GeneProof
Molecular diagnostics for your routine

DEVICE MANUAL

ThermoFisher

Applied Biosystems 7500 & 7500 Fast Real-Time PCR 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 devices 7500 Real-Time PCR System and 7500 Fast Real-Time PCR System.

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 and open them in the software 7500 Software.exe.

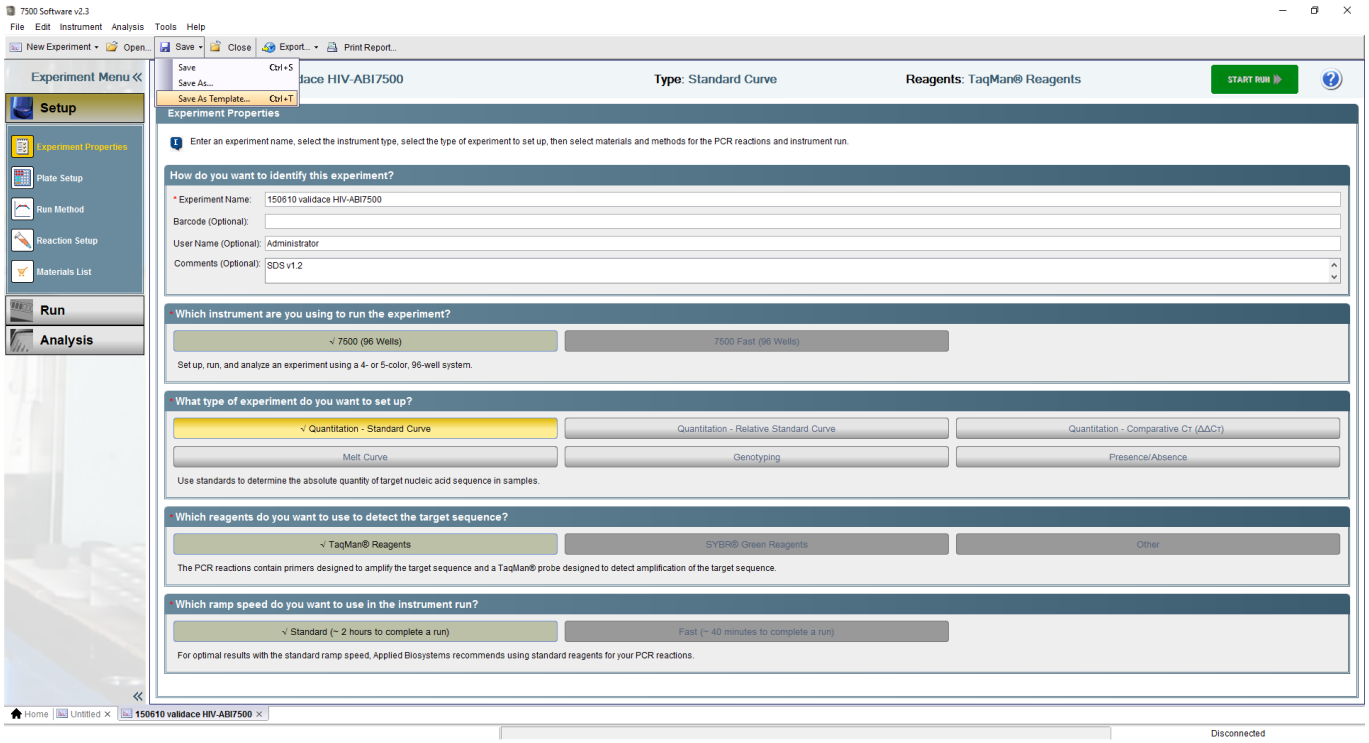


Fig. 1.1: Save template

After saving, the template can be opened from the file Templates**.** 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 Starting the software

1. *Start **the 7500 Software**.*
2. *In the **Set Up** column, select **Template.***
3. *Open template for the given GeneProof PCR kit.*

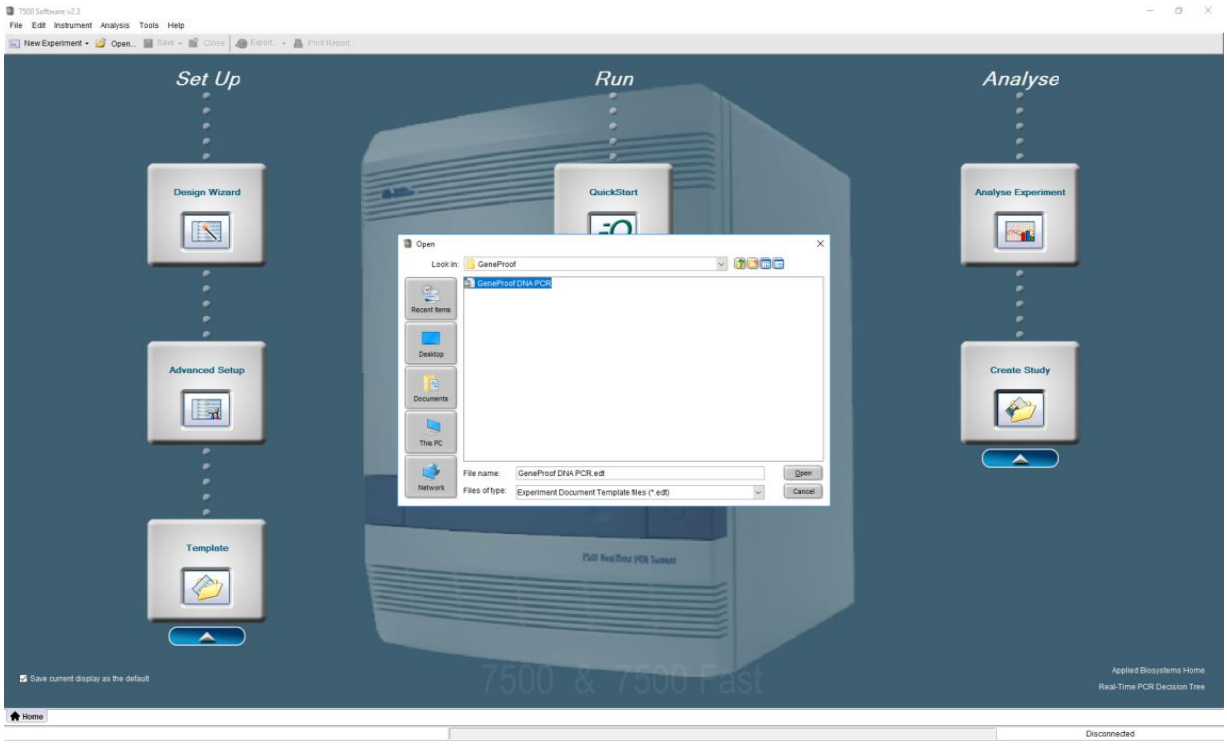


Fig. 1.2 Opening the template

1.3.2 PCR plate editing

1. In **Experiment Properties**, enter experiment name into the **Experiment Name** row.
2. In **Plate Setup**, use **Add New Target** to define targets according to the kits used in the experiment.

E.g. for HSV detection (3 channels) set Target Name: **HSV 1**, Reporter: **FAM**, Quencher: **None**; Target Name: **HSV IC**, Reporter: **JOE**, Quencher: **None** and Target Name: **HSV 2**, Reporter: **Cy5**, Quencher: **None**. For MT detection (2 channels) set Target Name: **MT**, Reporter: **FAM**, Quencher: **None** and Target Name: **MT IC**, Reporter: **JOE**, Quencher: **None**. Use **Save Target** and **Add Saved Target** to save and reuse targets.

3. Use **Add New Sample** to define samples.
4. In the case of qualitative detection, define positive control as a sample, e.g. **MT Positive Control**.

