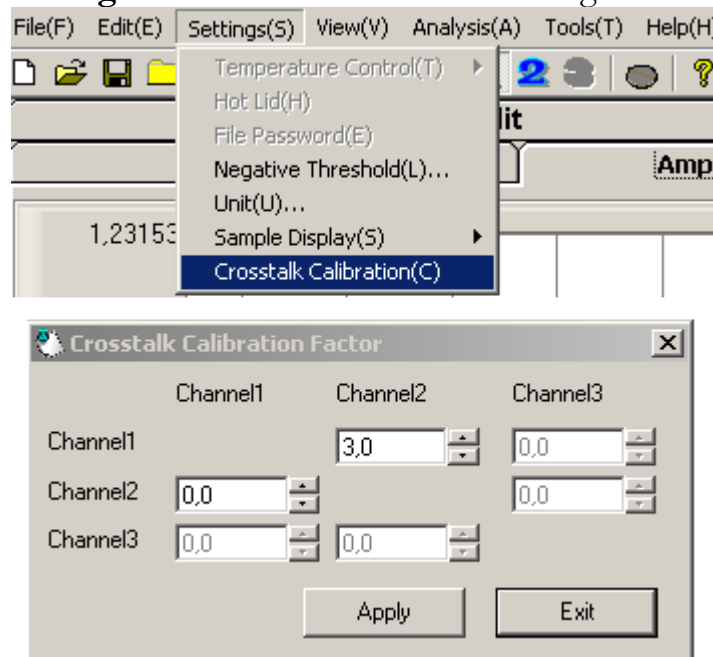
1. Select values for the detection qualitative analysis in the **Module Mode** tab. **Positive Control** and **Negative Control** are the minimum required samples for a valid assessment of a PCR reaction (Fig. 11).

Fig. 11. Sample description during qualitative detection.



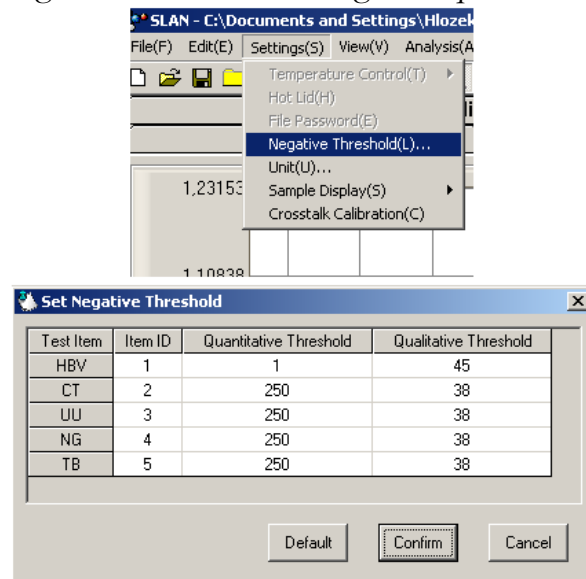
2. Click the **Amplification Curve** tab, then select **Settings(S)** and **Crosstalk Calibration(C)**. Type *3.0* into the **Channel2** field in the **Crosstalk Calibration Factor** box. Click **Apply** to confirm (Fig. 12).

Fig. 12. Crosstalk Calibration setting.



