

# GeneProof®

## PCR Kit

This manual is designed for the following kits:

*Mycobacterium tuberculosis* PCR Kit

*Mycoplasma pneumoniae* PCR Kit

*Legionella pneumophila* PCR Kit

*Chlamydia pneumoniae* PCR Kit

*Chlamydia trachomatis* PCR Kit

*Neisseria gonorrhoeae* PCR Kit

*Borrelia burgdorferi* PCR Kit

*Cytomegalovirus* (CMV) PCR Kit

*Epstein-Barr Virus* (EBV) PCR Kit

*Herpes Simplex Virus 1* (HSV-1) PCR Kit

*Herpes Simplex Virus 2* (HSV-2) PCR Kit

*Varicella-Zoster Virus* (VZV) PCR Kit

*Hepatitis B Virus* (HBV) PCR Kit

CE

*in vitro* Diagnostics

User manual for use with the following device:

## LightCycler® 2.0

VERSION K1-AJ-03

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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 fluorophor and the probe designated for the internal standard detection is marked by the JOE fluorophor).

### GeneProof PCR kits

- Use the “hot start” technology, minimizing non-specific reactions and assuring maximum sensitivity.
- Contain uracil-DNA-glycosylase (UDG), eliminating 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.
- 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.
- 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/*)

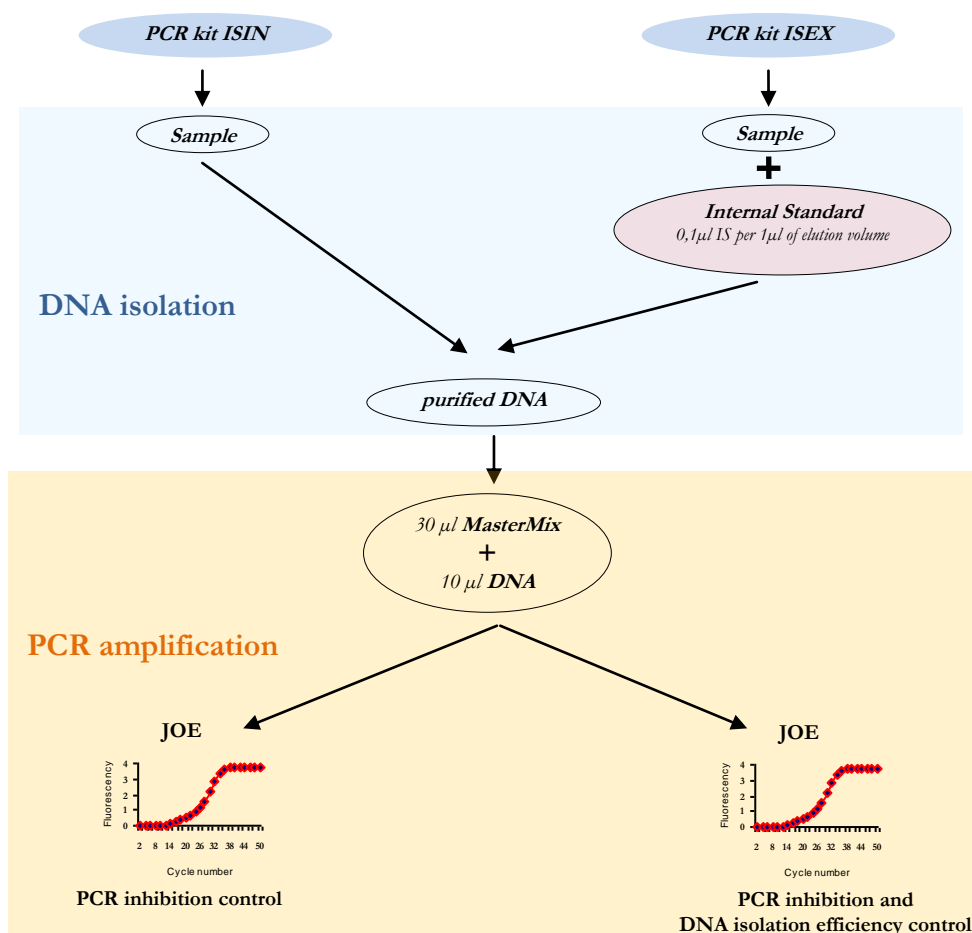
In this version of the PCR kit the Internal Standard is included in the MasterMix tube. This PCR kit version enables PCR inhibition control.

