GeneProof
Molecular diagnostics for your routine

DEVICE MANUAL

Bio-Rad

CFX ConnectTM Real-Time PCR Detection System

CFX96TM Real-Time PCR Detection System

Dx Real-Time System

Designed for GeneProof diagnostic kits

See www.geneproof.com for the current kits list

CONTENTS

1. Purpose

This device manual describes in detail the process of using GeneProof PCR kits for microbiological diagnostics with the CFX96 / CFX Connect Real-Time PCR Detection System and Dx Real-Time System devices.

1.1. PCR Reaction Preparation

Prepare PCR reaction according to the Instruction for use of the used GeneProof PCR kit.

1.2. Device Programming

In case the software does not include predefined templates, it is necessary, before the first use with GeneProof PCR kits, to programme them according to the Instruction for use of the used GeneProof kits , or download them from the product site of the used GeneProof PCR kits from the website of the company www.geneproof.com . Save the downloaded templates on your local disc to the ExpressLoad.

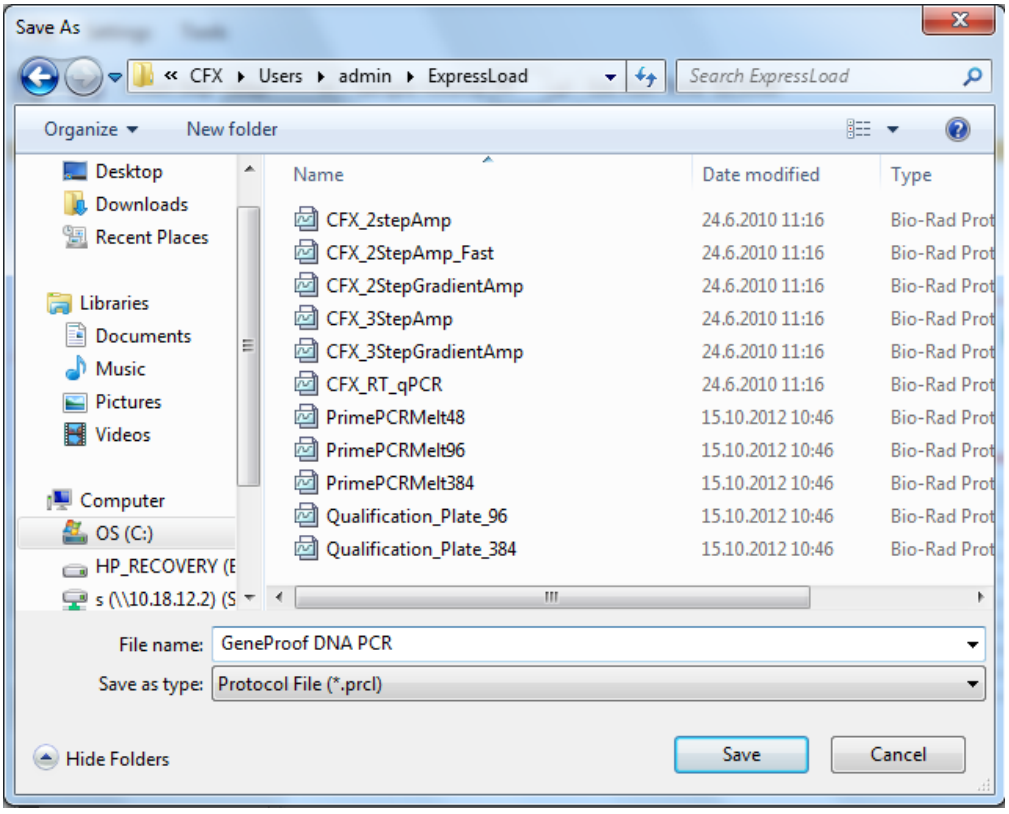


Fig. 1.1 Save template

With each next usage of GeneProof PCR kits continue from the chapter 1.3 Starting the PCR amplification.

1.3. PCR Amplification Start

1.3.1 Open a saved PCR profile template

1. Open **Bio-Rad CFX Manager**.
2. In the **Startup Wizard** box select **CFX96** and click **User-defined**.
3. Click **OK**.
4. In the **Protocol** tab of the **Run Setup** box, in section **Express Load** select file for the concrete type of examination.