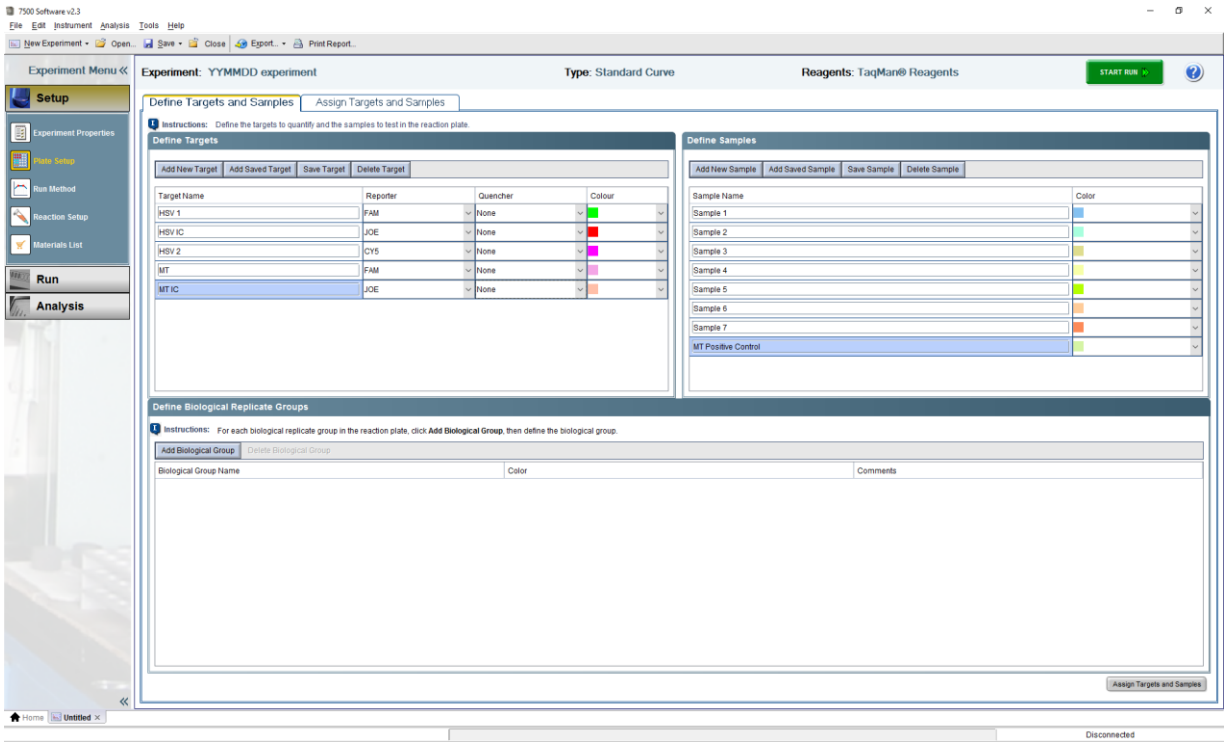


Fig. 1.3 Define targets and samples

5. Switch to the **Assign Targets and Samples** tab.
6. Assign the appropriate targets and samples (controls) for used wells by checking the boxes.
7. For Negative Controls set **N** in the **Task** column of pathogen target.
8. For calibrators (in the case of quantitative detection) set **S** in the **Task** column of pathogen target and enter the corresponding quantity in the **Quantity** column – according to the Package Insert of the used GeneProof PCR kit, e.g.: 10 000, 1 000, 100 a 10.
9. Select **ROX** passive reference (in the case of the Geneproof PCR kit with TEXAS RED detection, select **None**).

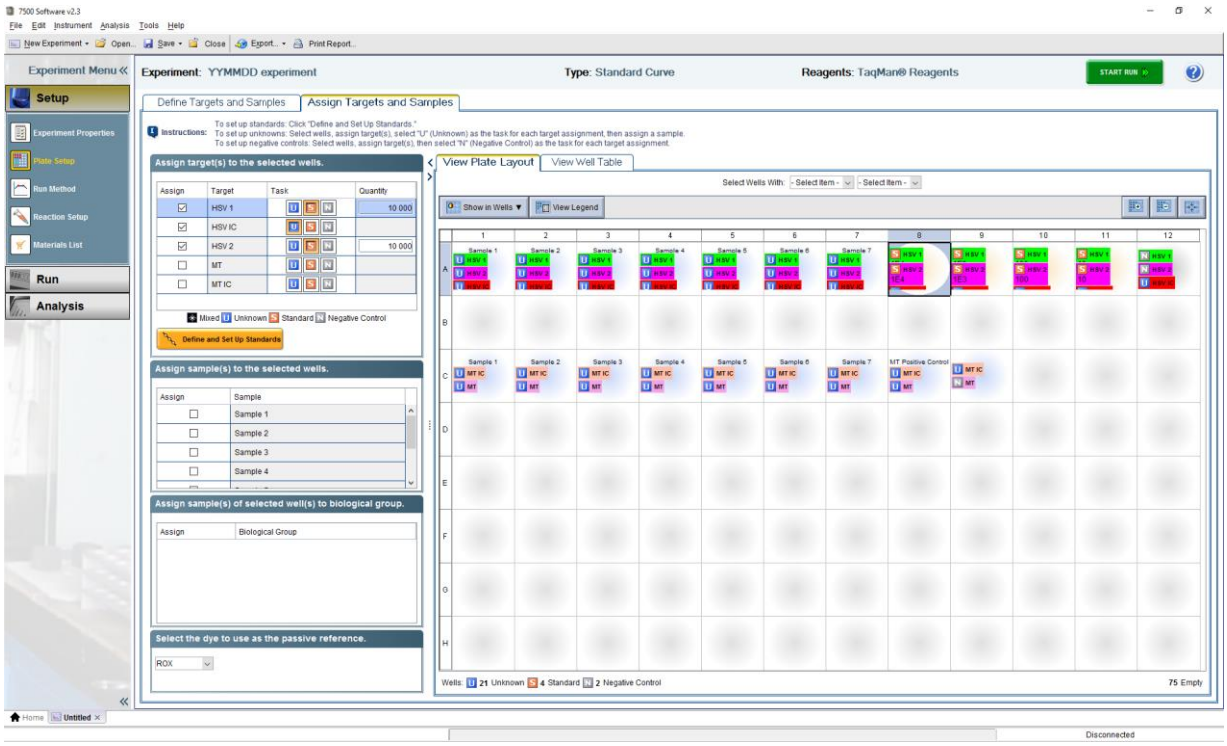


Fig. 1.4 Assign targets and samples

1.3.3 Starting the experiment

Save the experiment before starting the device.

1. Select Save in the main menu and save the created experiment as the **Experiment Document Single files (*.eds)** file type. To make search easier it is recommended to create the **Experiments folder**.

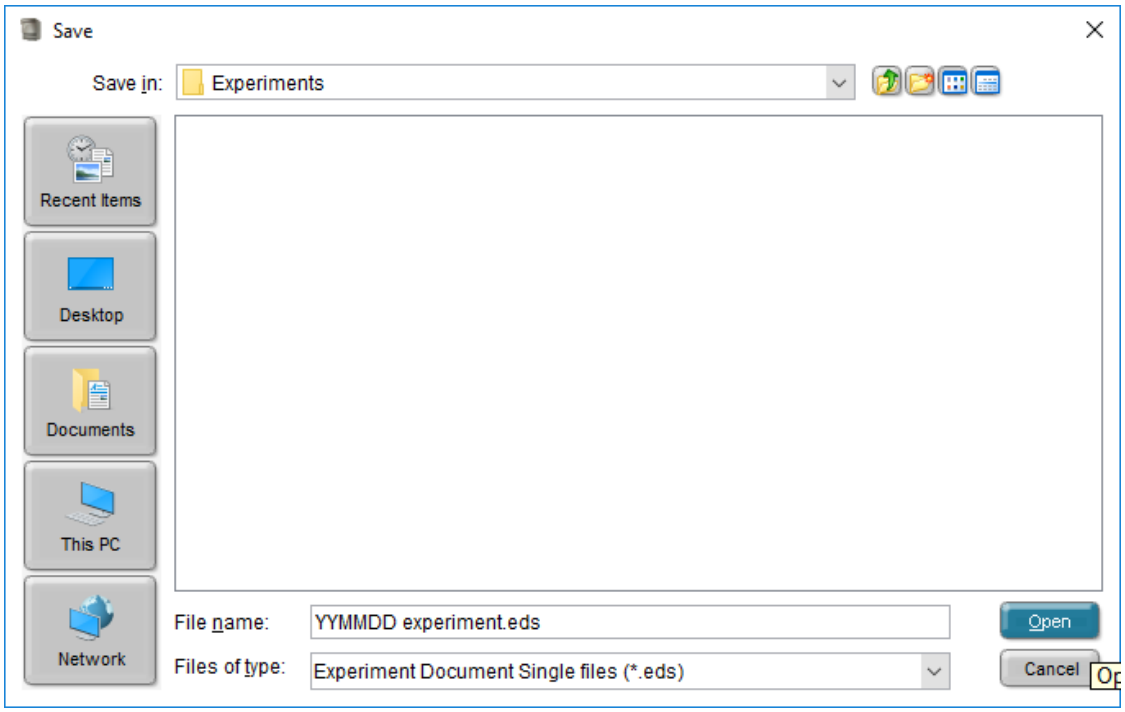


Fig. 1.5 Save experiment

2. Click 

button to start the experiment.