### 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  $\mu$ l of the Internal Standard per 1  $\mu$ l of elution volume:**

Elution Volume	25 $\mu$ l	50 $\mu$ l	100 $\mu$ l	200 $\mu$ l
Internal Standard	2.5 $\mu$ l	5 $\mu$ l	10 $\mu$ l	20 $\mu$ l



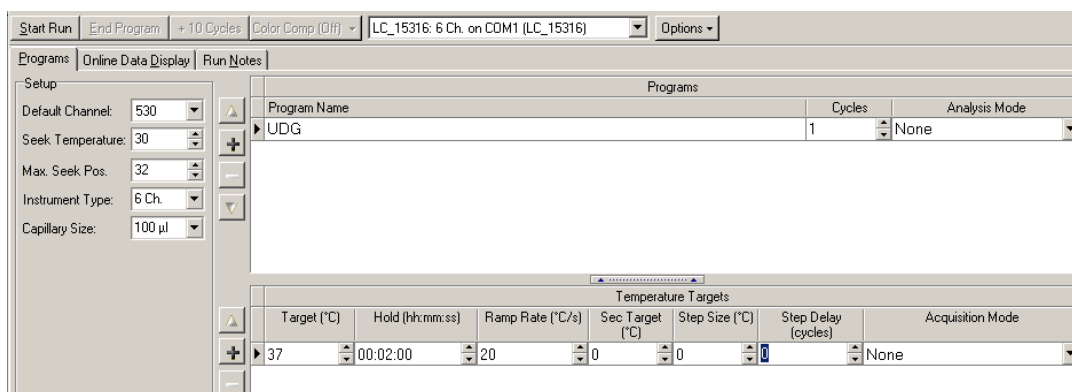
# User manual for LightCycler 2.0®

## PCR Reaction Preparation

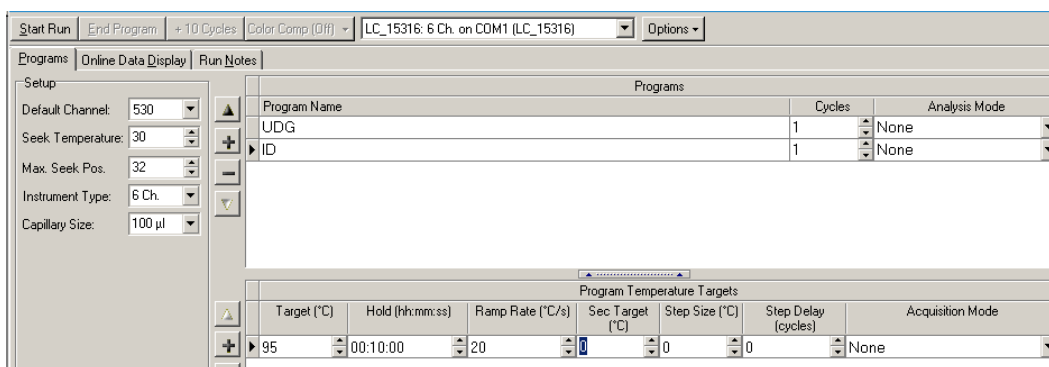
1. Add **30 µl** of the **MasterMix** and **10 µl** of the **DNA isolate** or **10 µl** of the **Positive Control** into a 100 µl capillary tube. The final reaction mix volume should be **40 µl**.
2. Close the capillary tubes, shortly centrifuge, insert into LightCycler® 2.0 and program according to the following table

## Device Programming

1. Activate **New** in the LightCycler main menu and select **LightCycler Experiment**
2. **Set UDG treatment step:**
  - a) Enter **UDG** into **Program Name**, **1** into **Cycles** and **None** into **Analysis Mode**.
  - b) Enter **37** into **Target (°C)**, **00:02:00** into **Hold (hh:mm:ss)**, **20** into **Ramp Rate (°C/s)** and **None** into **Acquisition Mode**.