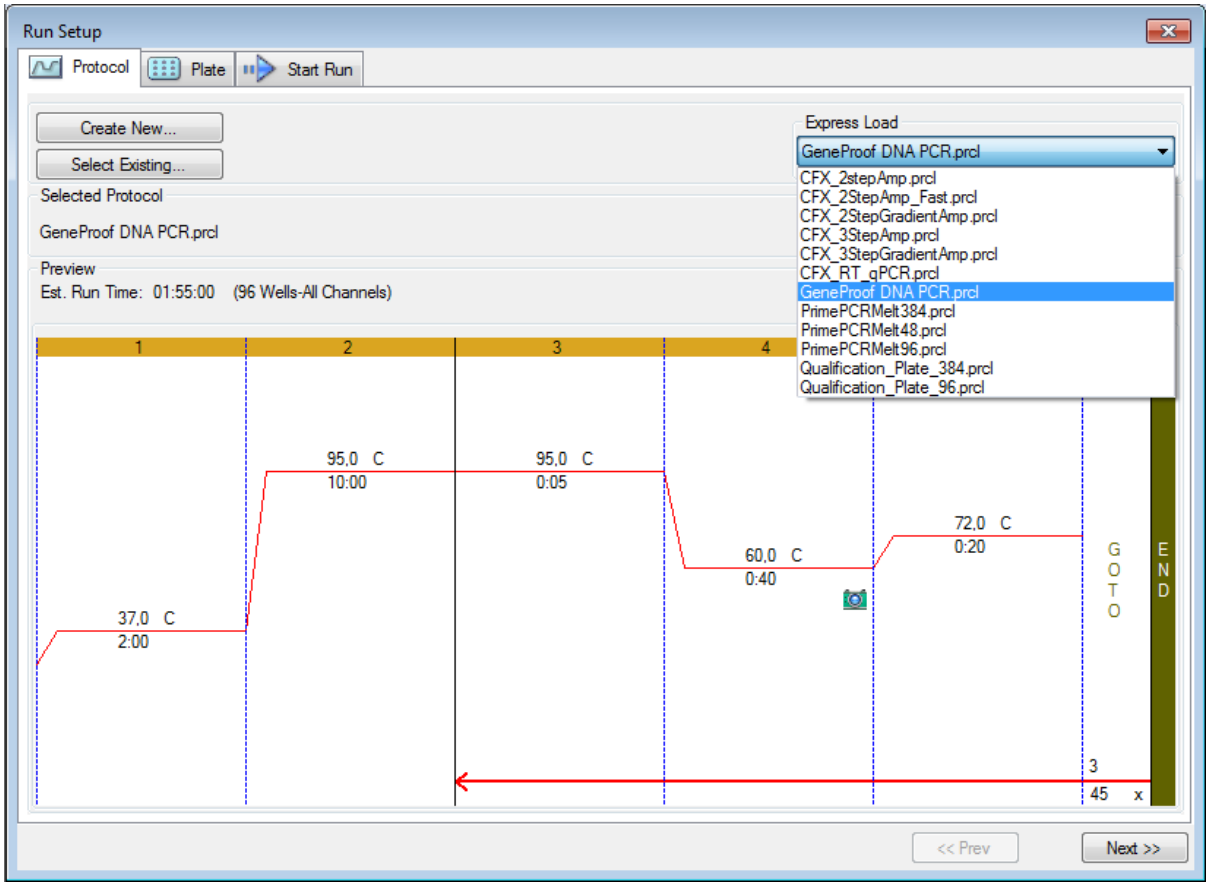


Fig. 1.2 Using the saved amplification profile

1.3.2 Using the saved plate

1. In the **Express Load** section of the **Plate** tab select **the type of examination**.

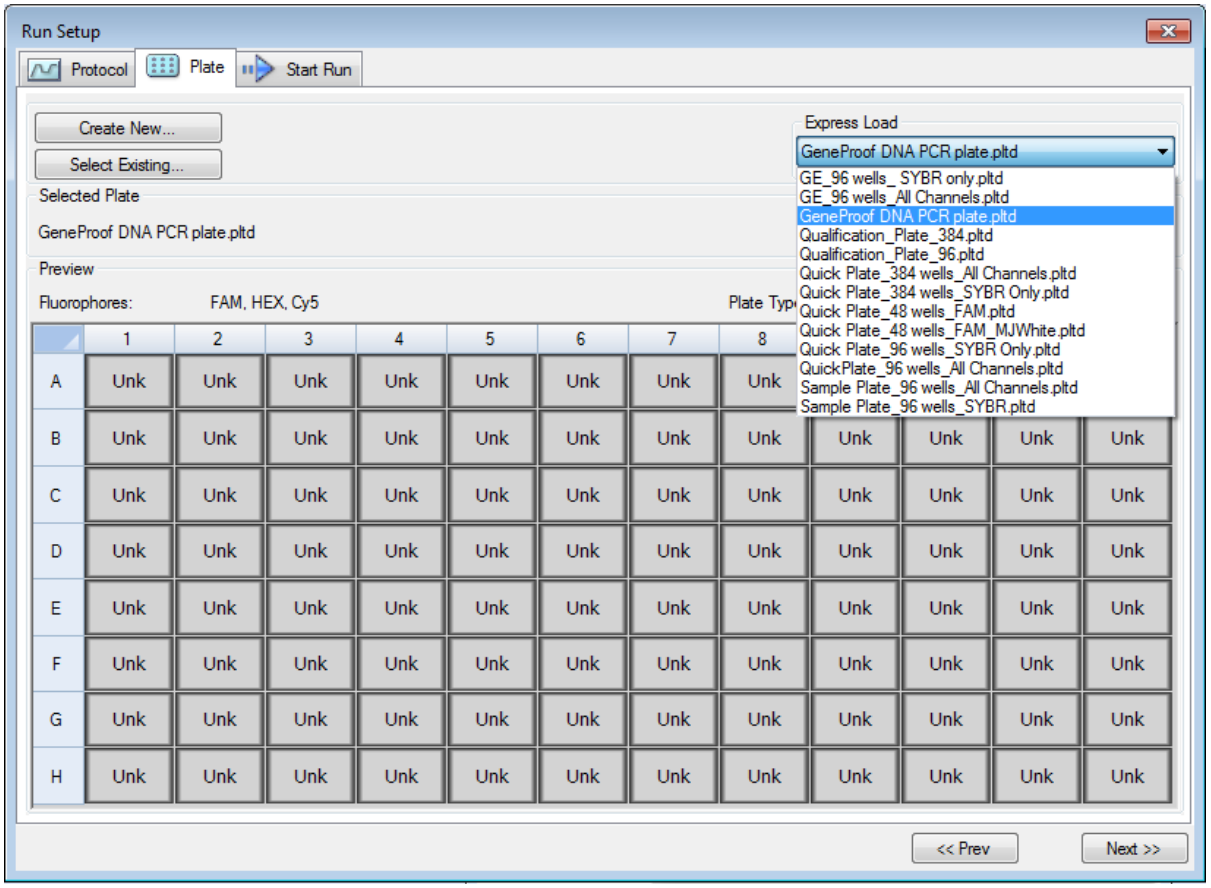


Fig. 1.3 Using the saved plate

1.3.3 PCR plate editing

1. Click the **Edit Selected** button to edit the PCR plate for the specific PCR examination.

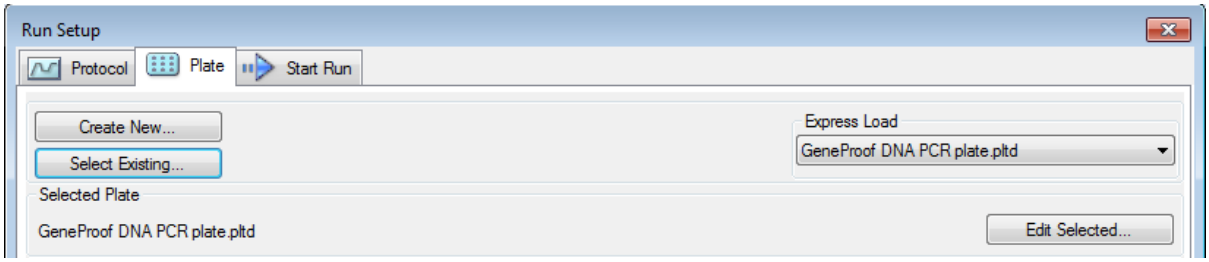


Fig. 1.4 Edit plate

2. Select all the wells that will not be filled in during the specific PCR examination and then click Clear Wells to delete them from the protocol.

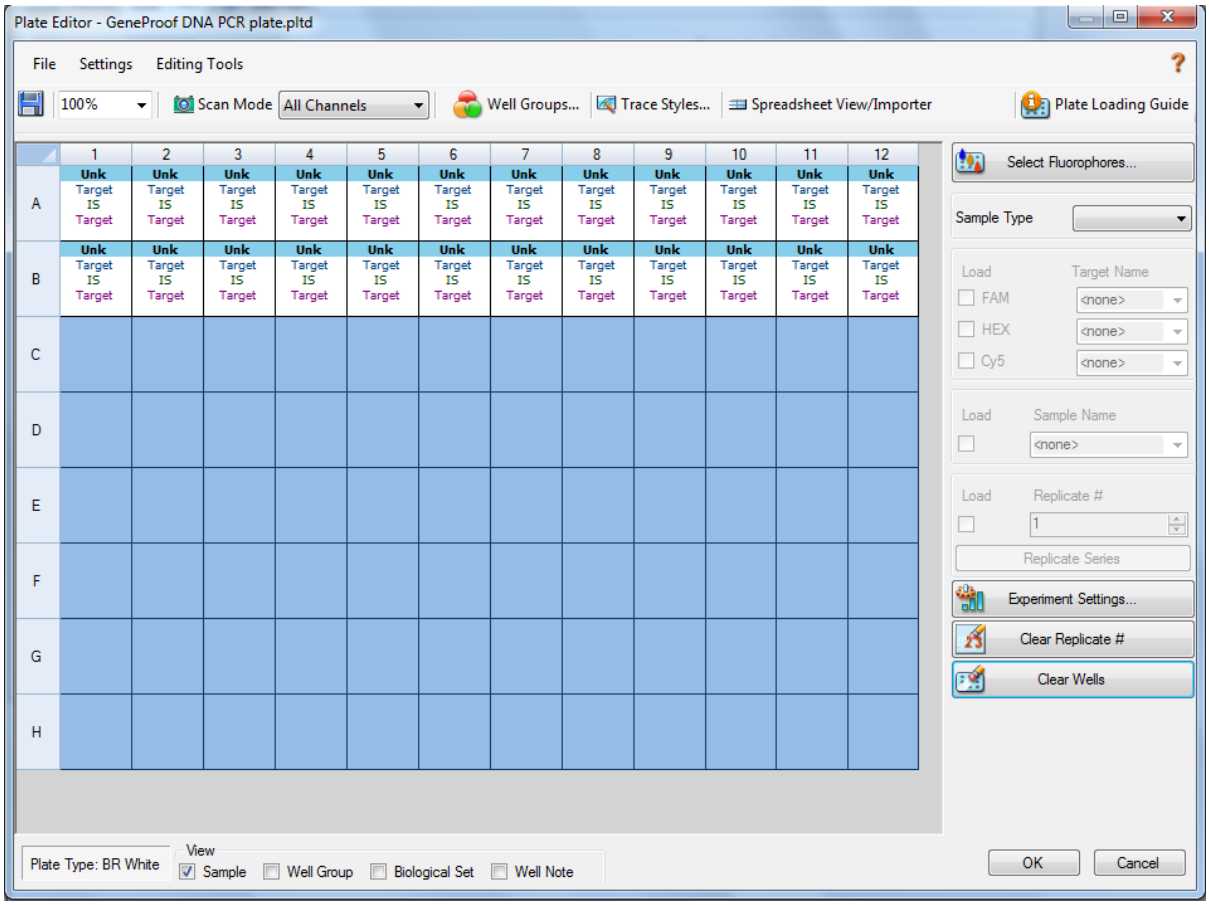


Fig. 1.5 Delete wells

1. Plate editing when using Qualitative Detection

1. Select the wells designated for positive control and then enter **Positive Control** into the **Sample Type** field.
2. Select the wells designated for negative control and then enter **Negative Control** into the **Sample Type** field.

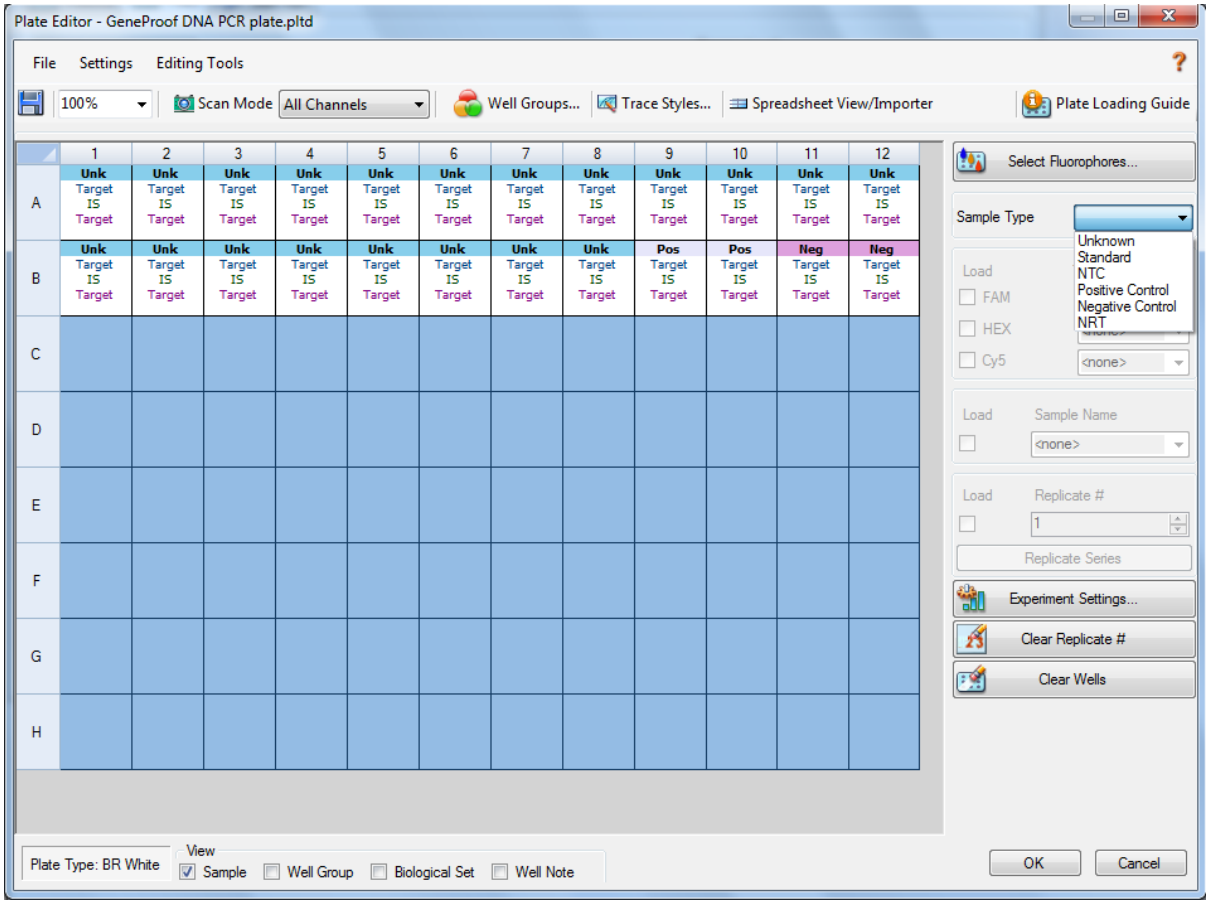


Fig. 1.6 Qualitative detection plate

2. Plate editing when using Quantitative Detection

1. Select wells designated for the calibration control series and then enter **Standard** into the **Sample Type** field and in the right column select the **target** detectors according to the Instruction for use of used GeneProof PCR kit..
2. For each calibration control in the **Concentration** field of the right column enter its concentration and check **Load** to confirm.
3. Select the cells designated for negative control and then enter **Negative Control** into the **Sample Type** field.