1.4. Qualitative analysis of the result and evaluation of detection for microbiological detection

PCR detection result 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.

When the experiment is finished, **Amplification Plot** is displayed.

Fig. 1.6 Amplification curves

1.4.4 Analysis settings

1. Open **Analysis Settings**.
2. Uncheck **Use Default Settings** for all targets.
3. Uncheck **Automatic Threshold** and leave original value.
4. Uncheck **Automatic Baseline** and leave **Start Cycle 3** and **End Cycle 15**.
5. Click **Apply Analysis Settings** to confirm.

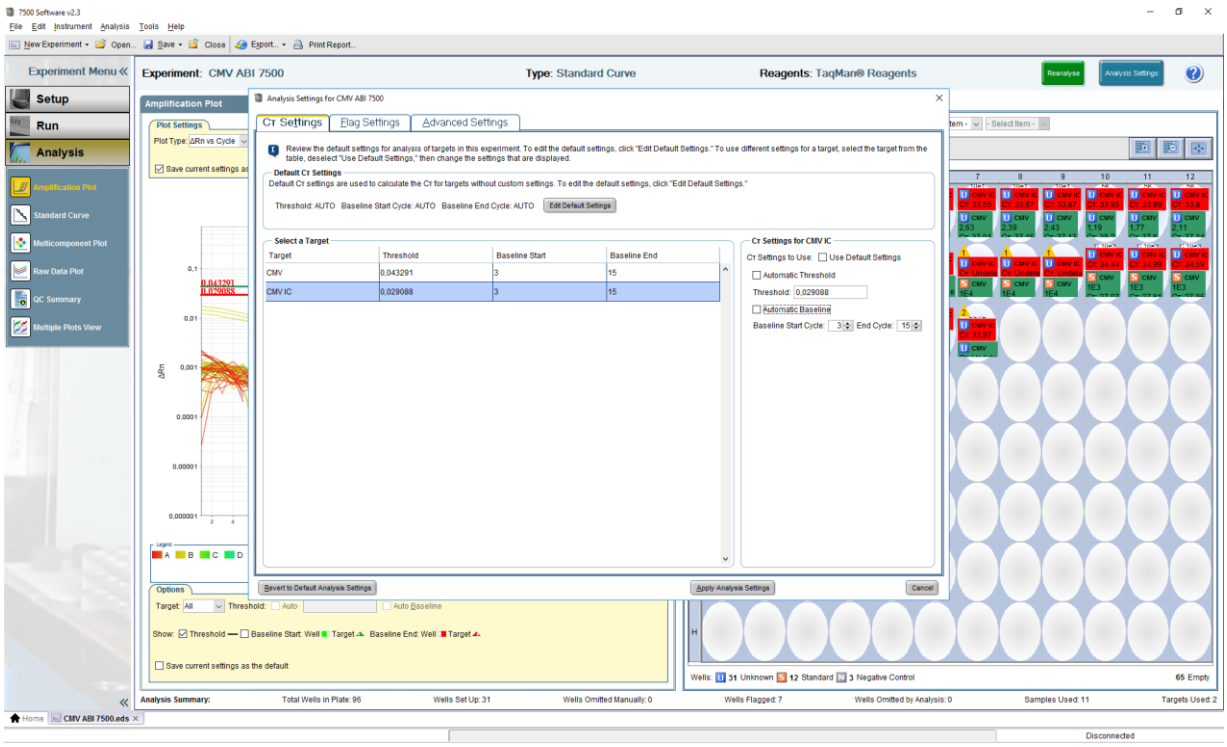


Fig. 1.7 Analysis settings

1.4.5 Detection analysis of the studied microorganism

1. In **Plot Settings,** select **Plot Type: ΔRn vs Cycle** and **Color: Target**.
2. In **Options**, select the target microorganism (e.g. CMV) in the **Target** combo box and move the Threshold line just above the reaction basal noise.
3. Click **Reanalyse**.

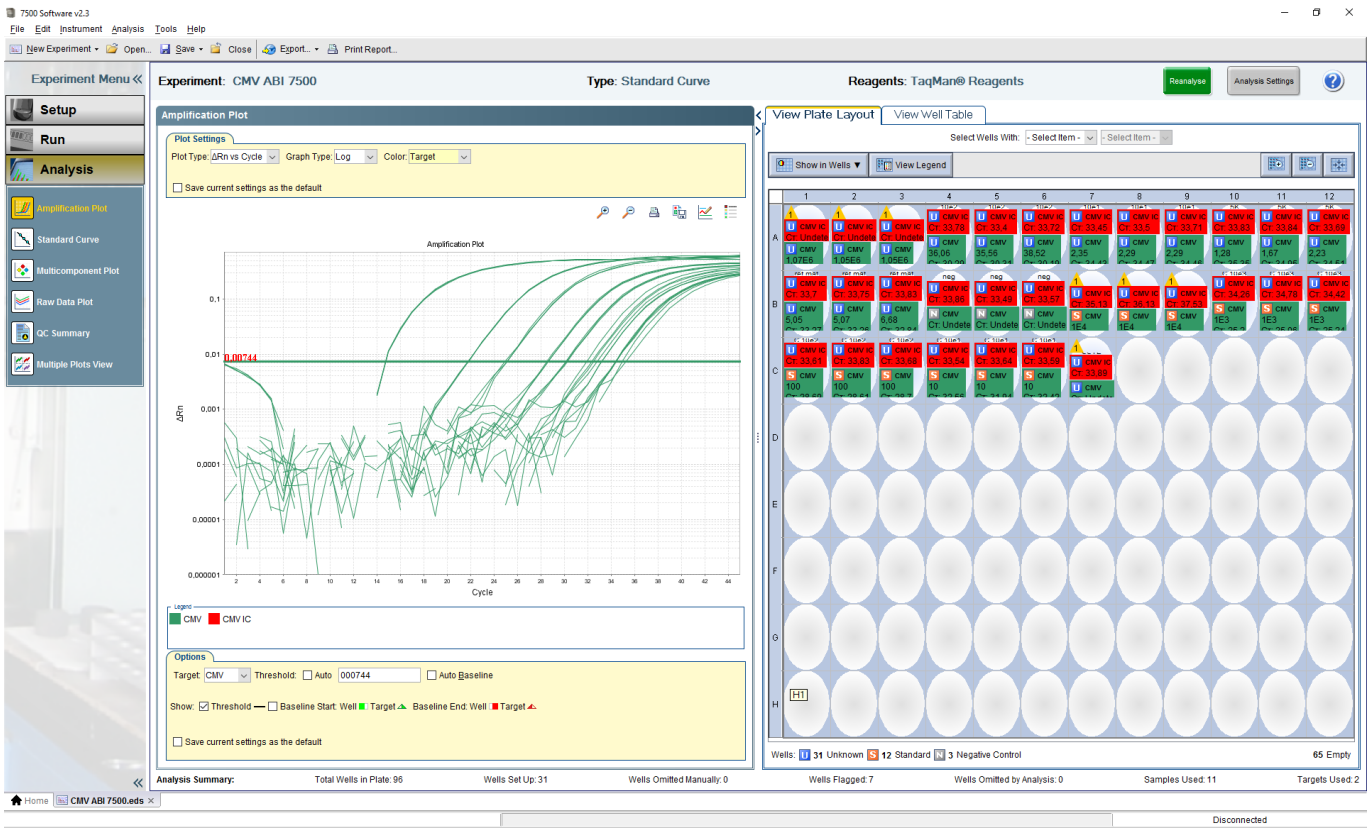


Fig. 1.8 Amplification curves of the studied microorganism

4. In **Plot Settings**, select **Graph Type: Linear**.

The curves display in a linear scale. The **Ct** values can be displayed in the **View Well Table** tab.