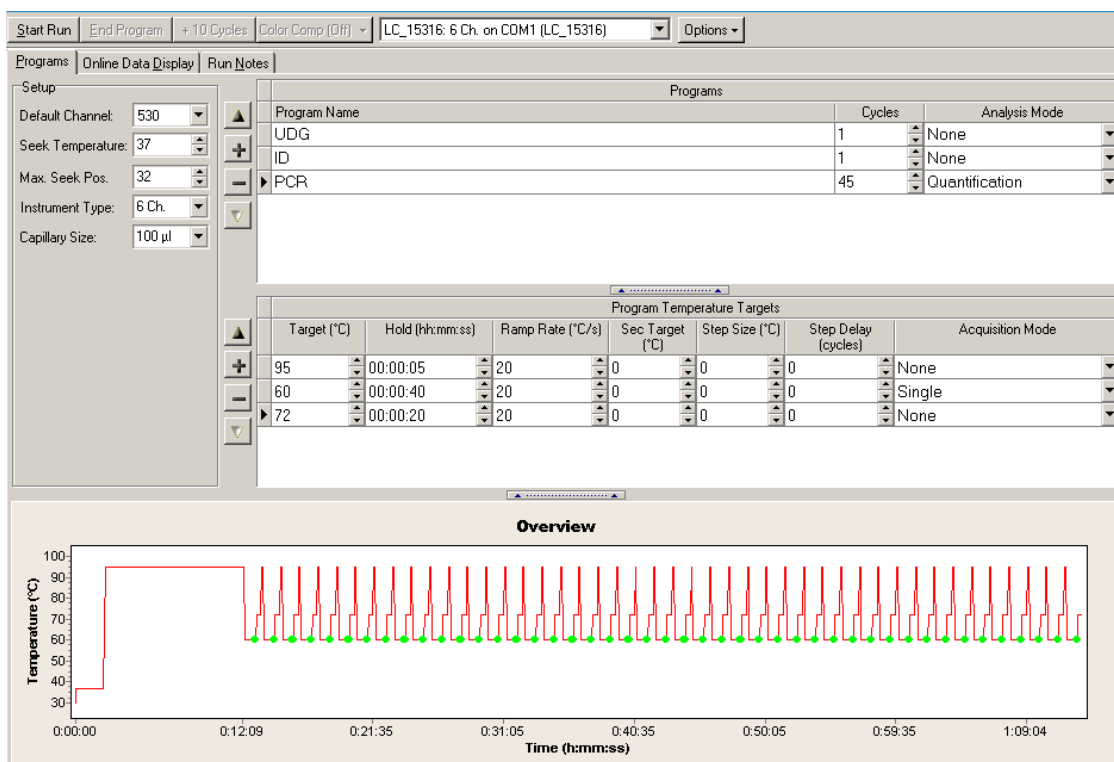


3. **Set initial denaturation step:**
  - a) Press + to add a new program block in the **Programs** window.
  - b) Enter **ID** into **Program Name**, **1** into **Cycles** and **None** into **Analysis Mode**.
  - c) Enter **95** into **Target (°C)**, **00:10:00** into **Hold (hh:mm:ss)**, **20** into **Ramp Rate (°C/s)** and **None** into **Acquisition Mode**.

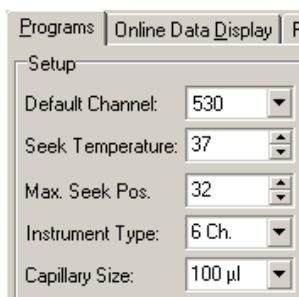


4. Set PCR cycling profile:

- Press + to add a new program block in the **Programs** window.
- Enter **PCR** into **Program Name**, **45** into **Cycles** and **Quantification** into **Analysis Mode**.
- Set PCR denaturation step:** Enter **95** into **Target (°C)**, **00:00:05** into **Hold (hh:mm:ss)**, **20** into **Ramp Rate (°C/s)** and **None** into **Acquisition Mode** in the **Program Temperature Targets** window.
- Set PCR annealing step:** Press + to add a new program block in the **Program Temperature Targets** window. Enter **60** into **Target (°C)**, **00:00:40** into **Hold (hh:mm:ss)**, **20** into **Ramp Rate (°C/s)** and **Single** into **Acquisition Mode**.
- Set PCR extension step:** Press + to add a new program block in the **Program Temperature Targets** window. Enter **72** into **Target (°C)**, **00:00:20** into **Hold (hh:mm:ss)**, **20** into **Ramp Rate (°C/s)** and **None** into **Acquisition Mode**.



5. **Setup parameter entering:** Enter **530** into **Default channel**. Enter **37** into **Seek Temperature**, enter **Max.Seek Pos.** according to the number of capillary tubes, enter **6** into **Instrument Type** and enter **100 µl** into **Capillary Size**.