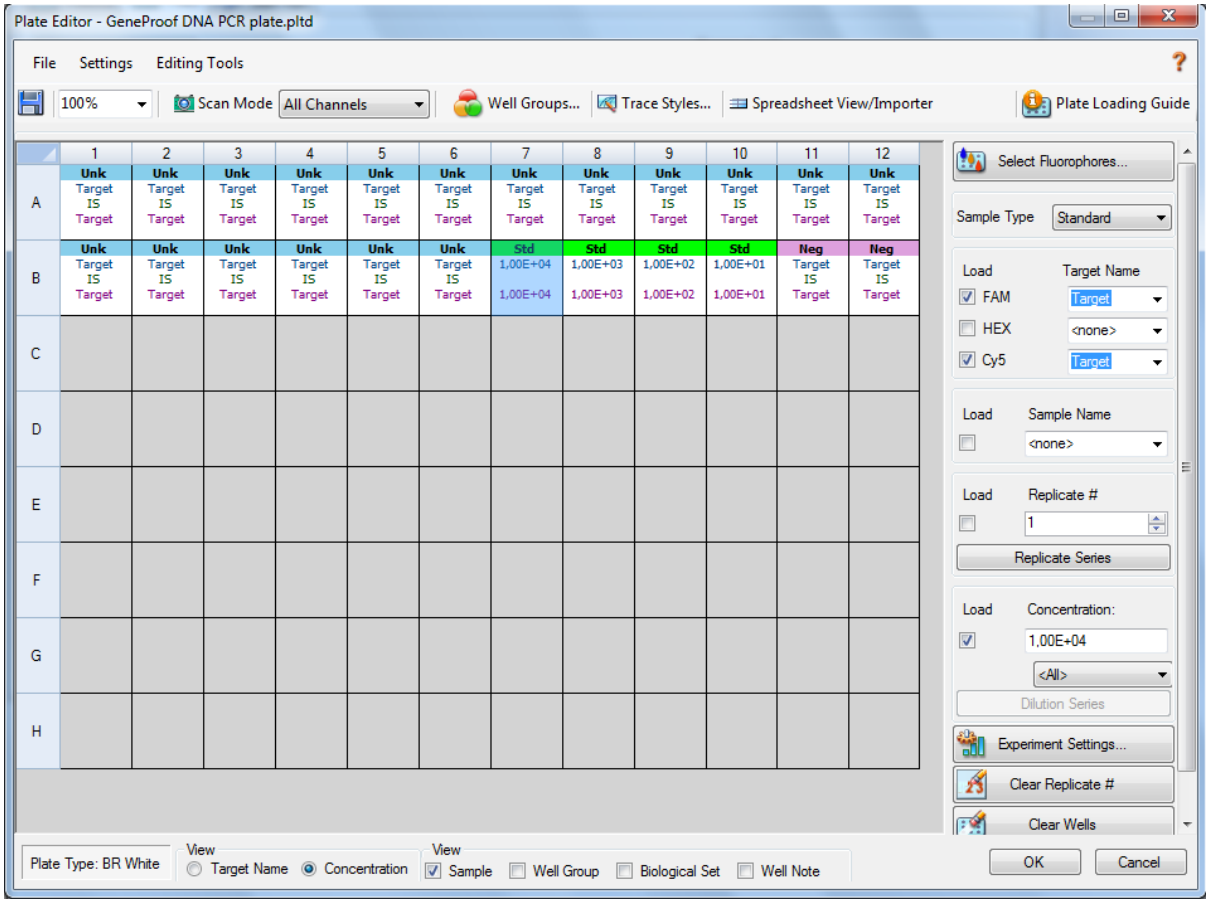


Fig. 1.7 Quantitative detection plate

1.3.4 Starting the experiment

1. Save the experiment before starting the device.

Select **File** in the main menu, click **Save As** and save the created protocol under the name **GP PCR-YYMMDD** as a **Plate File (*.pltd)** type into the **RealTimeProtocols** folder.

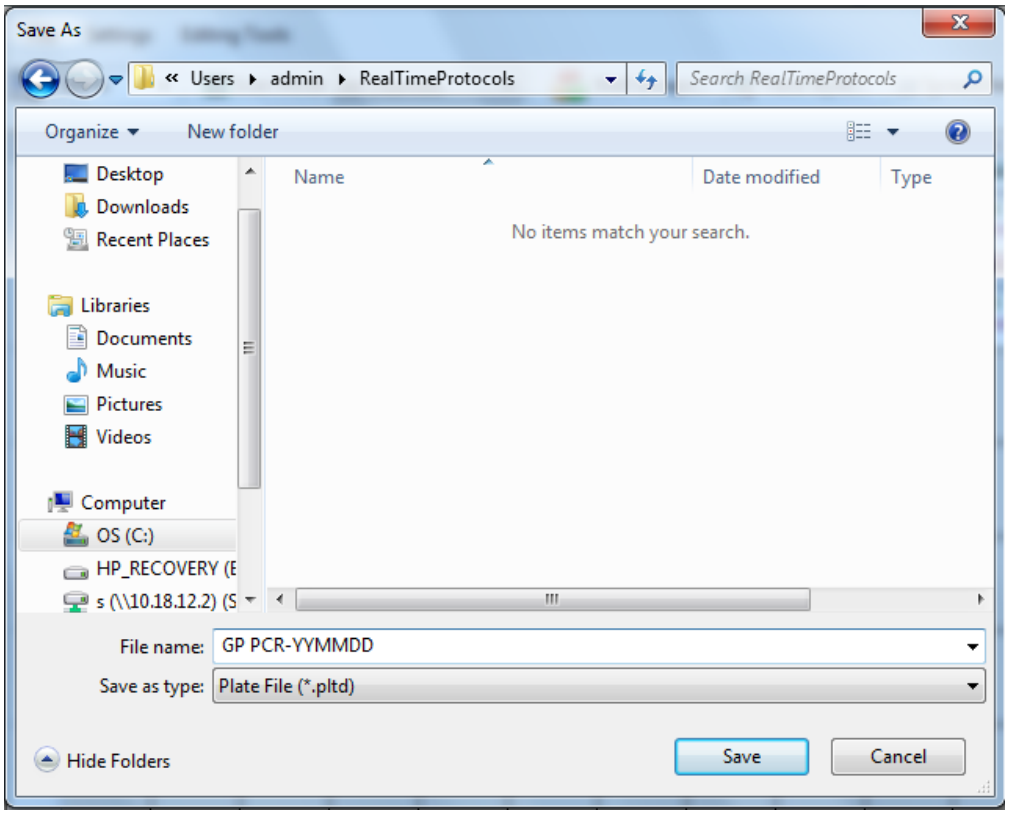


Fig. 1.8 Save edited plate

2. Starting PCR test

1. Select the **Start Run** tab in the **Run Setup** window.
2. Use the **Close Lid** button to close the device lid.
3. Use the **Start Run** button to start the test.

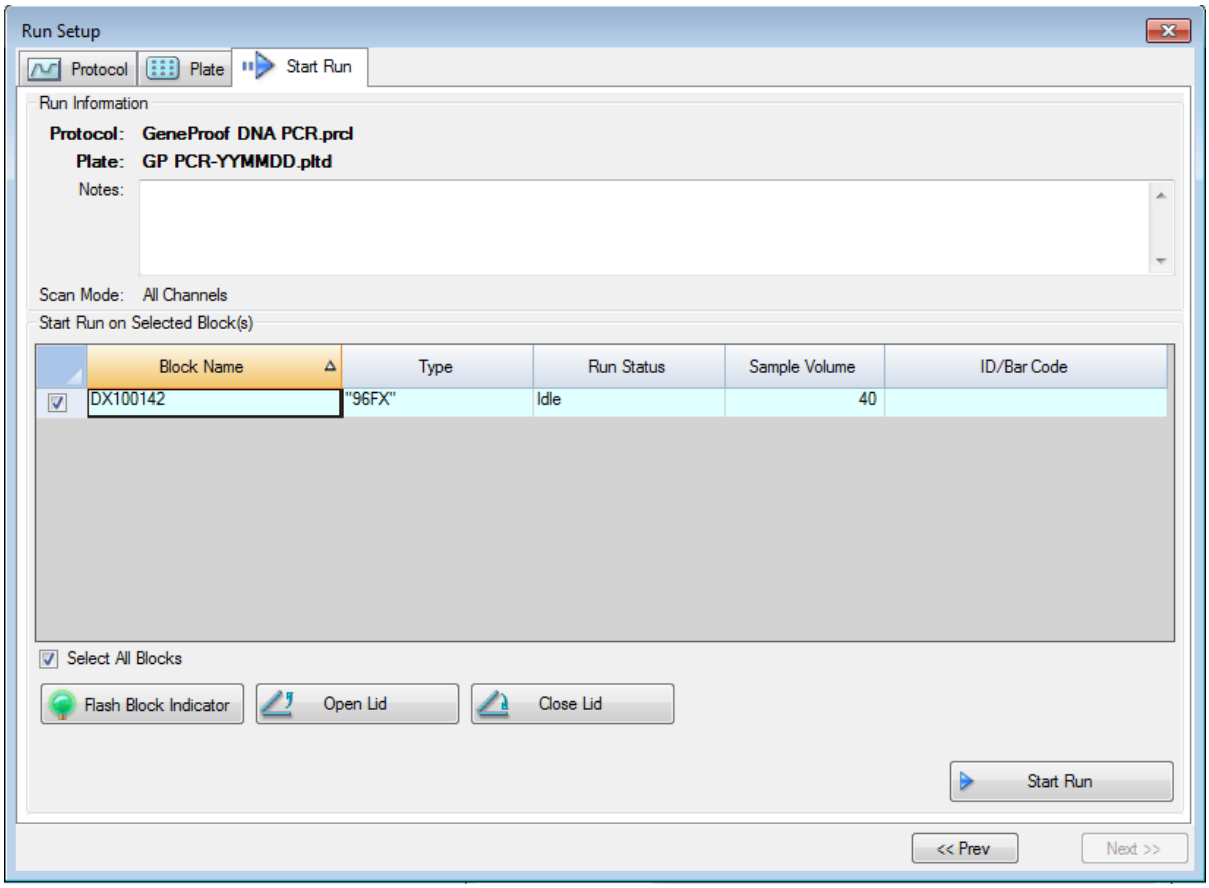


Fig. 1.9 Starting PCR test

1.4. Qualitative analysis of the result and evaluation of detection

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.

The **Data Analysis** box will automatically open at the end of the program.

1.4.5 Detection analysis of the studied microorganism

The first tab, Quantification, is designated for manual analysis.