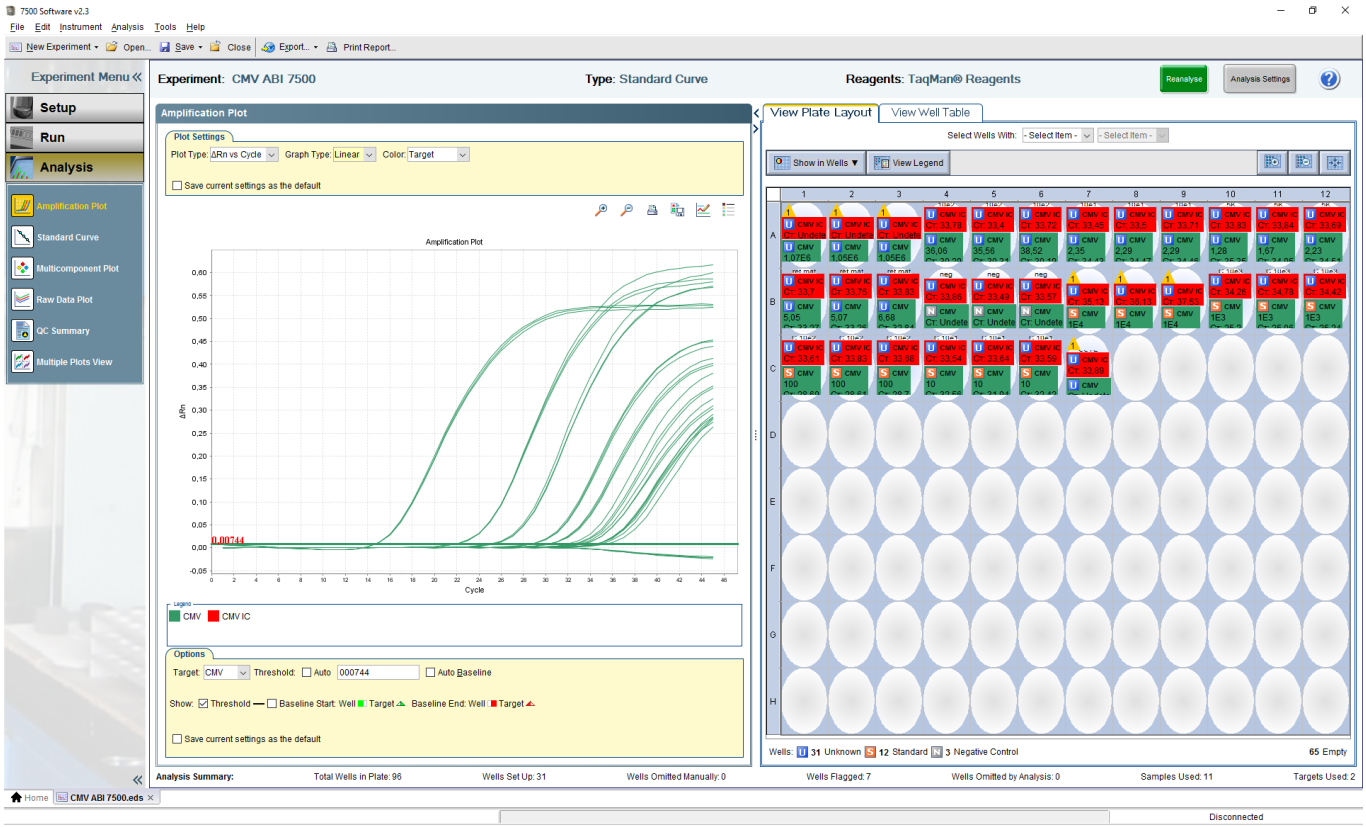


Fig. 1.9 *Amplification curves of the studied microorganism in linear scale*

In the case of multiplex kit, follow the instructions for all the studied microorganisms.

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

1.4.6 Internal Standard detection analysis

1. In **Plot Settings,** select **Graph Type: Log**.
2. In **Options**, select the internal standard (e.g. CMV IC) in the **Target** combo box and move the Threshold line just above the reaction basal noise.
3. Click **Reanalyse**.

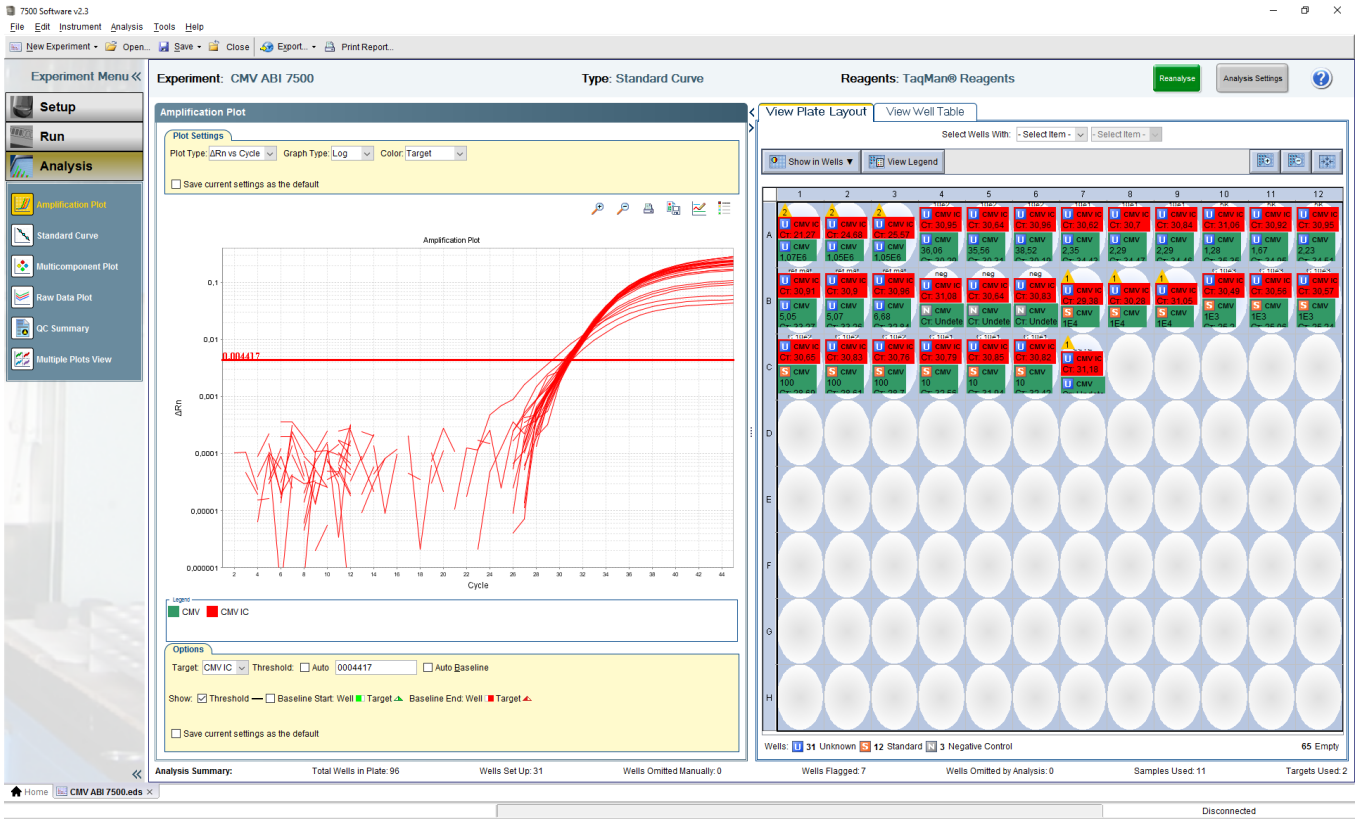


Fig. 1.10 Amplification curves of the internal standard

4. In **Plot Settings**, select **Graph Type: Linear**.

The curves display in a linear scale. The **Ct** values can be displayed in the **View Well Table** tab.