## Samples Describing and Program Running

- Switch into the **Samples** menu and describe the samples according to the following chart:

- Select **Absolute Quantification** in **Analysis Type**.
- Select the **Capillary View** tab and describe the inserted samples in the **Sample Name** column.
- Enter **530** and **560** in the **Selected Channels** row.

**Sample data**

Analysis Type ▾ Reset Samples... Import SAM... Auto Copy... Selected Channels 530 560 610 640 670 705 Print Samples

Capillary View | Abs Quant

Sample Count 82 LC Carousel ID \_\_\_\_\_ MPLC Batch ID \_\_\_\_\_

Assay Cat. No. \_\_\_\_\_ Assay Lot No. \_\_\_\_\_ Color Comp ID \_\_\_\_\_

Pos	Sample Name	Repl. Of	Sample Note
1	Sample 1		
2	Sample 2		
3	Sample 3		
4	Sample 4		
5	Sample 5		
6	Sample 6		
7	Sample 7		
8	Calibrator I		
9	Calibrator II		
10	Calibrator III		
11	Calibrator IV		
12	Negative Control		

- Click the **Abs. Quant.** Tab:

- fill in „sample“ in case of a sample into the **Channel 530** rows.
- fill in „standard“ in case of calibration control into the **Channel 530** rows.
- fill in “IS” into the **Channel 560** rows.
- In the **Sample Type** column enter **Standard** for capillary tubes selected as calibration controls and in the **Concentration** column enter the particular concentration of the entered calibrator.
- Make sure the **Enable Controls** parameter **is not** entered.

**Sample data**

Analysis Type ▾ Reset Samples... Import SAM... Auto Copy... Selected Channels 530 560 610 640 670 705 Print Samples

Capillary View | Abs Quant

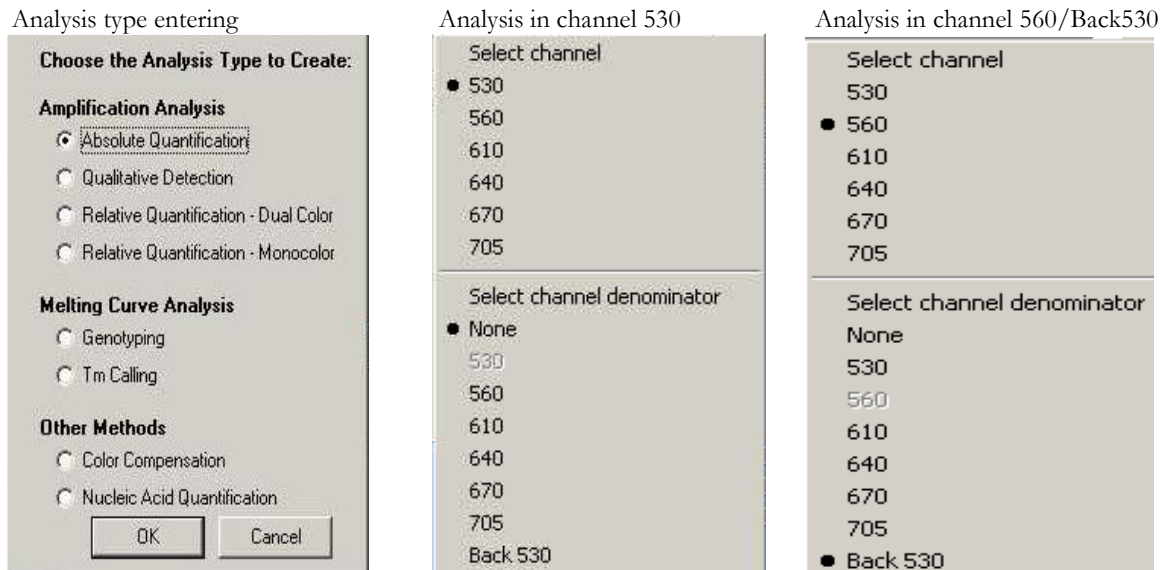
Enable Controls Unit \_\_\_\_\_

Pos	Sample Name	Channel	Target Name	Sample Type	Concentration
1	Sample 1	530	1	Unknown	
		560	IS	Unknown	
2	Sample 2	530	2	Unknown	
		560	IS	Unknown	
3	Sample 3	530	3	Unknown	
		560	IS	Unknown	
4	Sample 4	530	4	Unknown	
		560	IS	Unknown	
5	Sample 5	530	5	Unknown	
		560	IS	Unknown	
6	Sample 6	530	6	Unknown	
		560	IS	Unknown	
7	Sample 7	530	7	Unknown	
		560	IS	Unknown	
8	Calibrator I	530	Calibrator 0704	Standard	1.00E4
		560	IS	Unknown	
9	Calibrator II	530	Calibrator 0704	Standard	1.00E3
		560	IS	Unknown	
10	Calibrator III	530	Calibrator 0704	Standard	1.00E2
		560	IS	Unknown	
11	Calibrator IV	530	Calibrator 0704	Standard	1.00E1
		560	IS	Unknown	
12	Negative Control	530	NC	Unknown	
		560	IS	Unknown	