1. In the options below the chart select the **FAM** channel and then click **Settings** in the main menu and select **Baseline Threshold**.

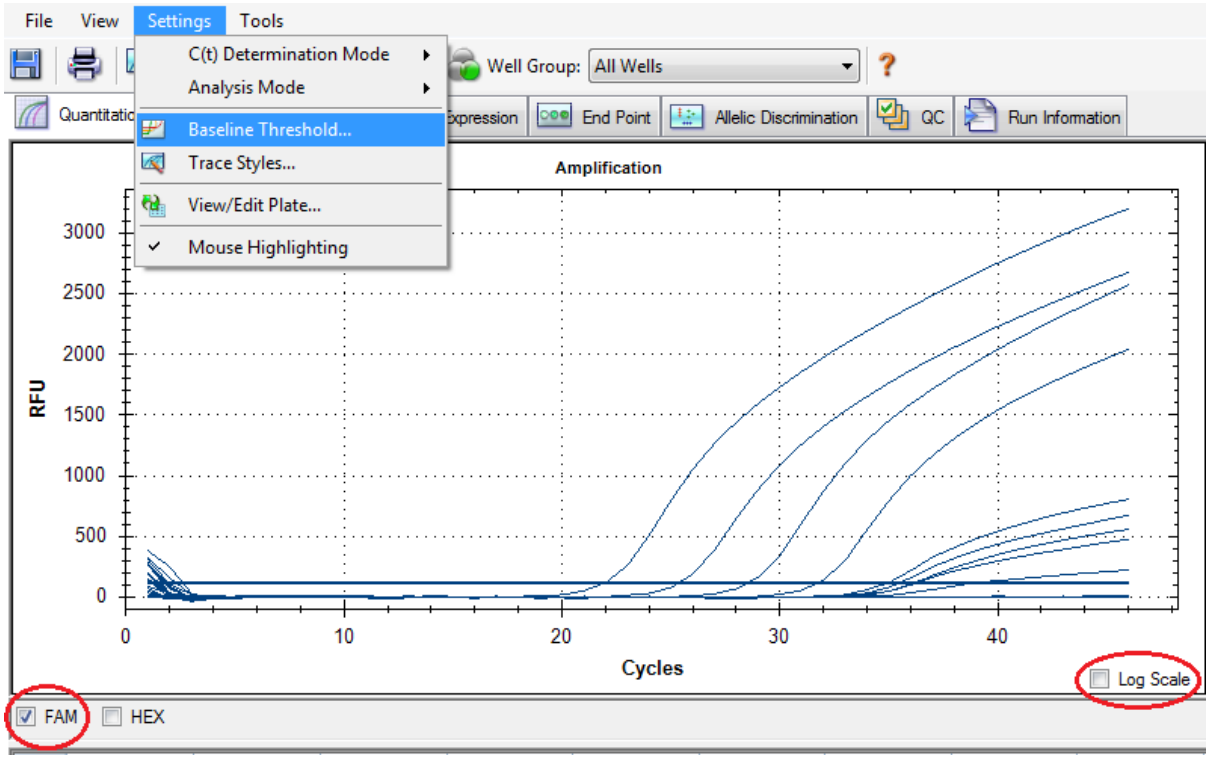


Fig. 1.10 Detection analysis of the studied microorganism

2. Select **Auto Calculated** for the **Baseline Cycles** and **Single Threshold** parameters.

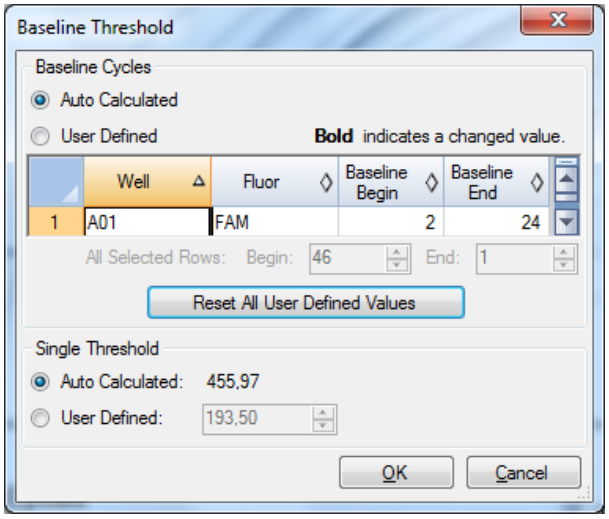


Fig. 1.11 Baseline Threshold setting

3. When needed you can also adjust the **Threshold** by moving the threshold line in the chart – for example if the Threshold is automatically set above the weakly positive curve. For easier identification of weakly positive samples use the logarithmic measure of the **Log Scale** chart to set the **Threshold**. For **Cq** values for the individual positive samples see the **table** beneath the chart.

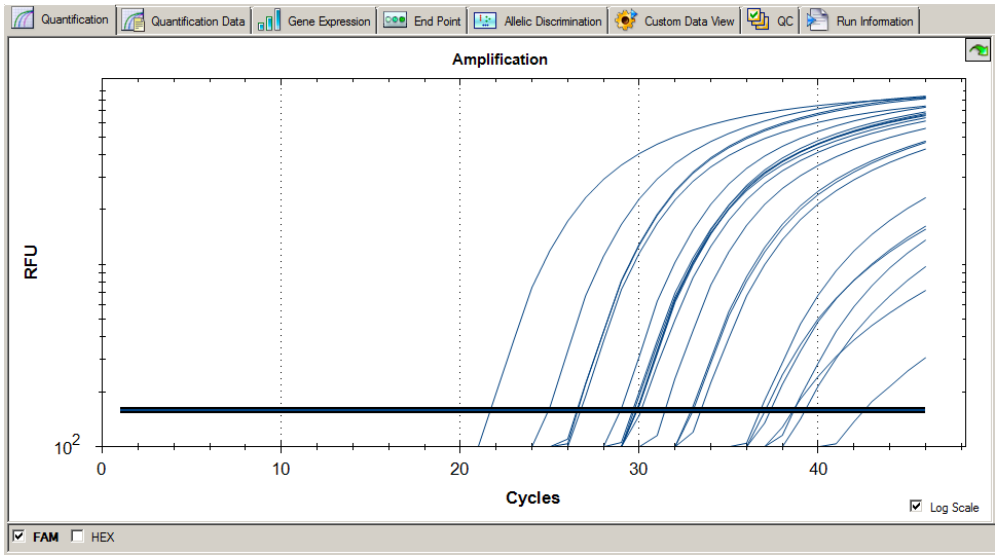


Fig. 1.12 Adjusting Threshold

Perform the same for the other **target** channels when using a multiplex PCR kit.
Perform evaluation according to the Instruction for use of the used GeneProof PCR kit.

1.4.6 Internal Standard detection analysis

1. In the options below the chart select the **HEX** channel and then click **Settings** in the main menu and select **Baseline Threshold**.

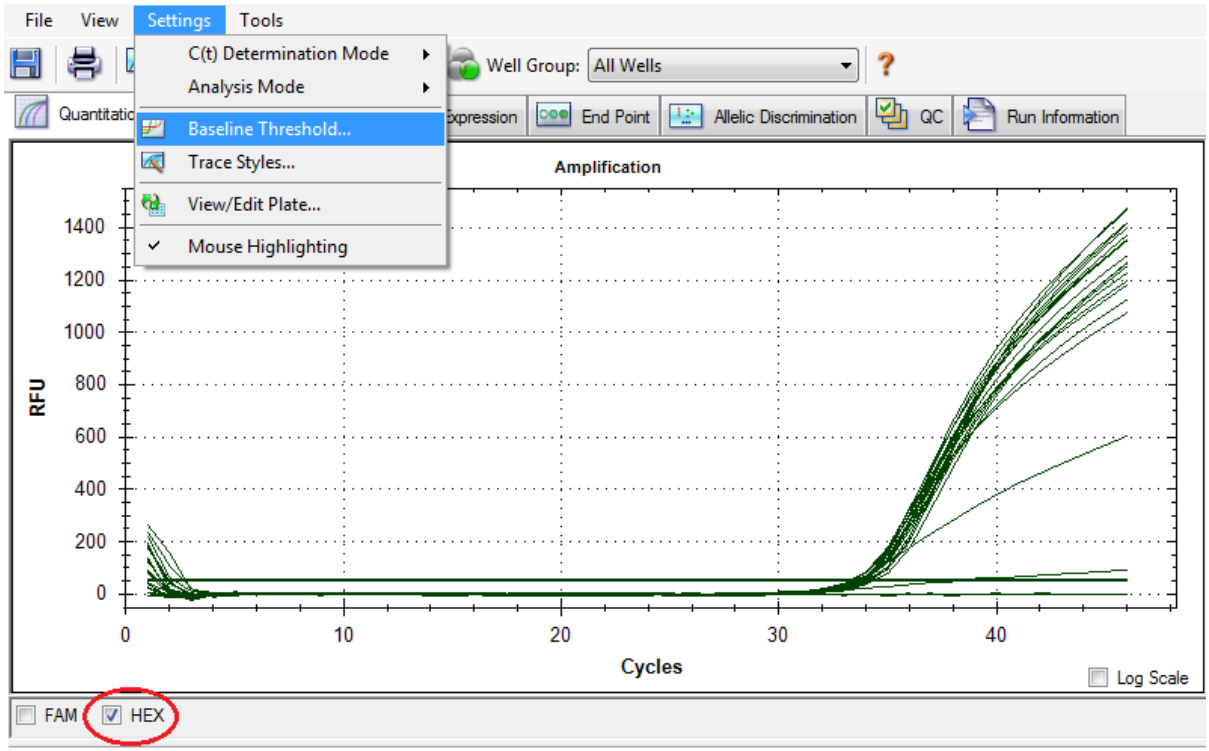


Fig.1.13 Internal Standard detection analysis

2. Select **Auto Calculated** for the **Baseline Cycles** and **Single Threshold** parameters.
3. When needed you can also adjust the **Threshold** by moving the threshold line in the chart – for example if the Threshold is automatically set above the weakly positive curve.

Perform evaluation according to the Instruction for use of the used GeneProof PCR kit.

1.5. Result quantitative analysis and detection evaluation

1. Evaluate the calibration quality. Calibration parameters are located under the **Standard Curve** calibration curve chart. The **R^2** parameter in a well-performed calibration achieves a minimum value of **0.98** or higher. If the **R^2** parameter is lower than **0.98**, move the **Threshold** and repeat the analysis.