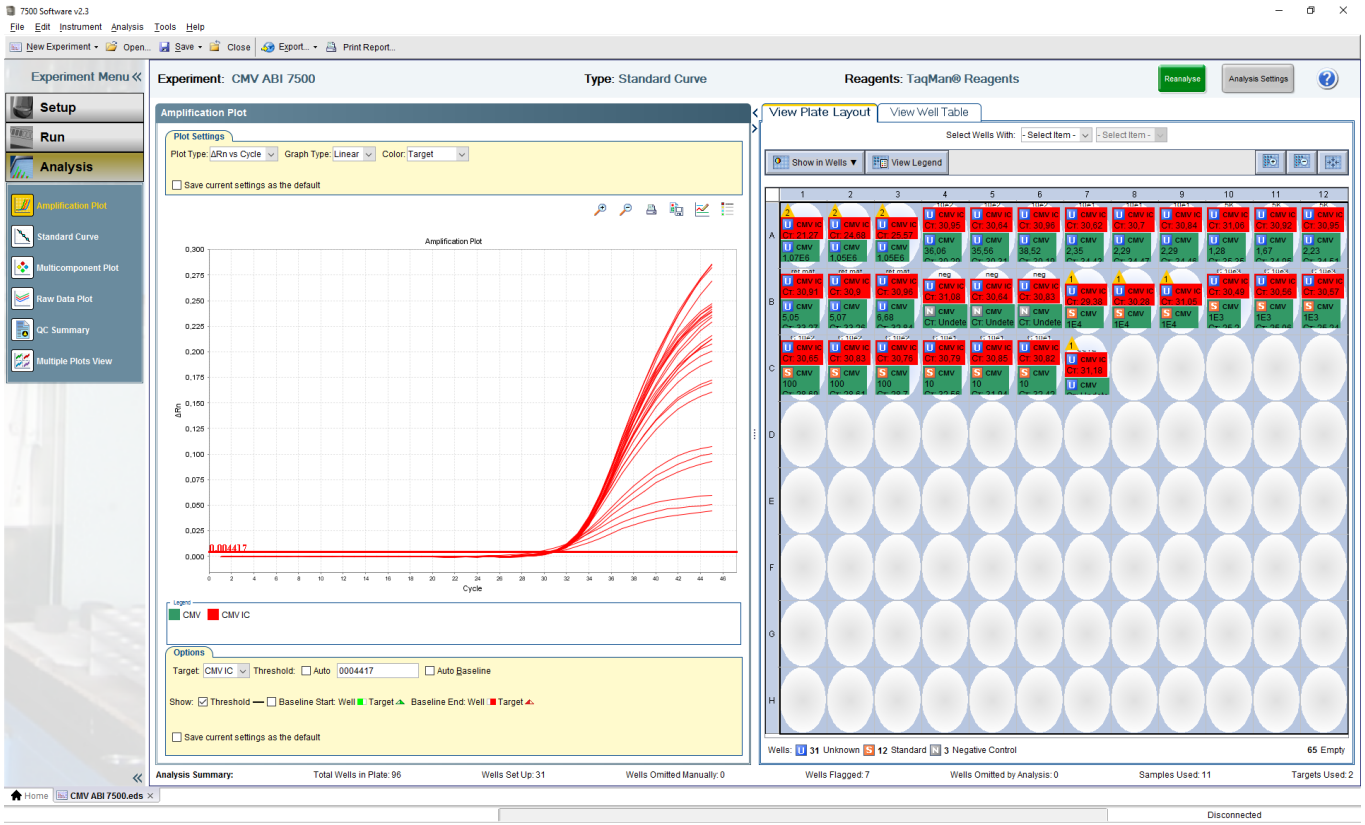


Fig. 1.11 Amplification curves of the internal standard in linear scale

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

1.5. Result quantitative analysis and detection evaluation

1. In **Standard Curve**, evaluate the calibration quality. 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.

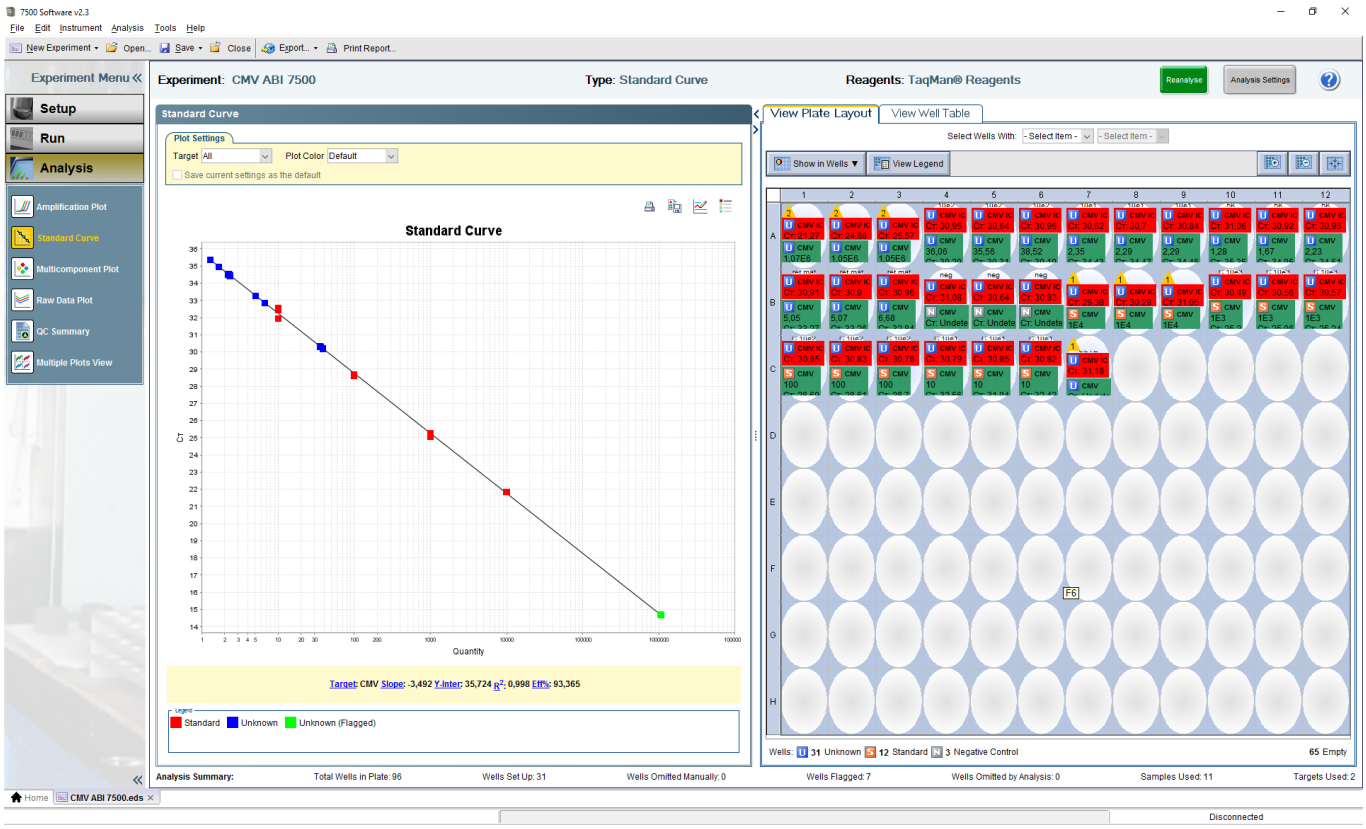


Fig. 1.12 Calibration curve

2. Switch to the **View Well Table** tab. Concentrations of positive samples are displayed in the **Quantity** column of the table.