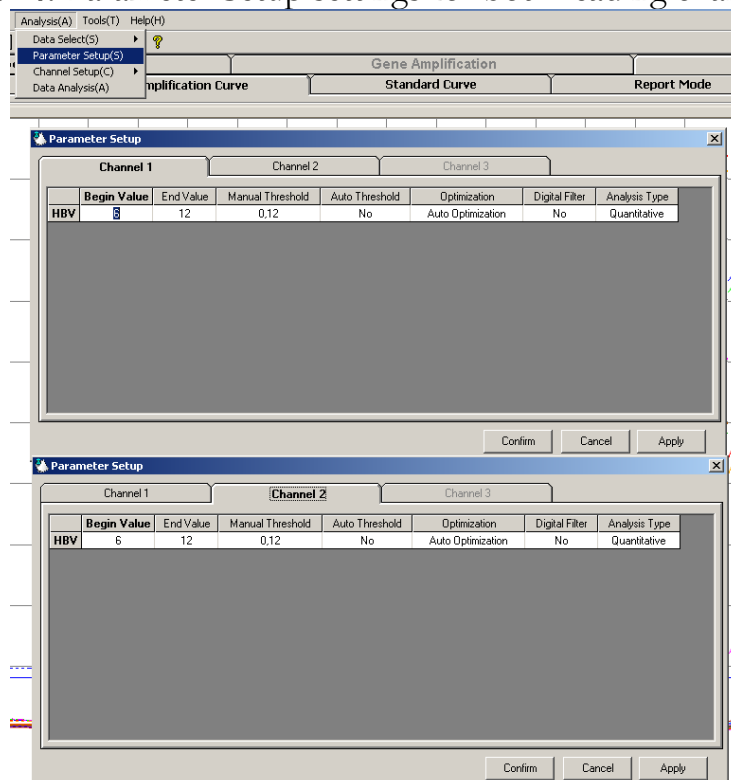
- Set threshold for qualitative detection. Select **Settings(S)** and **Negative Threshold(L)**. Enter **1** into the **Quantitative Threshold** column and **45** into the **Qualitative Threshold** column. Click **Confirm** to continue (Fig. 13).

Fig. 13. Negative threshold settings for qualitative detection.



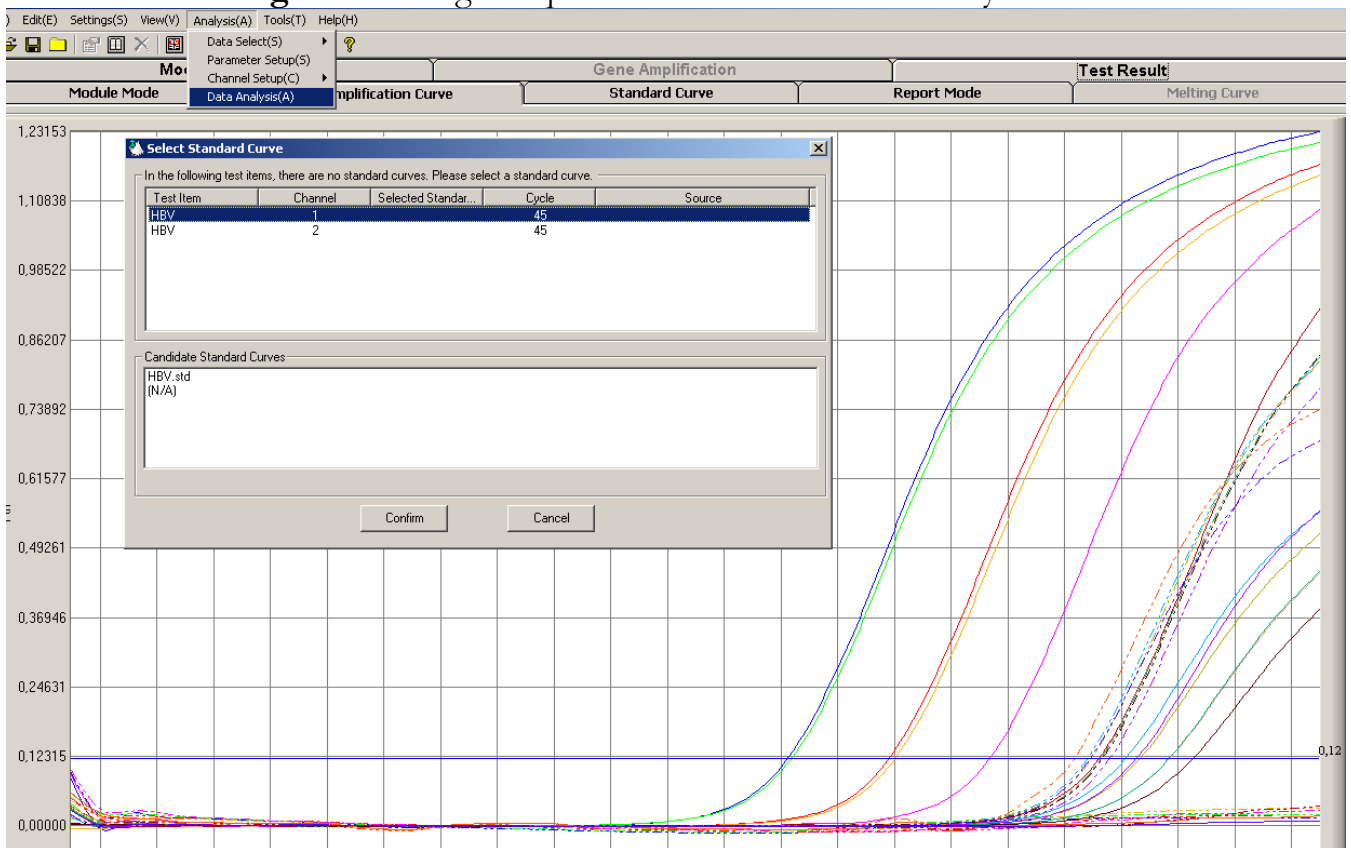
- Select **Parameter Setup(S)** in the upper bar. Set values for detection qualitative analysis in **Channel 1: Manual Threshold 0.12, Auto Threshold No** and **Analysis Type Qualitative**. Leave other parameters without any changes. Set values for detection qualitative analysis in **Channel 2: Manual Threshold 0.12, Auto Threshold No** and **Analysis Type Qualitative**. Leave other parameters without any changes. Click **Apply** and **Confirm** to apply the settings for both detection channels (Fig. 14).