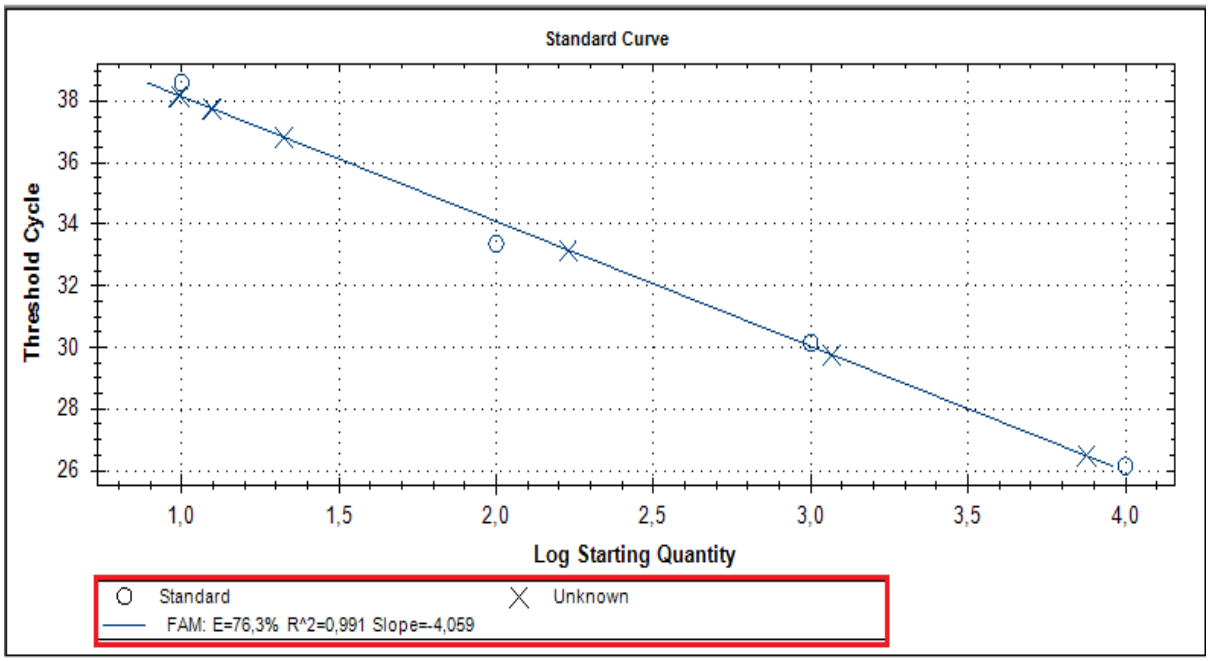
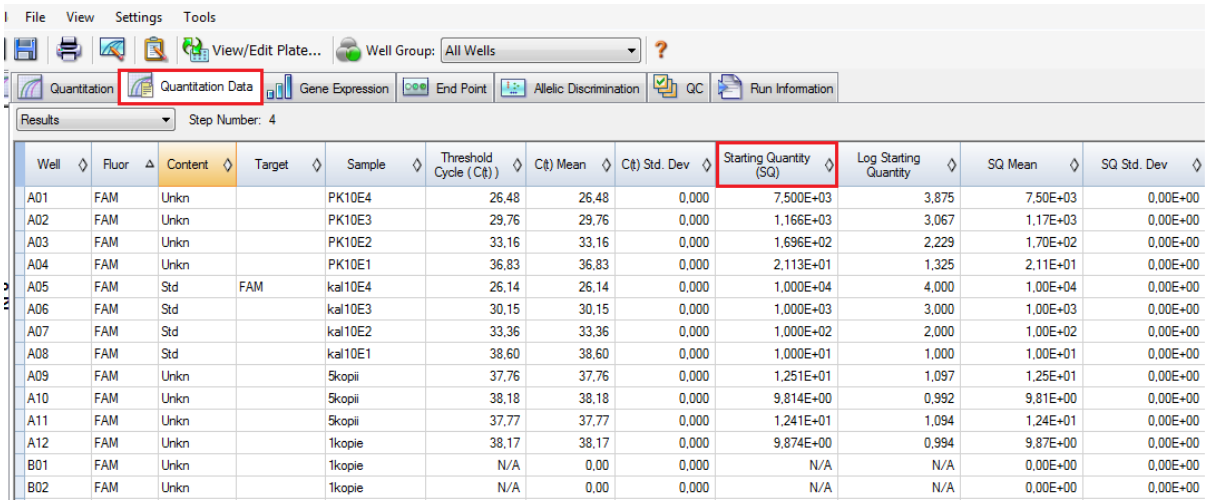


Fig. 1.14 Calibration curve

2. For details of the quantitative evaluation of the positive signal presence in the channel for the detected organism see the **Quantitation Data** tab.

   Perform evaluation, including the virus concentration calculation according to the Instruction for use of the used GeneProof PCR kit.



Well	Fluor	Content	Target	Sample	Threshold Cycle (C(t))	C(t) Mean	C(t) Std. Dev	Starting Quantity (SQ)	Log Starting Quantity	SQ Mean	SQ Std. Dev
A01	FAM	Unkn		PK10E4	26,48	26,48	0,000	7,500E+03	3,875	7,50E+03	0,00E+00
A02	FAM	Unkn		PK10E3	29,76	29,76	0,000	1,166E+03	3,067	1,17E+03	0,00E+00
A03	FAM	Unkn		PK10E2	33,16	33,16	0,000	1,696E+02	2,229	1,70E+02	0,00E+00
A04	FAM	Unkn		PK10E1	36,83	36,83	0,000	2,113E+01	1,325	2,11E+01	0,00E+00
A05	FAM	Std	FAM	kal10E4	26,14	26,14	0,000	1,000E+04	4,000	1,00E+04	0,00E+00
A06	FAM	Std		kal10E3	30,15	30,15	0,000	1,000E+03	3,000	1,00E+03	0,00E+00
A07	FAM	Std		kal10E2	33,36	33,36	0,000	1,000E+02	2,000	1,00E+02	0,00E+00
A08	FAM	Std		kal10E1	38,60	38,60	0,000	1,000E+01	1,000	1,00E+01	0,00E+00
A09	FAM	Unkn		5kopii	37,76	37,76	0,000	1,251E+01	1,097	1,25E+01	0,00E+00
A10	FAM	Unkn		5kopii	38,18	38,18	0,000	9,814E+00	0,992	9,81E+00	0,00E+00
A11	FAM	Unkn		5kopii	37,77	37,77	0,000	1,241E+01	1,094	1,24E+01	0,00E+00
A12	FAM	Unkn		1kopie	38,17	38,17	0,000	9,874E+00	0,994	9,87E+00	0,00E+00
B01	FAM	Unkn		1kopie	N/A	0,00	0,000	N/A	N/A	0,00E+00	0,00E+00
B02	FAM	Unkn		1kopie	N/A	0,00	0,000	N/A	N/A	0,00E+00	0,00E+00

Fig. 1.15 Quantitative evaluation details

2. Genetic diagnostics

This chapter describes in detail the process of using GeneProof PCR kits for genetic diagnostics using the CFX96 / CFX Connect Real-Time PCR Detection System a Dx Real-Time System.

2.1. Device Programming

In case the software does not include predefined templates, it is necessary, before the first use with GeneProof PCR kits, to programme them according to the Instruction for use of the used GeneProof kits , or download them from the product site of the used GeneProof PCR kits from the website of the company www.geneproof.com .
Save the downloaded templates on your local disc to the ExpressLoad file.

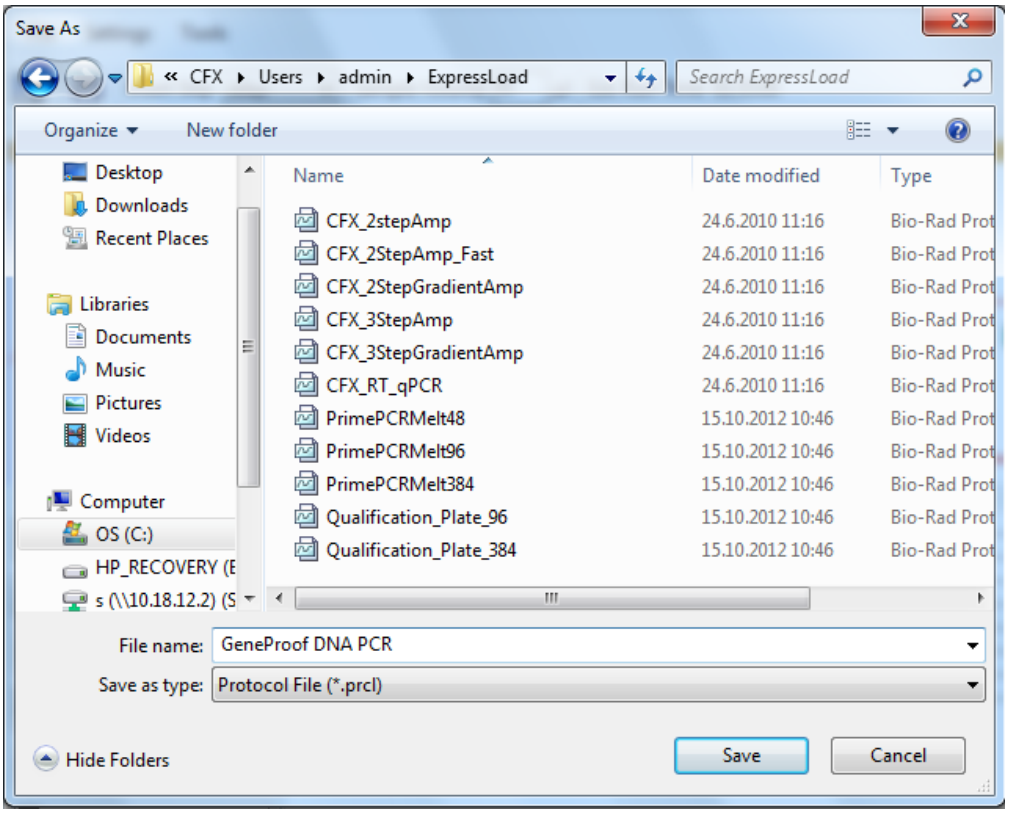


Fig. 2.1 Save template

With each next usage of GeneProof PCR kits continue from the chapter 2.2 Starting the PCR amplification.