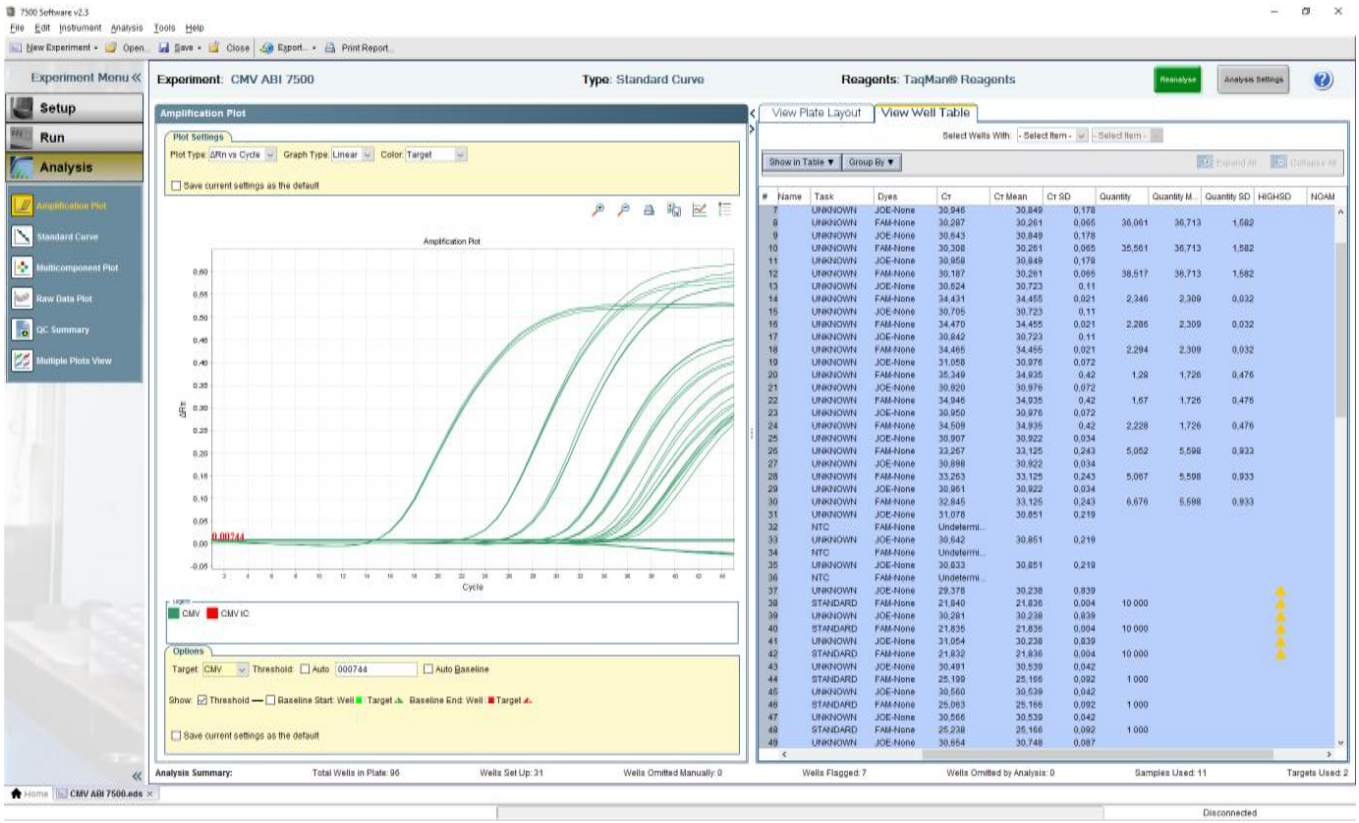


Fig. 1.13 Results table

Perform evaluation, including the virus concentration calculation in copies/ml (cp/ml) according to the Instruction for use of the used GeneProof PCR kit.

2. Genetic diagnostics

This chapter describes in detail the process of using GeneProof PCR kits for genetic diagnostics using the instruments 7500 Real-Time PCR Systém a 7500 Fast Real-Time PCR 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 and open them in the software 7500 Software.exe.

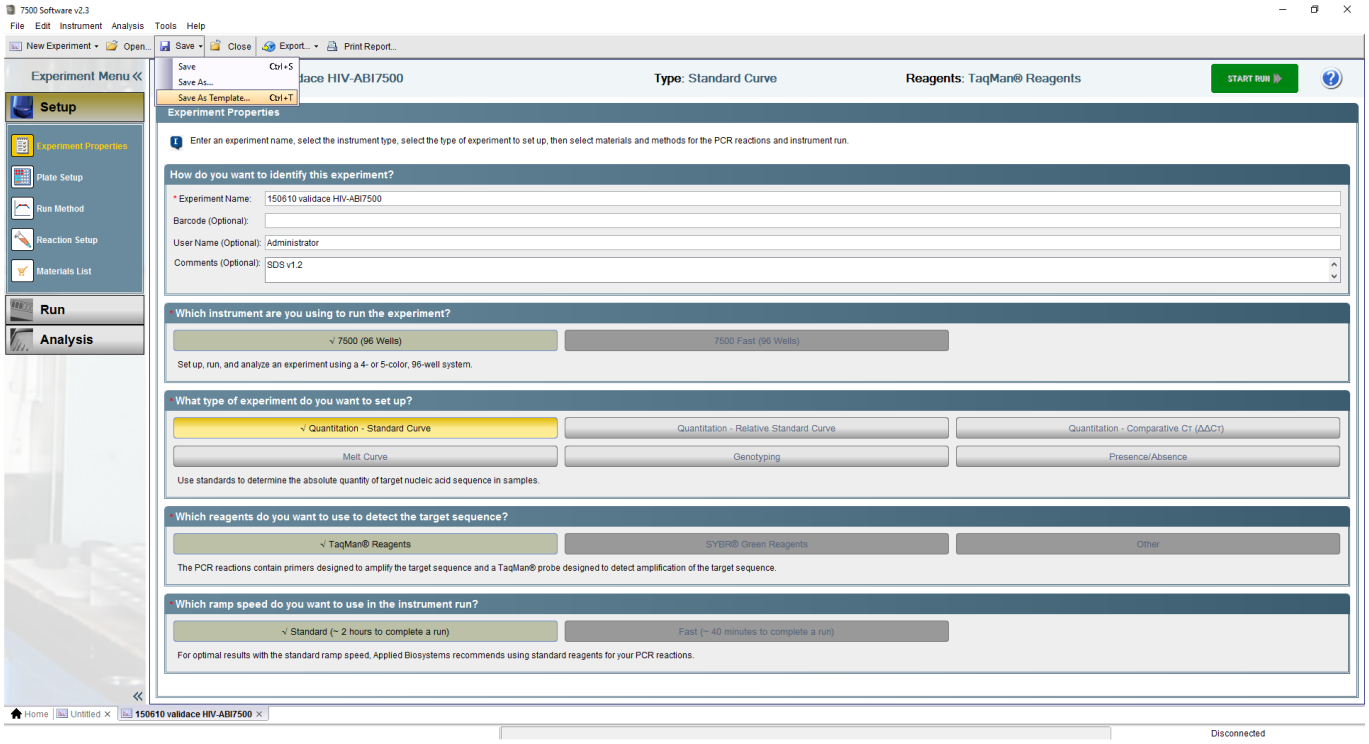


Fig. 2.1 Save template

After saving, the template can be opened from the file **Templates.** With each next usage of GeneProof PCR kits continue from the chapter **1.3 Starting the PCR amplification.**

2.2. *Starting the software*

2.2.1 Opening of the saved template

*1. Start the **7500 Software**.*
*2. In the **Set Up** column, select **Template**.*
3. Open template for the given GeneProof PCR kit.