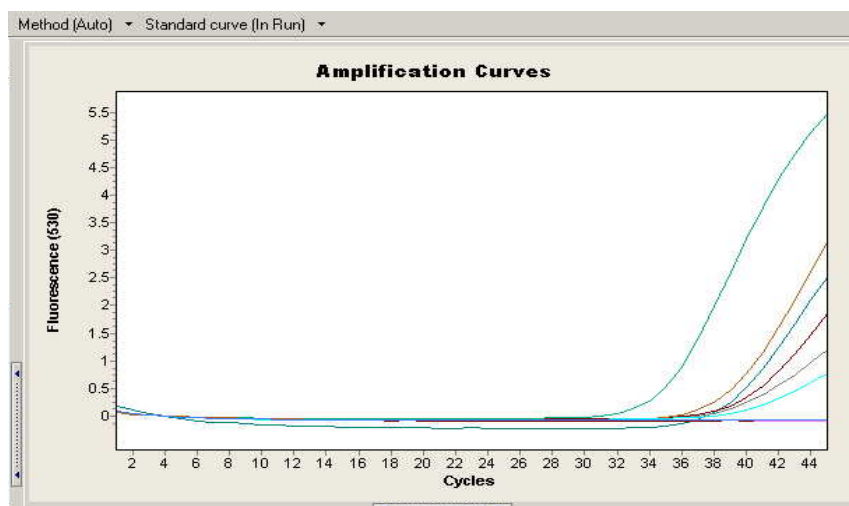
- Save the program.
- Press the **Run** button to switch into the main window.
- Press **Start Run** to run the PCR program.

## Result evaluation;

1. Open the **Analysis tab** and enter the **Absolute Quantification**.
2. Enter the **Channel 530** (for the purpose of the 530 nm channel signal analysis corresponding to the specific pathogen detection) or the **Channel 560/back530** (for the purpose of the 705 nm channel signal analysis corresponding to the Internal standard amplification).

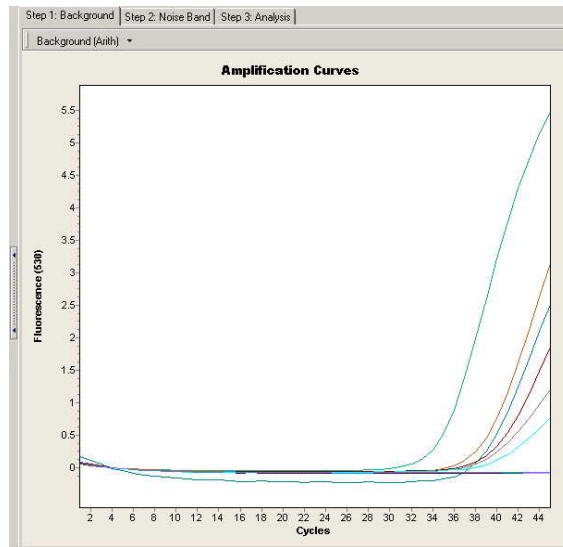


3. Open the **Color Compensation** tab and enter the color-compensation protocol (not included in the kit).
4. Enter **Method (Auto)** for the result analysis in the 530 nm channel.

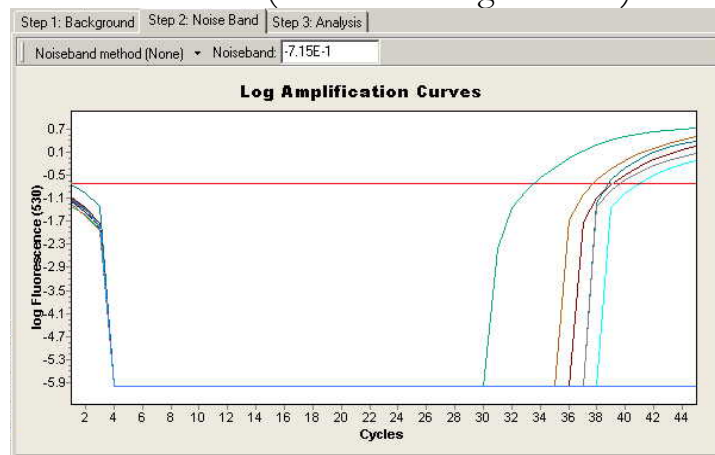


5. Samples testing positive by the automatic method in the 530 nm channel should be verified by the **Fit Points** method and the following chart should be observed during evaluation.