2.2. PCR Amplification start

2.2.1 Open a saved PCR template profile

1. Open **Bio-Rad CFX Manager**.
2. In the **Startup Wizard** box select **CFX96** and click **User-defined**.
3. Click **OK**.
4. In the **Protocol** tab of the **Run Setup** box, in section **Express Load** select file for **the concrete type of examination.**

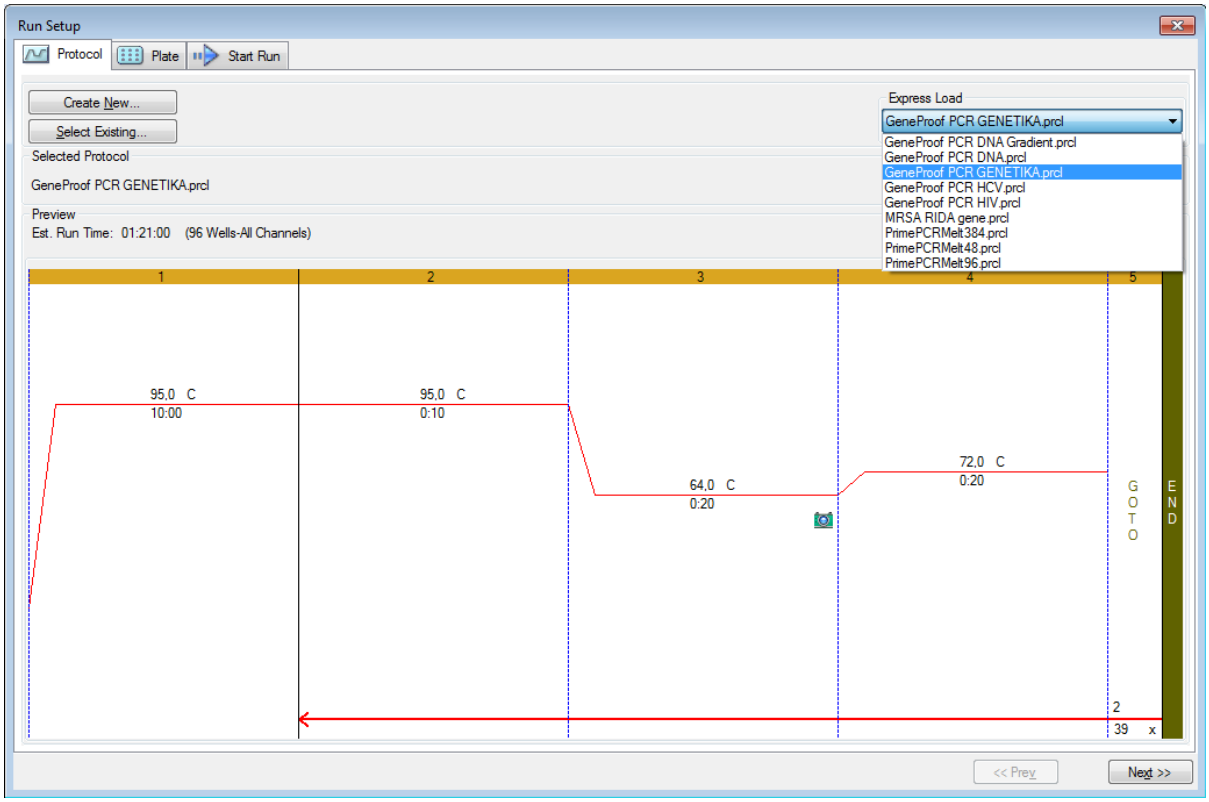


Fig. 2.2 Using the saved amplification profile

2.2.2 Using the saved plate

1. In the **Express Load** section of the **Plate** tab select **the type of examination**.

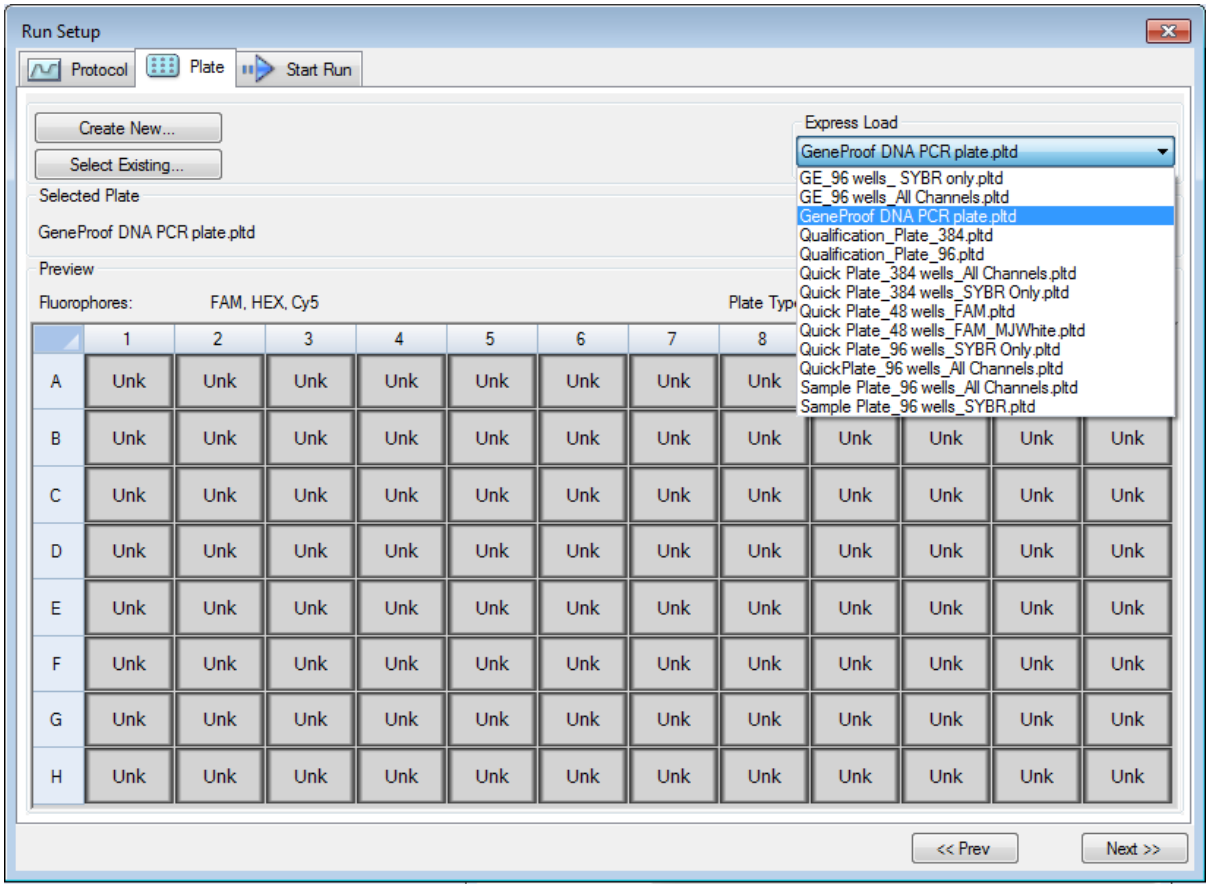


Fig. 2.3 Using the saved plate

2. Click the **Edit Selected** button to edit the PCR plate for the specific PCR examination.

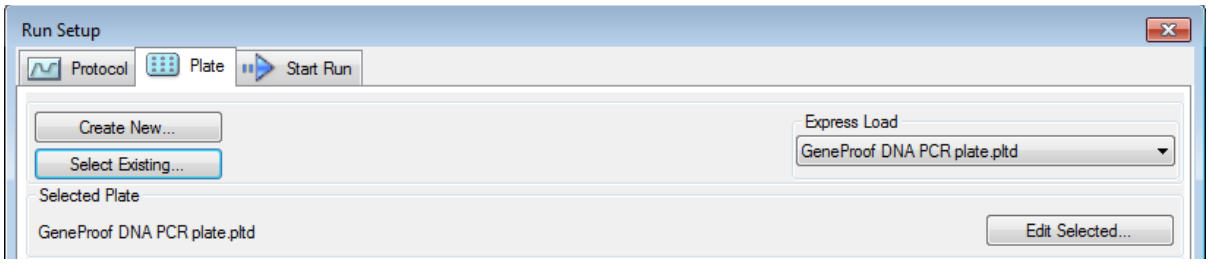


Fig. 2.4 Edit plate

3. Select all the wells that will not be filled in during the specific PCR examination and then click **Clear Wells** to delete them from the protocol.