Fig. 14. Parameter Setup settings for both reading channels.



- Starting the result analysis with preset values. Select **Data Analysis(A)** in the upper bar and click **Confirm** to confirm the result evaluation for the selected detection channels (Fig. 15.).

Fig. 15. Starting the qualitative detection result analysis.



- Evaluation of the qualitative detection results (Fig. 16.).

Positive sample amplification

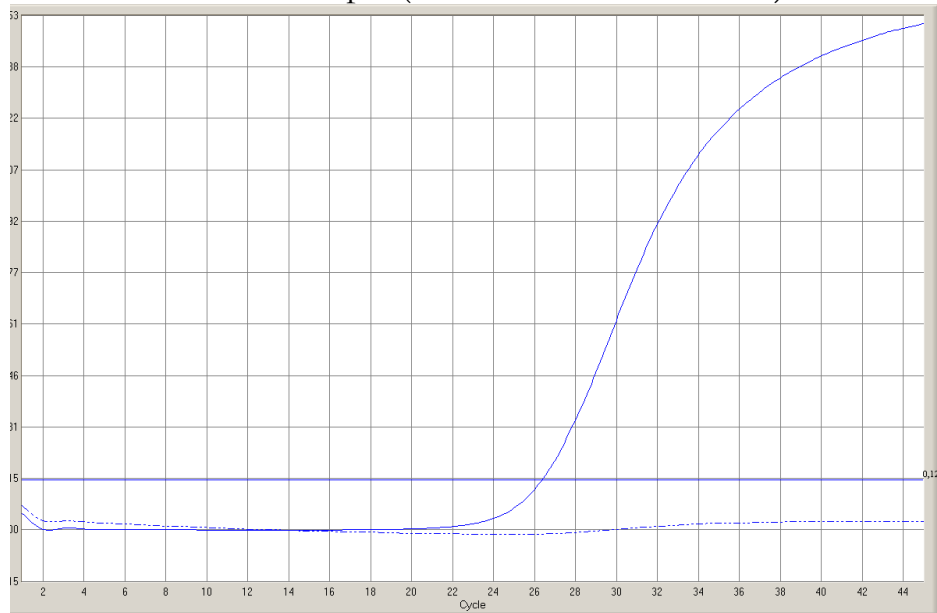
In the graphic display for **Channel 1** (or **Channel 3**, if available) you can see an ascending curve intersecting the manually set threshold. A numeric value **Ct** is assigned to this curve in the assessment table (**Report Mode** tab).

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).