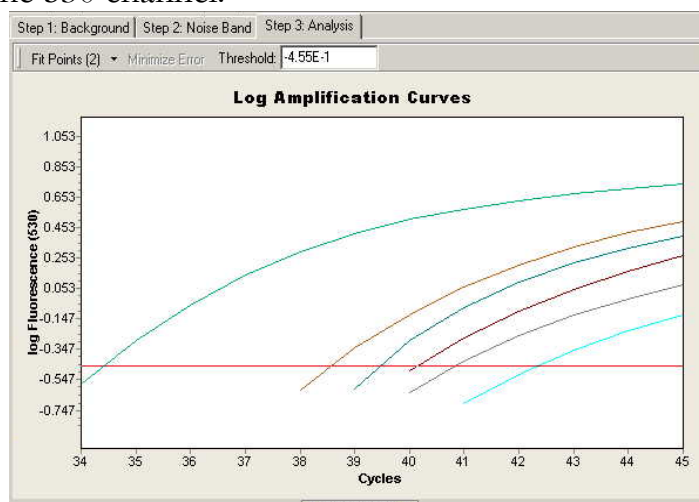
**Step 1: Background:** You can see the amplification result for the 530 channel on the screen.



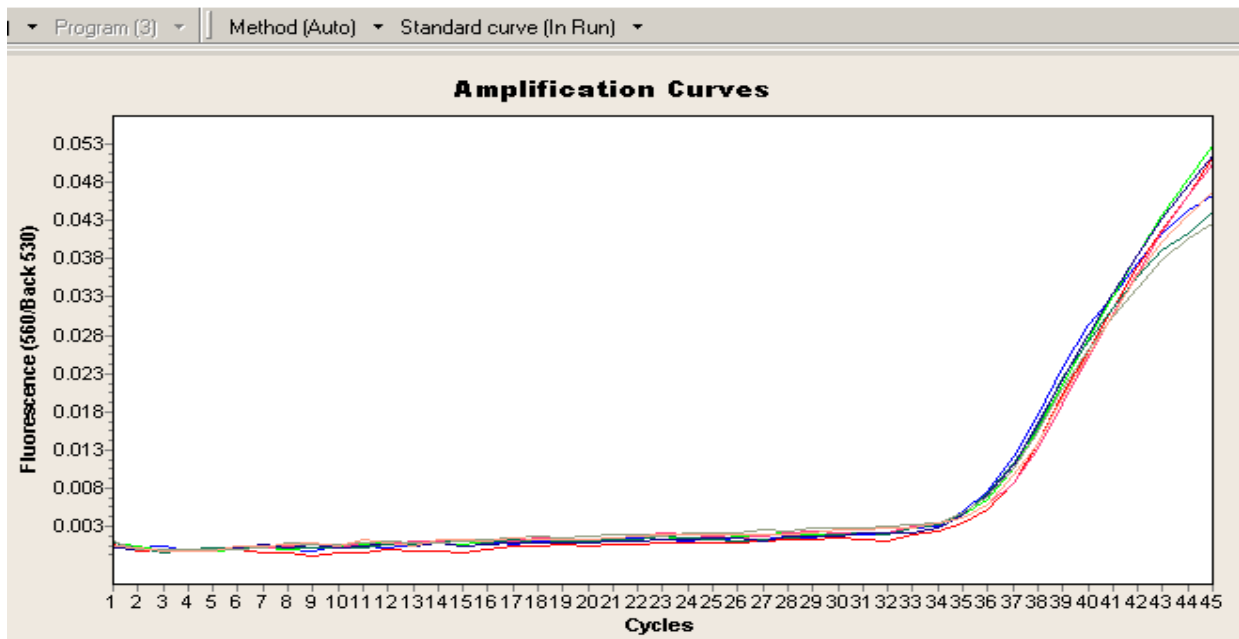
**Step 2: Noise band:** Click the red line and move it immediately above the basal reaction noise (in the left image section)