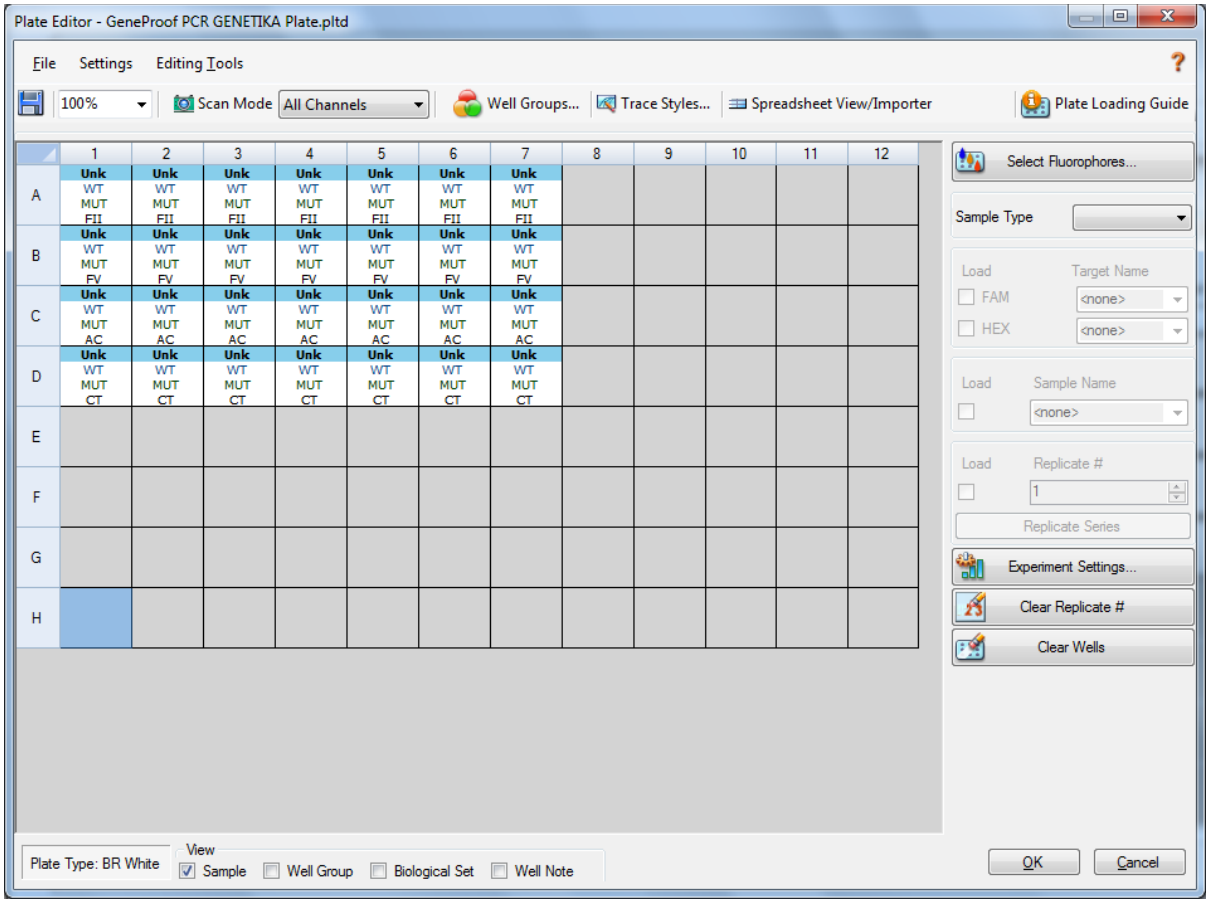


Fig. 2.5 Delete wells

2.2.3 Plate editing

1. Select the wells designated for positive control and then enter **Positive Control** into the **Sample Type** field.
2. Select the wells designated for negative control and then enter **Negative Control** into the **Sample Type** field.
3. Samples can be named by entering a name in the **Sample Name** field of the appropriate well and selecting Load.

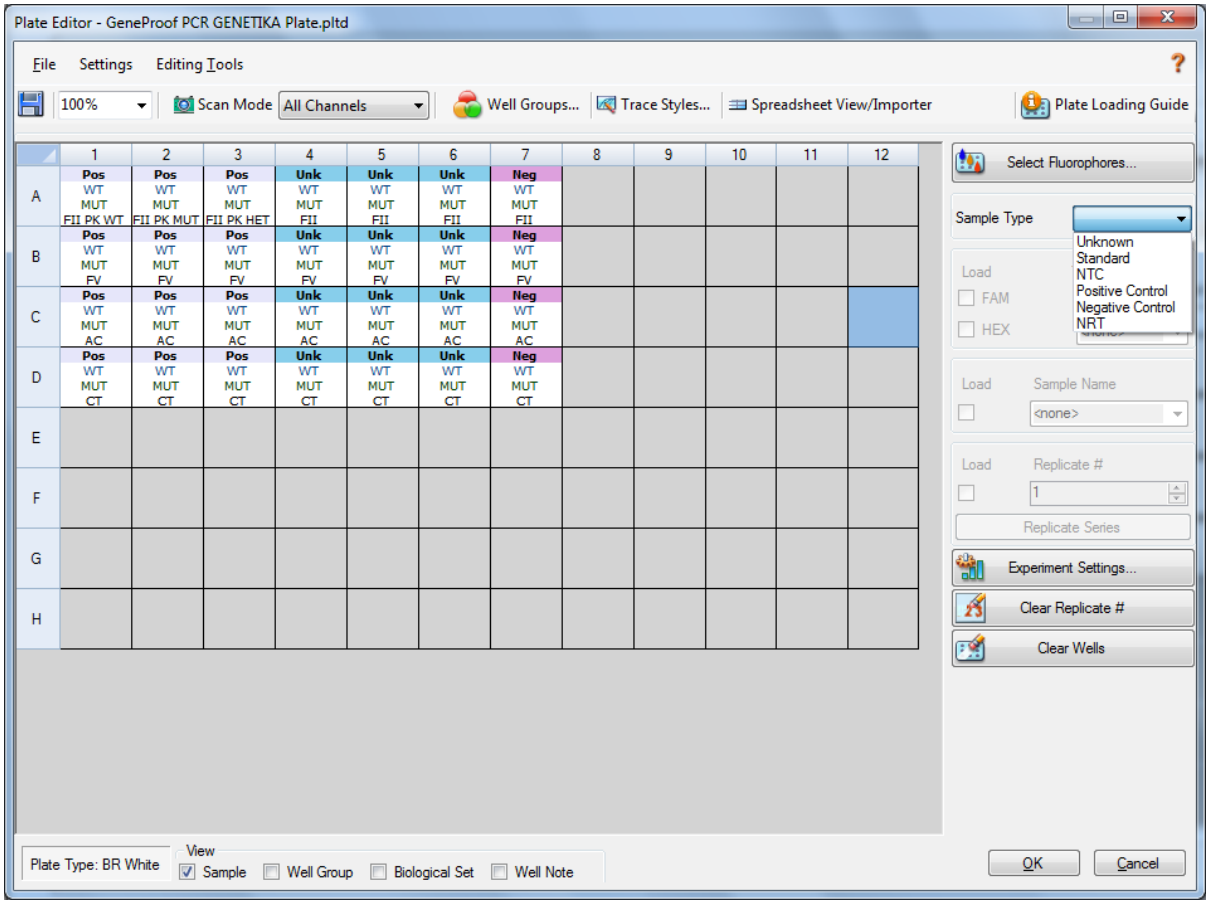


Fig. 2.6 Plate editing

2.2.4 Edited plate saving

1. Select File in the main menu, click Save As and save the created protocol under the name **GP PCR-YYMMDD** as a **Plate File (*.pltd)** type into the RealTimeProtocols folder.

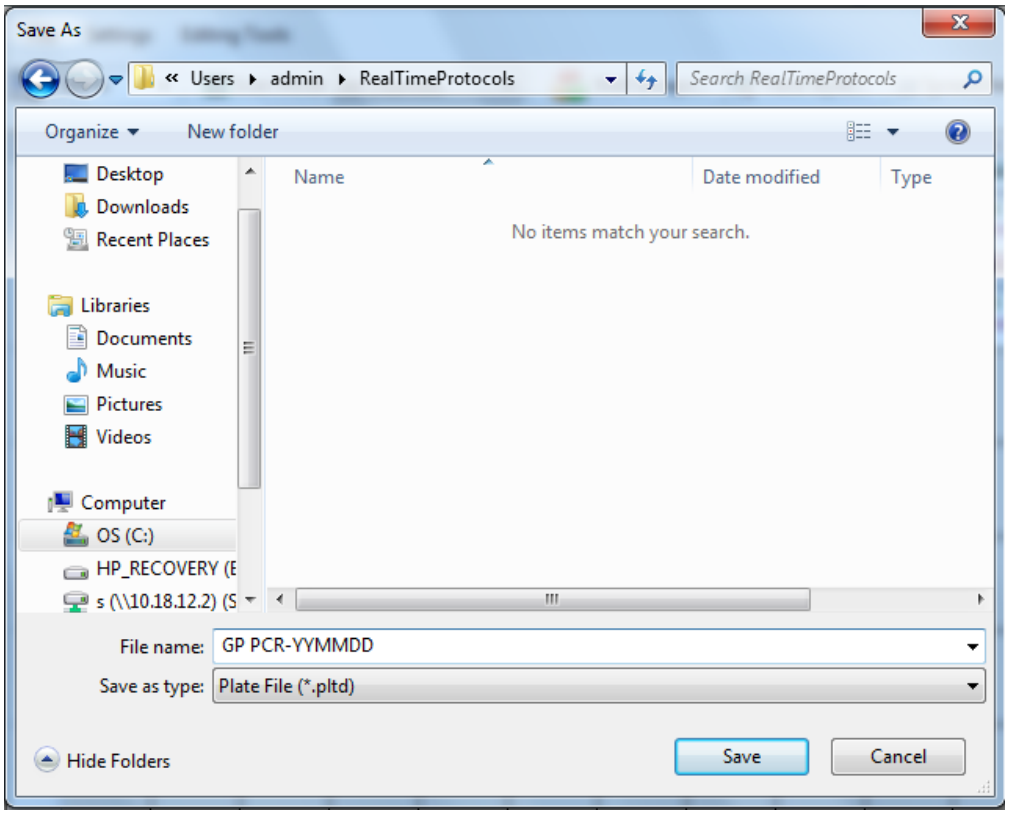


Fig. 2.7 Save edited plate

2.2.5 Starting the PCR test

1. Select the **Start Run** tab in the **Run Setup** window.
2. Use the **Close Lid** button to close the device lid.
3. Use the **Start Run** button to start the test.