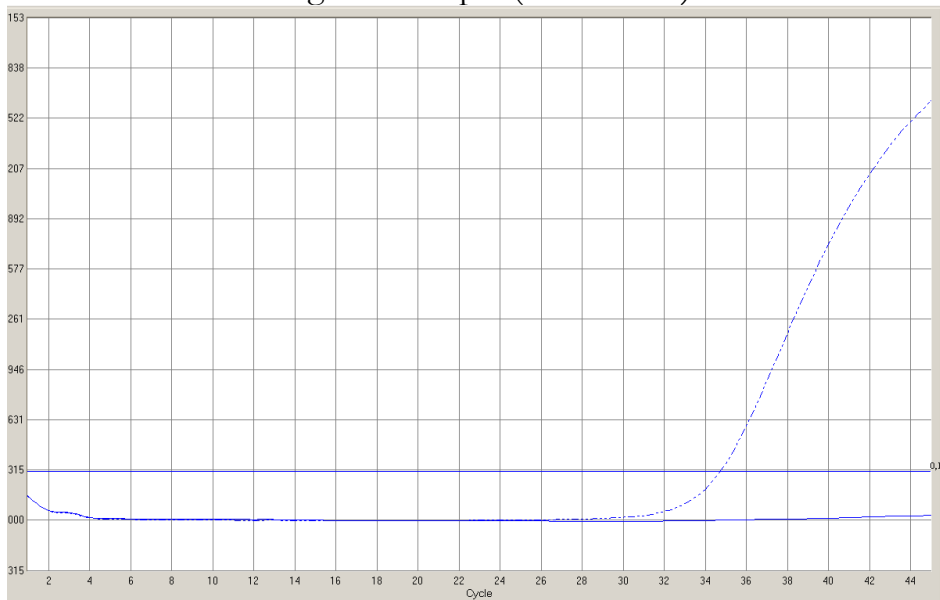
Fig. 16. Graphic representation of the qualitative detection result.

Positive sample (**Channel 1 or Channel 3**)



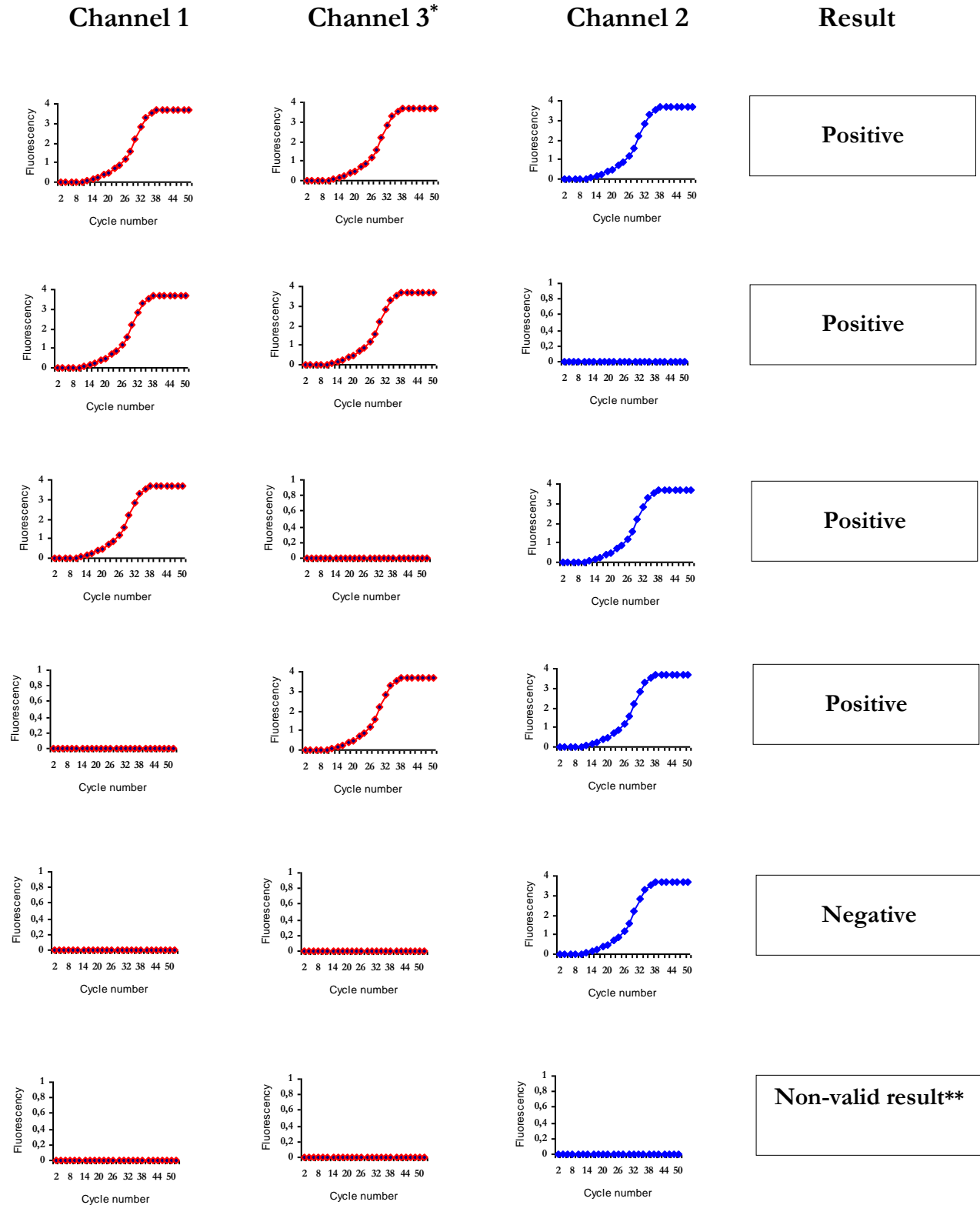
Well	Channel	Type	Test Item	Property	CT
A3	1	Sample	HBV		26,34
	2	Sample	HBV		No Ct