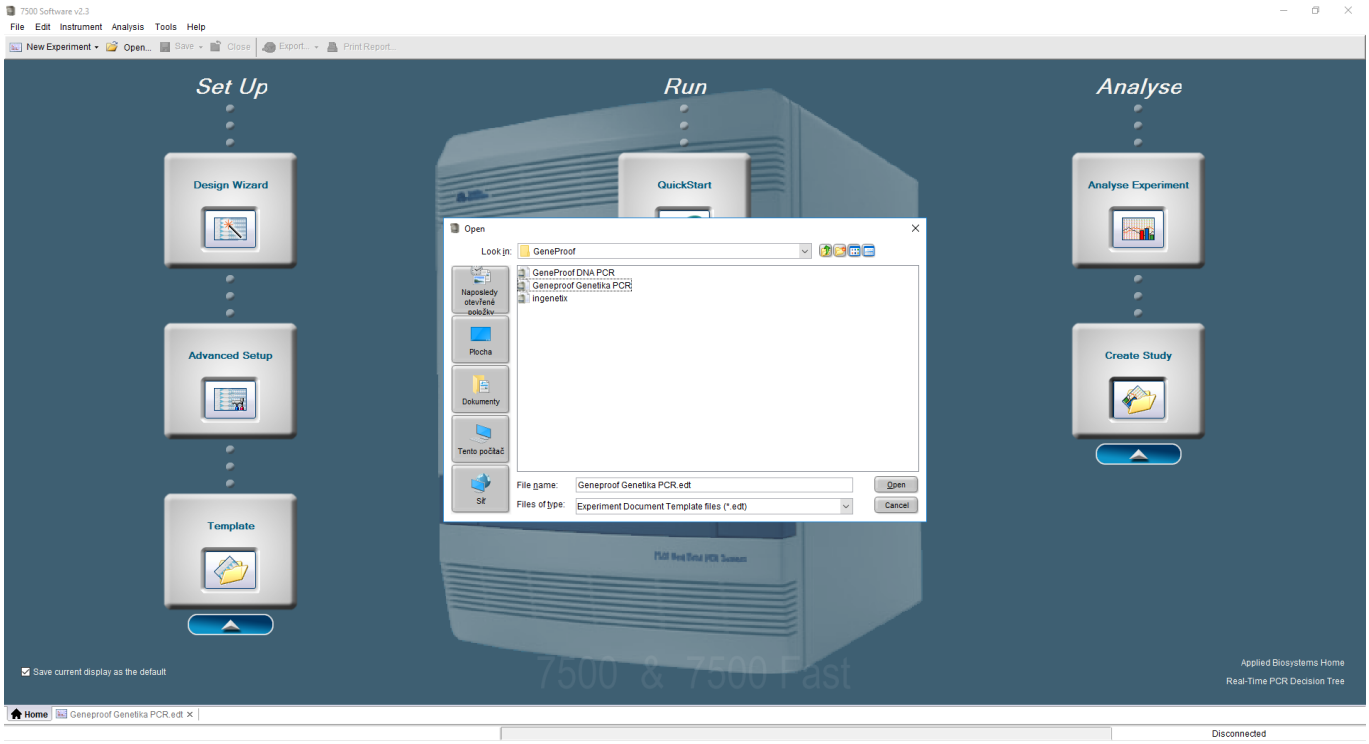


Fig. 2.2 Open template

1. In **Experiment Properties**, enter experiment name into the **Experiment Name** row.
2. In **Plate Setup**, use **Add New Target** to define targets according to the kits used in the experiment.

E.g. for FII detection (3 channels) set Target Name: **FII WT**, Reporter: **FAM**, Quencher: **None**; Target Name: **FII MUT IC**, Reporter: **JOE**, Quencher: **None.** Use **Save Target** and **Add Saved Target** to save and reuse targets.

3. Use **Add New Sample** to define samples.
4. Define positive controls as a sample, e.g. **FII WT, FII MUT and FII HET**.

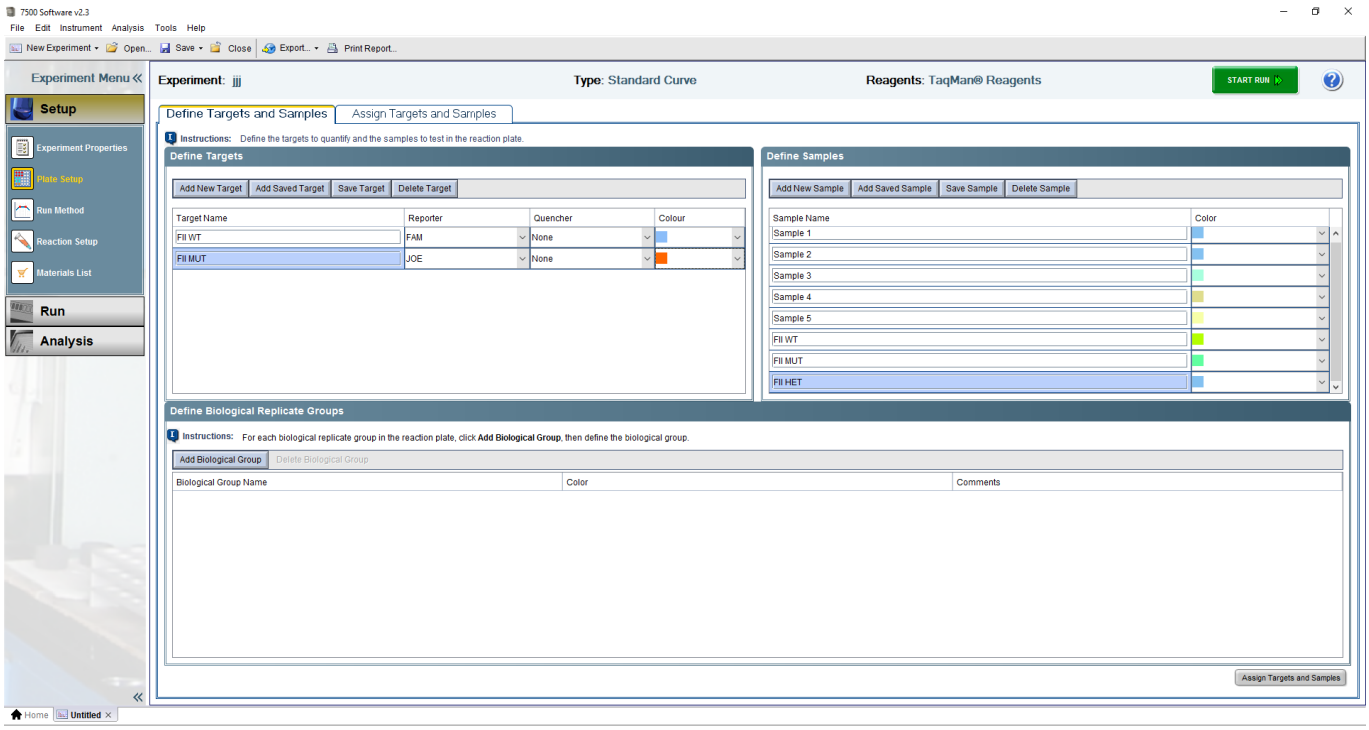


Fig. 2.3 Define targets and samples

4. Switch to the **Assign Targets and Samples** tab.
5. Assign the appropriate targets,samples and controls for used PCR wells by checking the boxes.
6. Select **ROX** passive reference.

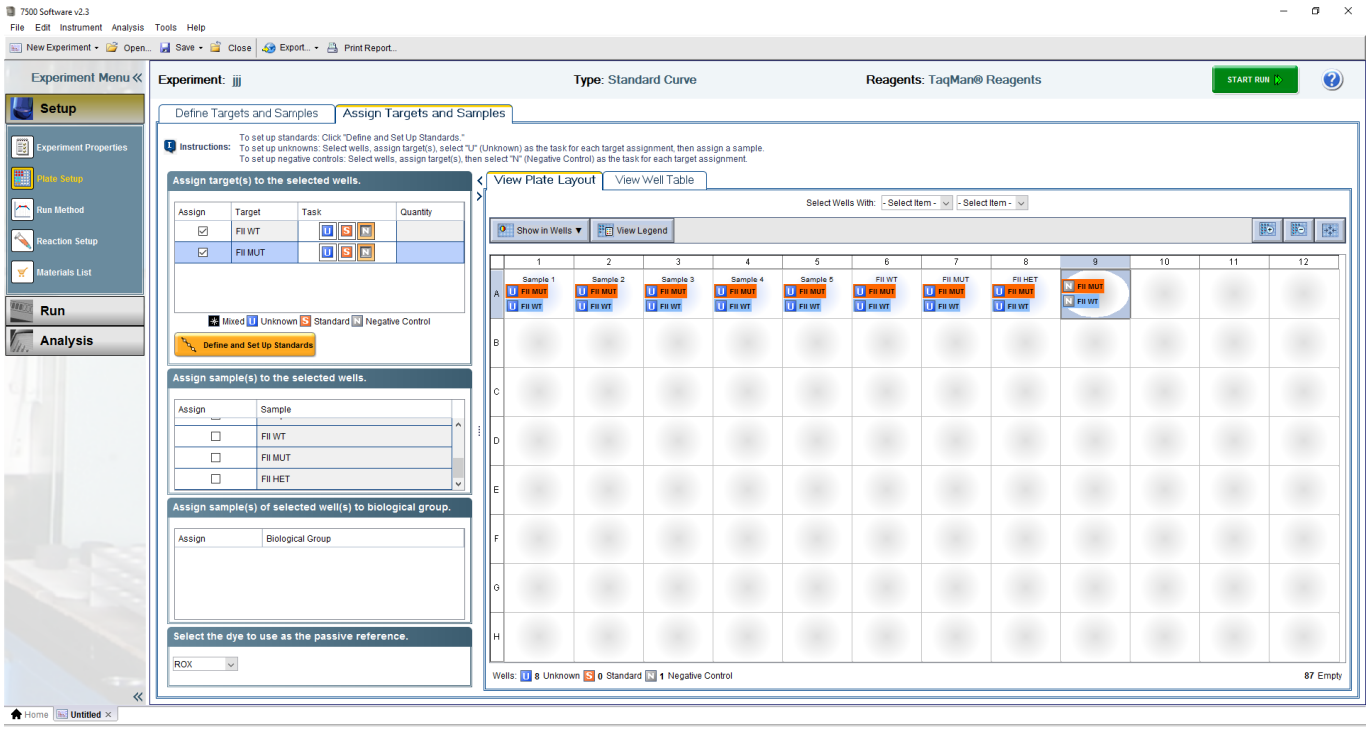


Fig. 2.4 Assign targets and samples

2.2.2 Starting the experiment

Save the experiment before starting the device.