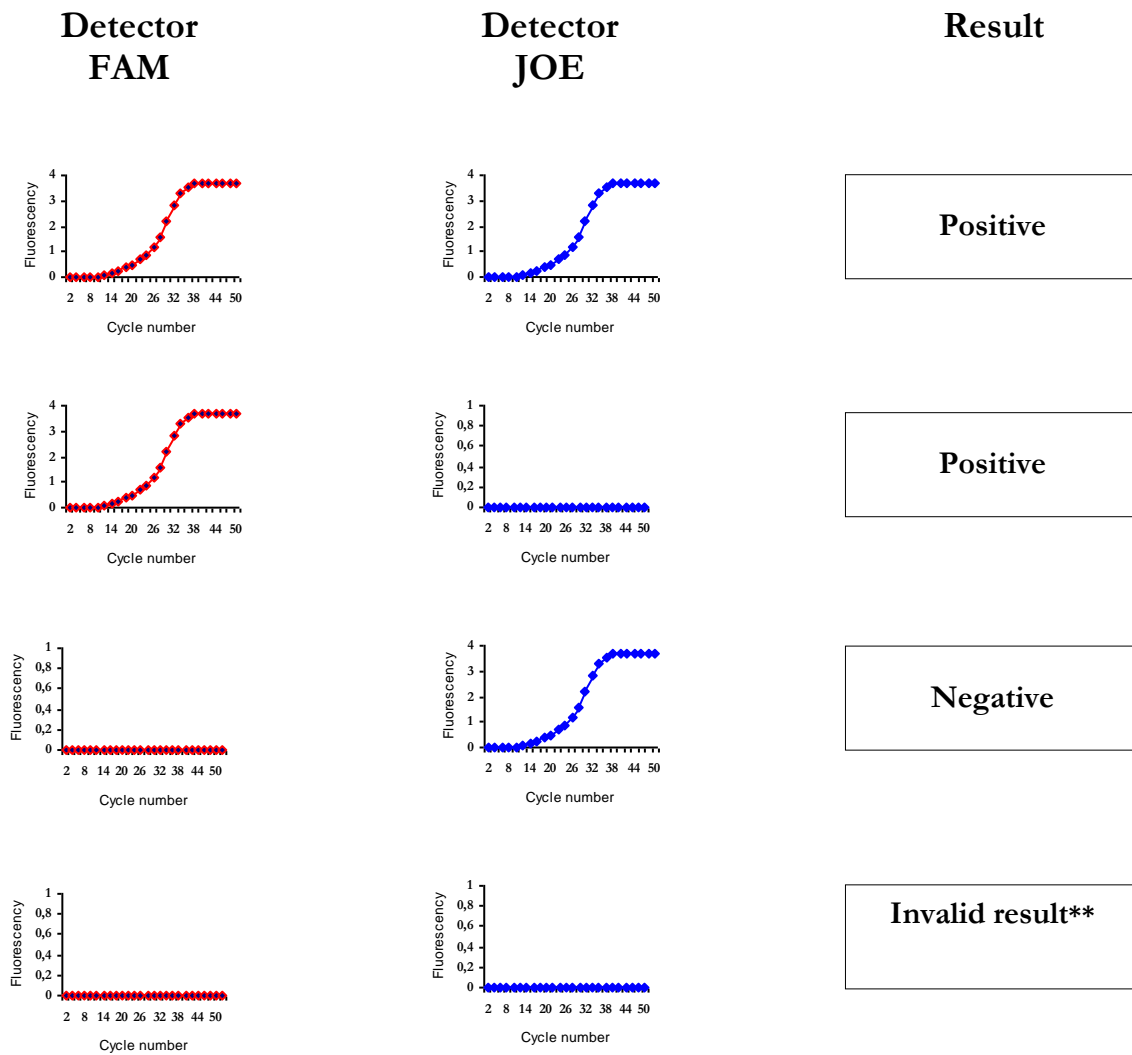
**Step 3: Analysis:** This step contains only curves for the positive signal for the 530 channel.



6. Internal standard presence (channel 560/Back 530) should be always evaluated by the **Method (Auto)**.



## Qualitative detection evaluation



\* A curve with clear fluorescence increase and Ct numerically defined in the result is considered a positive signal in the 530nm and 530/Back 560 nm channels.