Negative sample (**Channel 2**)



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

7. Qualitative analysis of the acquired results:



* if available
 ** see Detection Troubleshooting, page 20

Quantitative analysis of the detection results

1. Select values for the detection quantitative analysis in the **Module Mode** tab. **Calibration Control** and **Negative Control** are the minimum required samples for a valid quantitative assessment of a PCR reaction. Select a **Standard** item in the **Type** column for each calibration control in the reaction (**Channel 1**) and then select a concentration value in relevant units/ μl in the **Property** column. Select a **Negative** item in the **Type** column for the negative control (**Channel 1**) and then select a type of the negative sample in the **Property** column (e.g. sterile water, TE buffer...). **Channel 2** is reserved for the Internal Standard amplification detection. Click **Confirm** to confirm the selected parameters (Fig. 17.).

Fig. 17. Sample description during quantitative detection

HBV Standard 1000	HBV Standard 100	HBV Standard 10	HBV Standard 1	HBV Negative TE
HBV Control Internal standard	HBV Control Internal standard	HBV Control Internal standard	HBV Control Internal standard	HBV Control Internal standard

Module Edit ✕

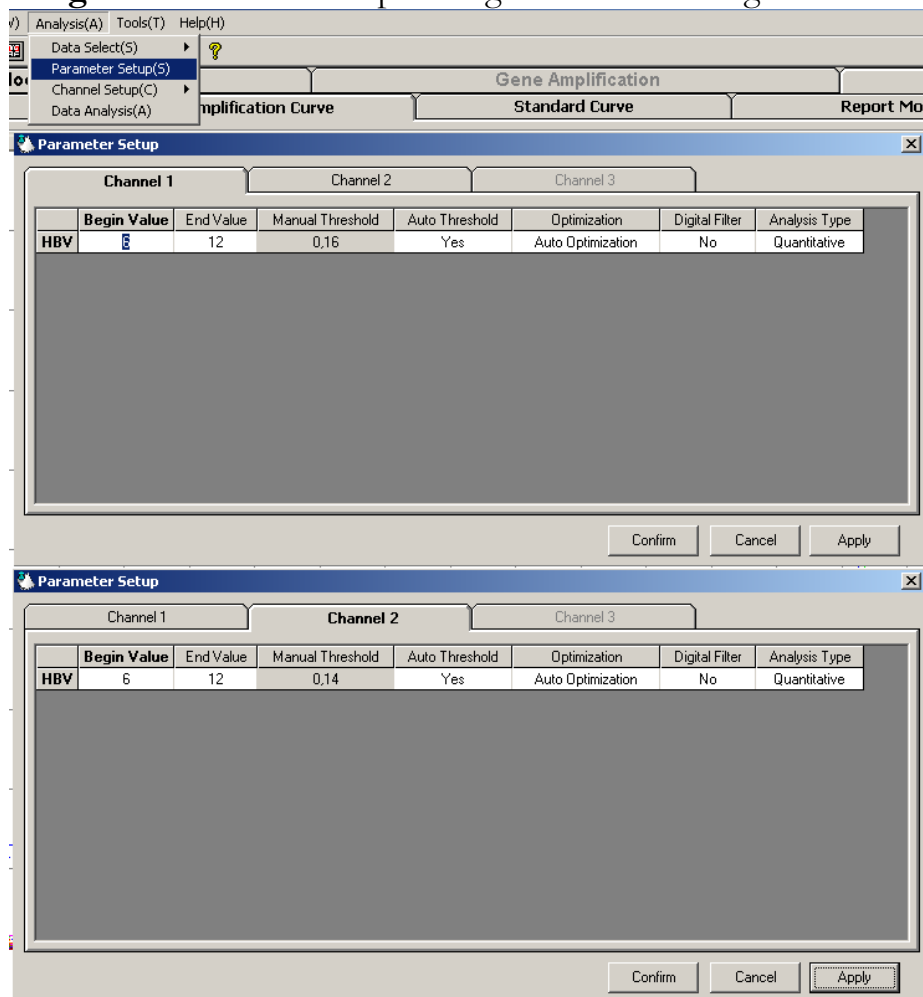
	Type	Test Item	Property
Channel1	Standard	HBV	1000
Channel2	Control	HBV	Internal standard

Module Edit ✕

	Type	Test Item	Property
Channel1	Negative	HBV	TE
Channel2	Control	HBV	Internal standard

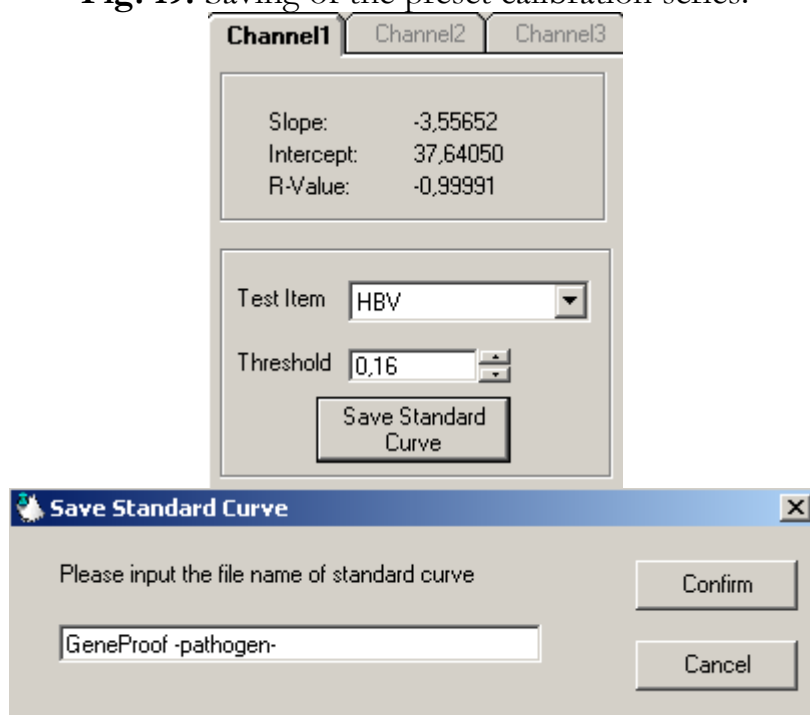
2. Click the **Amplification Curve** tab, then select **Settings(S)** and **Crosstalk Calibration(C)**. Type *3.0* into the **Channel2** field in the **Crosstalk Calibration Factor** box. Click **Apply** to continue (see Fig. 12).
3. Set a threshold for quantitative detection. Select **Settings(S)** and **Negative Threshold(L)**. Enter *1* into the **Quantitative Threshold** column and *45* into the **Qualitative Threshold** column. Click **Confirm** to continue (see Fig. 13.).
4. Select **Parameter Setup(S)** in the upper bar. Set values for detection quantitative analysis in **Channel 1**: Enter **Auto Threshold** *Yes* and **Analysis Type** *Quantitative*. Leave other parameters without any changes. Set values for the detection quantitative analysis in **Channel 2**: Enter **Auto Threshold** *Yes* and **Analysis Type** *Quantitative*. Leave other parameters without any changes. Click **Apply** and **Confirm** to apply the settings for both detection channels (Fig. 18.).