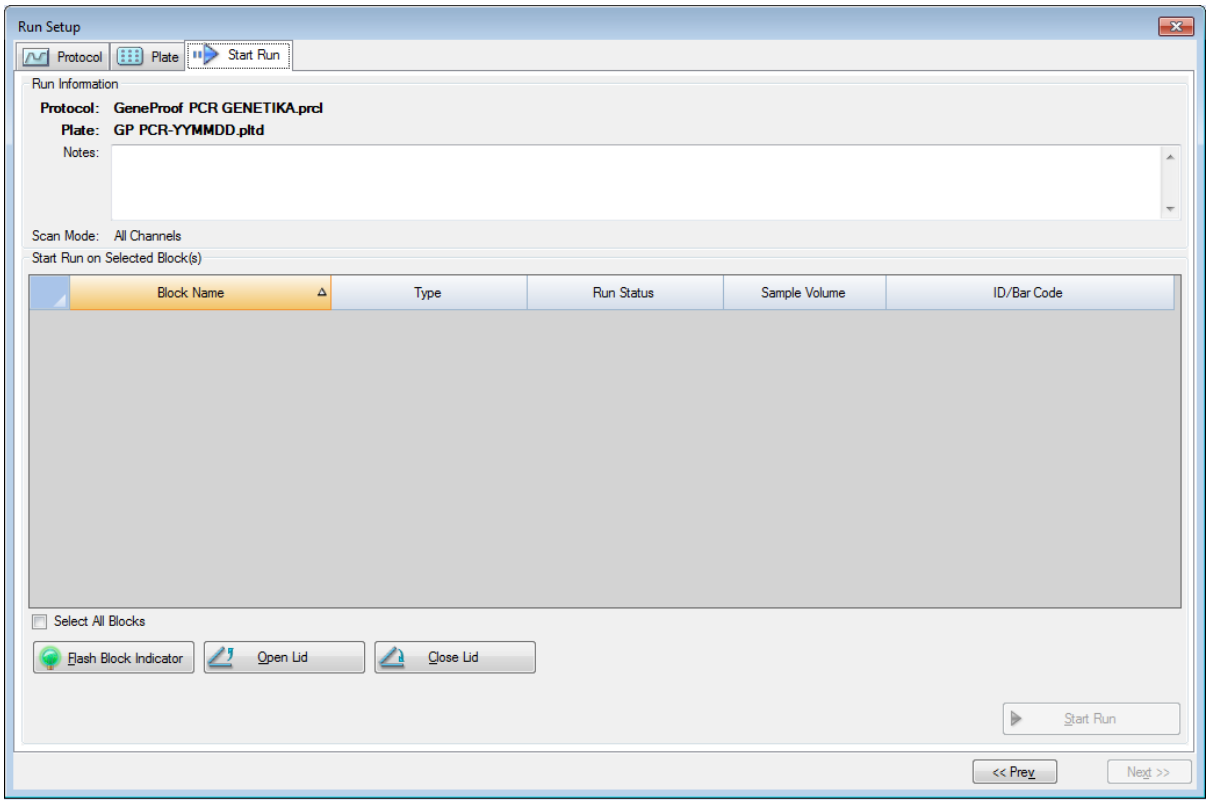


Fig. 2.8 Starting PCR test

2.3. Analysis of the result and evaluation of detection

2.3.6 Setting the Baseline and Treshold parametrs

The **Data Analysis** box will automatically open at the end of the program.

1. Mark 3 positive controls in the PCR plate schema and use your mouse to move the **Threshold** slider to a position when **PK WT** is positive only in the **FAM** channel, **PK MUT** is positive only in the **HEX** channel and **PK HET** is positive **in both** channels.

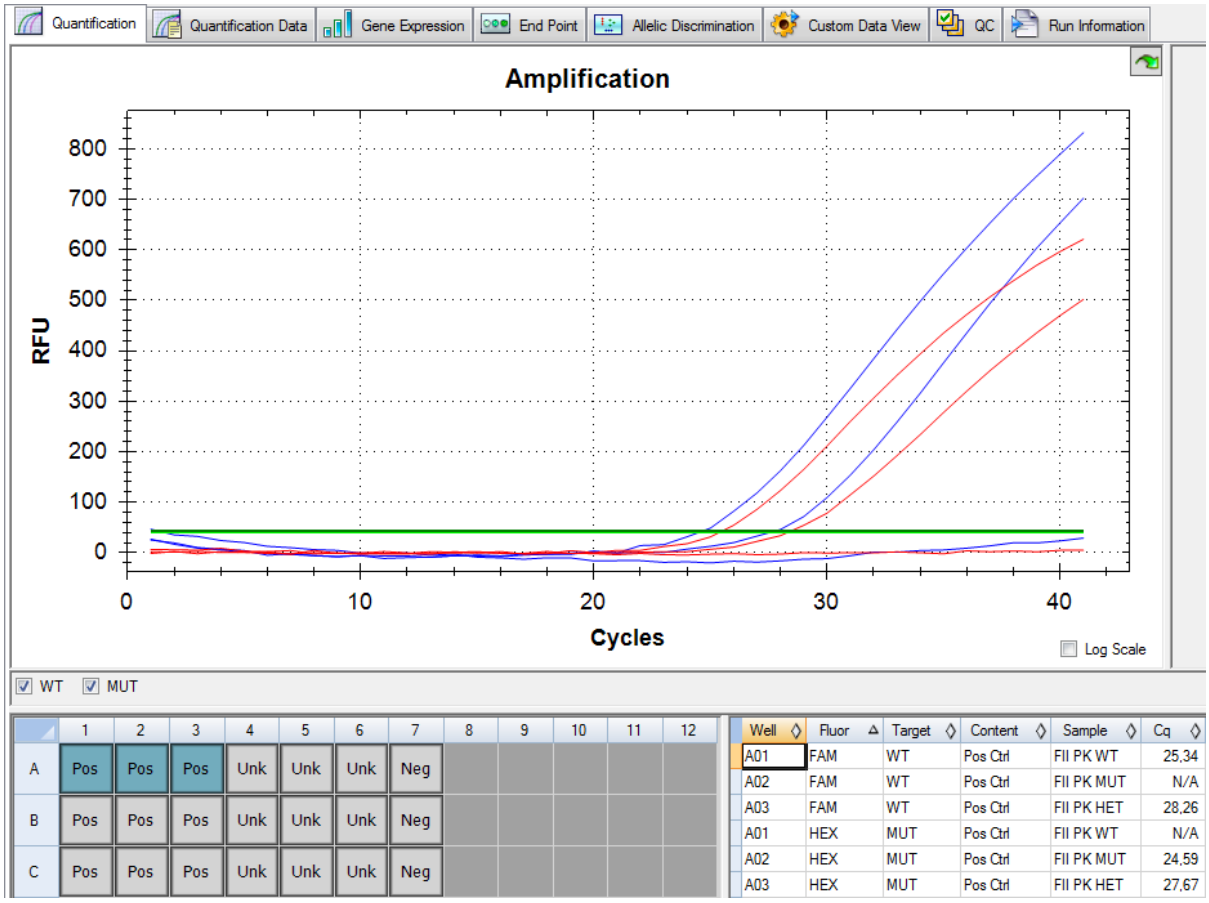
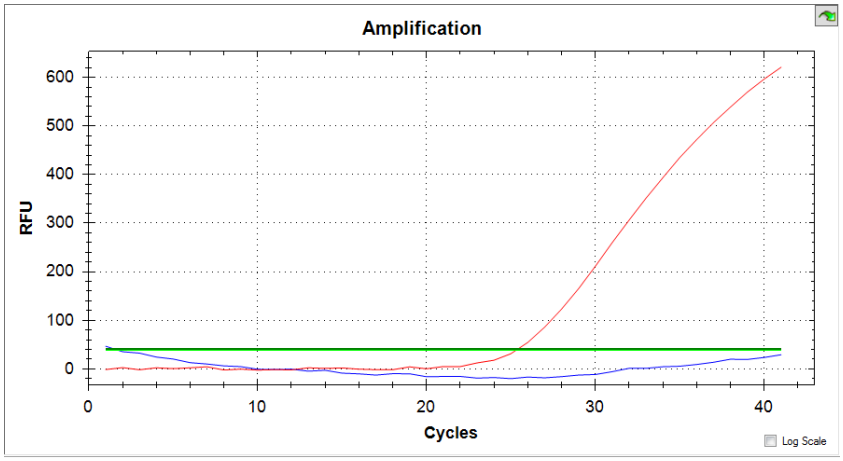


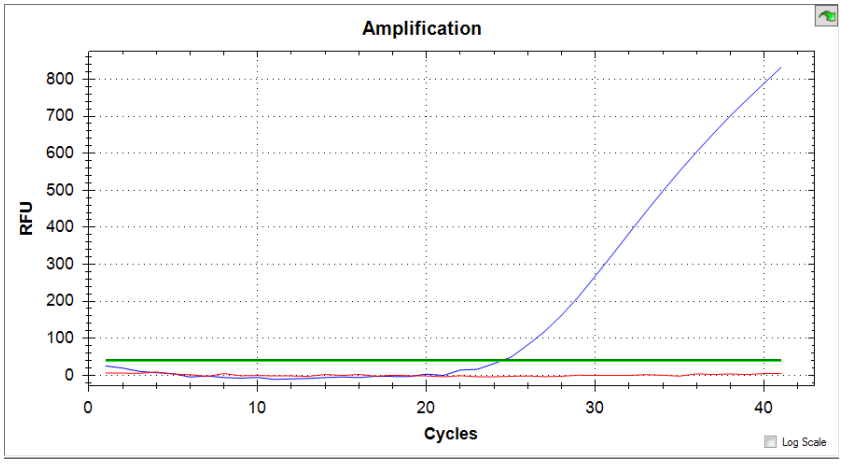
Fig. 2.9 Treshold settings

Perform evaluation according to the Instruction for use of the used GeneProof PCR kit.

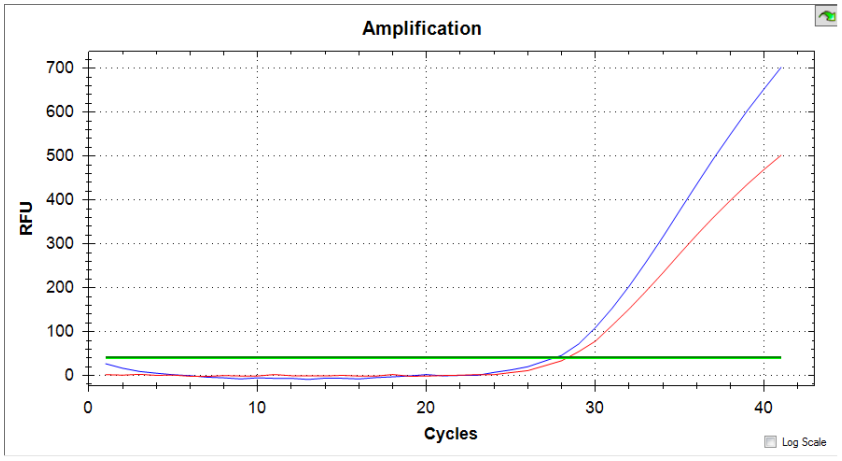
2.3.7 Examples of typical curves



Obr. 2.1 Typical WT curve



Obr. 2.2 Typical MUT curve



Obr. 2.3 Typical HET curve

3. Customer Service

We appreciate all our customers and besides high-quality products we provide, in cooperation with our partners, above-standard customer service including the following:

- Demonstration PCR kits
- Express deliveries
- Quick solution of issues related to the supplied products – service guaranteed within 24 hours from the time of report
- Consultations concerning technological and clinical interpretations

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

- Kit name
- Issue definition
- Kit lot - specified on the kit package
- Used device
- File with the examination log from the used device, if available

4. Contact Information

Support and customer care

Phone: +420 730 176 222
e-mail: support@geneproof.com

Orders

Phone: +420 543 211 679
e-mail: sales@geneproof.com