\*\* See Detection Troubleshooting, page 13

## Quantitative detection evaluation

***Quantitative analysis should be performed for samples evaluated as positive in the course of the qualitative analysis procedure!***

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:

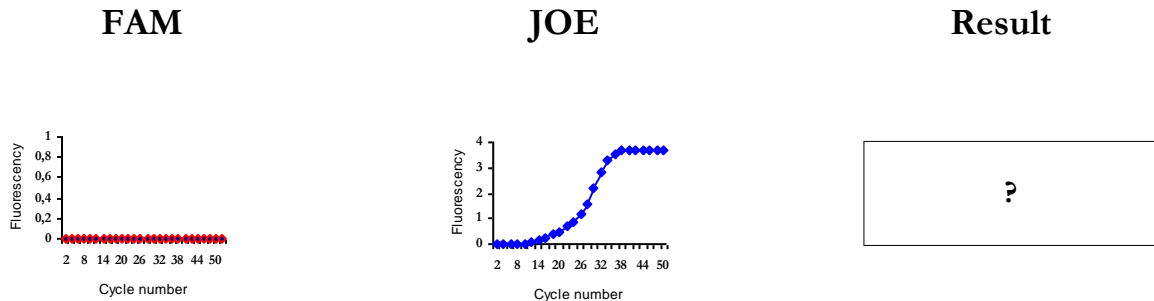
$$\text{Concentration/ml} = \frac{\text{cVZ} \times \text{EO}}{\text{I}}$$

cVZ = sample concentration in units/ $\mu\text{l}$   
EO = selected elution volume in  $\mu\text{l}$   
I = material volume used for isolation in ml

If you have any questions please contact our Product Support Department at: [support@geneproof.com](mailto:support@geneproof.com)

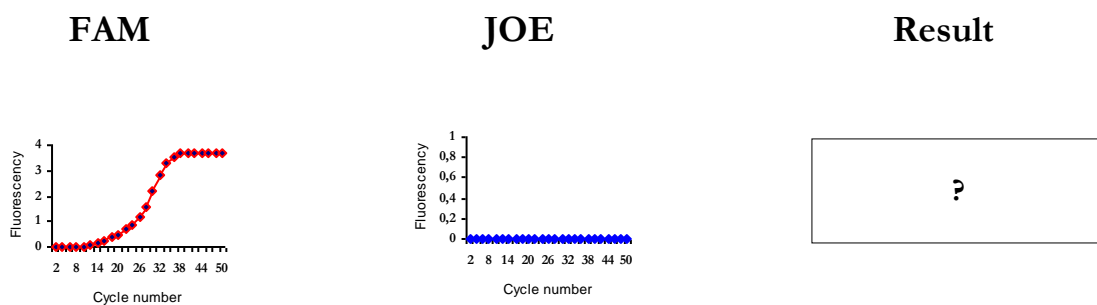
# Troubleshooting

## Invalid result of a POSITIVE CONTROL analysis



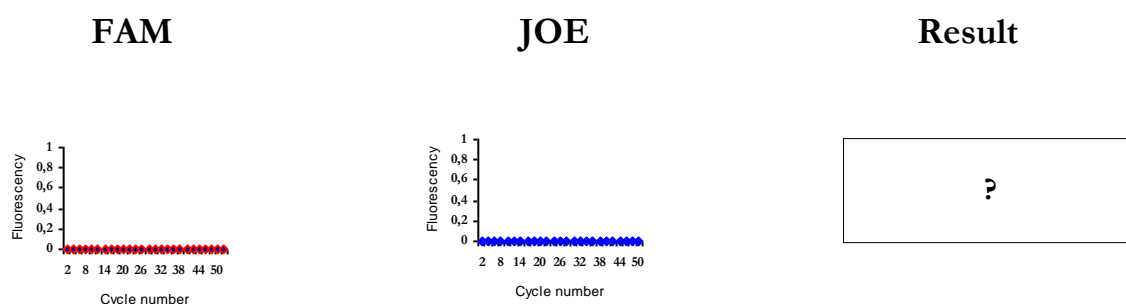
- ❖ Problem: *Incorrect programming of the PCR amplification*  
Problem resolution:
  1. Check device programming according to the manual
  2. Check correct temperature settings in the individual program blocks
- ❖ Problem: *Positive control incorrectly held in storage* (see Storage and transportation 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. Ad 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.com](mailto:support@geneproof.com)

## Notes:

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