Fig. 18. Parameter Setup settings for both reading channels.



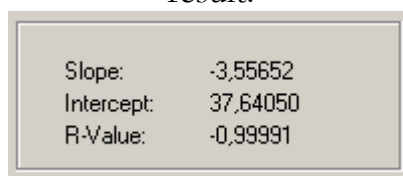
- Saving of the preset calibration series. Click the **Standard Curve** tab. In the right section select **Channel 1** for the calibration curve and click **Save Standard Curve** to save the settings. Click **Confirm** to continue (Fig. 19).

Fig. 19. Saving of the preset calibration series.



- Starting the result analysis with preset values. Select **Data Analysis(A)** in the upper bar and click **Confirm** to confirm the result evaluation for the selected detection channels (see Fig. 15).
- Evaluation of the calibration curve quality and reading of the detection quantitative result. Verify the correlation coefficient of the calibration series (**R-Value**) in the **Standard Curve** tab. The correlation coefficient must be 0.99 or higher. After reading the measured values click the **Report Mode** tab to display the numerical result of the unknown sample detection (Fig. 20).

Fig. 20. Assessment of the calibration curve quality and of the detection quantitative result.



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	

Only concentrations in the range specified by the calibration curve may be measured for a quantitative evaluation of the results.

Quantification of samples where concentration exceeds the upper measuring threshold determined by the calibration curve range (a calibrator with the highest concentration) is of reference value only. You can dilute these samples and repeat the assessment to achieve a precise quantification.

Samples with lower concentrations than the lowest concentrated calibrator can be quantified approximately only.

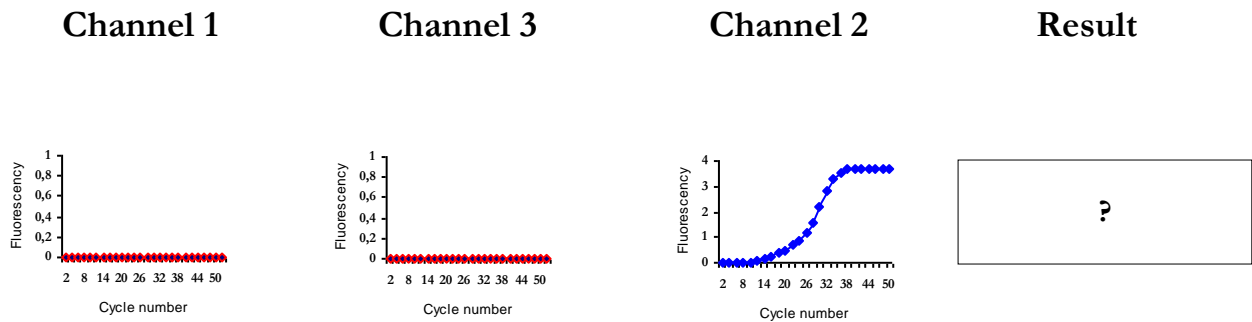
The following formula can be used to convert sample concentrations to *units/ml* taking into account the isolation procedure:

$$\text{Concentration/ml} = \frac{cVZ \times EO}{I}$$

cVZ = sample concentration in units/ μ l
EO = selected elution volume in μ l
I = material volume used for isolation ml

Troubleshooting

Invalid result of a Positive Control analysis



❖ Problem: *Incorrect programming of the PCR amplification*

Problem resolution:

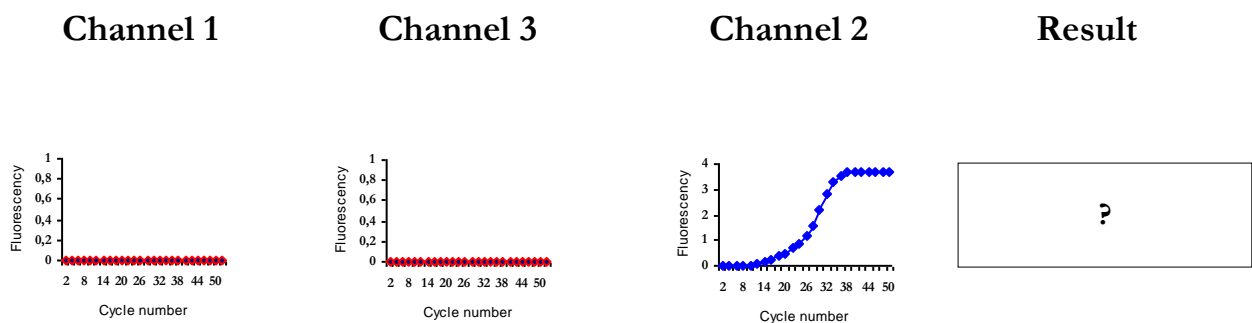
1. Check device programming according to the manual
2. Check correct temperature settings in the individual program blocks

❖ Problem: *Positive control incorrectly held in storage* (see Storage and transportation conditions)

Problem resolution:

1. Check whether kit component storage is in harmony with manufacturer's recommendations
2. Submultiple the Positive control and do not freeze and thaw it

Invalid result of a Negative Control analysis

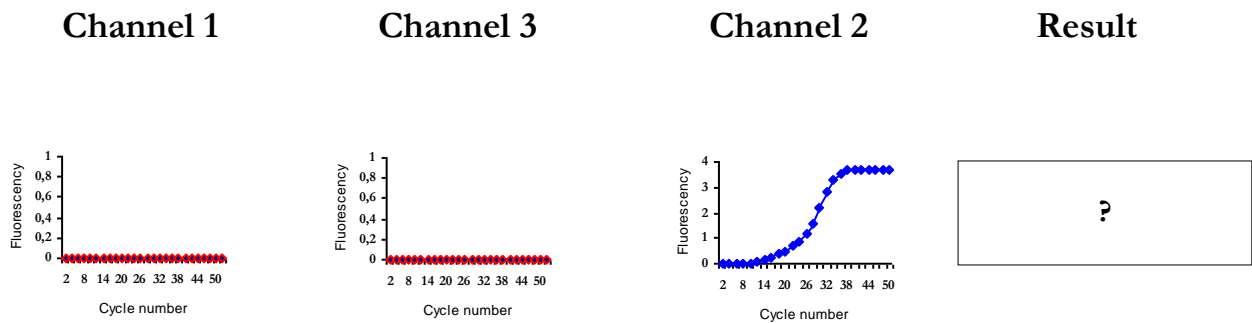


❖ Problem: *PCR reaction contamination*

Problem resolution:

1. Check the process of preparation and pipetting of the PCR mix into tubes
2. Check the handling of sterile plastics and filtered tips
3. Clean the PCR box
4. Add uracil-DNA-glycosylase (UDG) into the reaction

Invalid result of an Unknown Sample analysis



- ❖ Problem: *PCR reaction inhibition* (PCR kit ISIN and ISEX)
Problem resolution:
 1. Repeat DNA isolation
 2. Check the process of preparation and pipetting of the PCR mix into tubes

- ❖ Problem: *Invalid process of DNA isolation* (PCR kit ISEX)
Problem resolution:
 3. Repeat DNA isolation
 4. Check the process of preparation and pipetting of the Internal Standard at the beginning of the isolation process.

- ❖ Problem: *Incorrect storage of the MasterMix* (see Storage and transportation conditions)
Problem resolution:
 1. Check whether MasterMix storage is in harmony with manufacturer's recommendations
 2. Submultiple the MasterMix and do not freeze and thaw it

If you have any questions please contact our Product Support Department at:
support@geneproof.cz

Notes:

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