1. Select Save in the main menu and save the created experiment as the **Experiment Document Single files (*.eds)** file type. To make search easier it is recommended to create the **Experiments** folder.

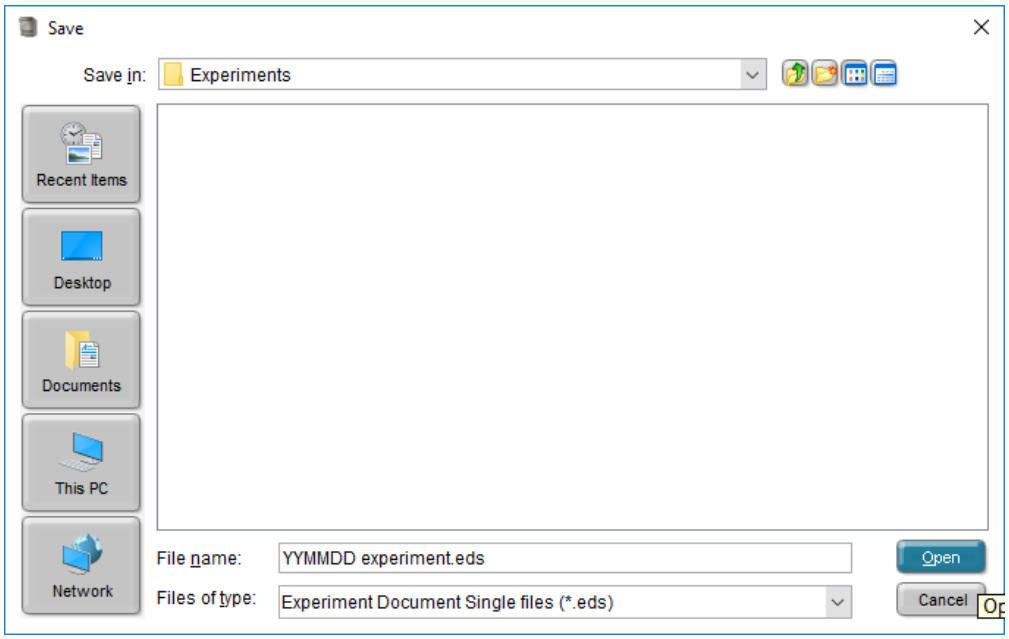


Fig. 2.5 Save experiment

2. Click 

button to start the experiment.

2.3. *Analysis of the result and evaluation of detection for genetic detection*

For correct parameter setting and evaluation when using multiple MasterMixes in one experiment, for example FII and FV, it is necessary to evaluate the results in groups (separately FII, separately FV…).

1. After the protocol is done, swich to the **Analysis** tab and select the **Amplification Plot** tab.
2. **Plot settings** pane set the **Linear**.

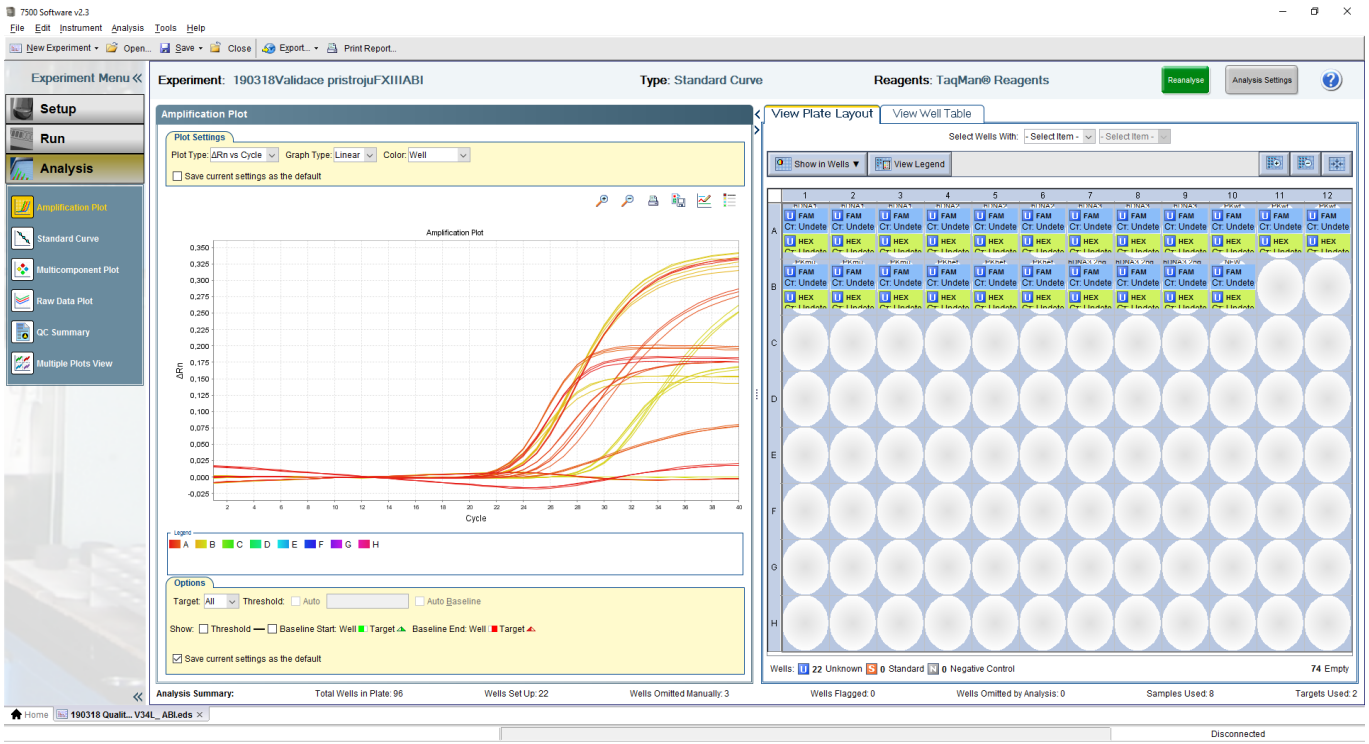
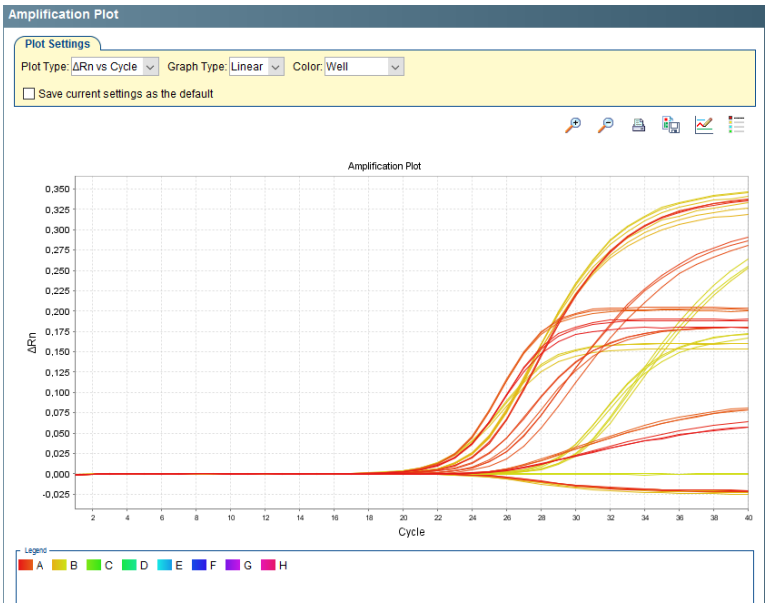


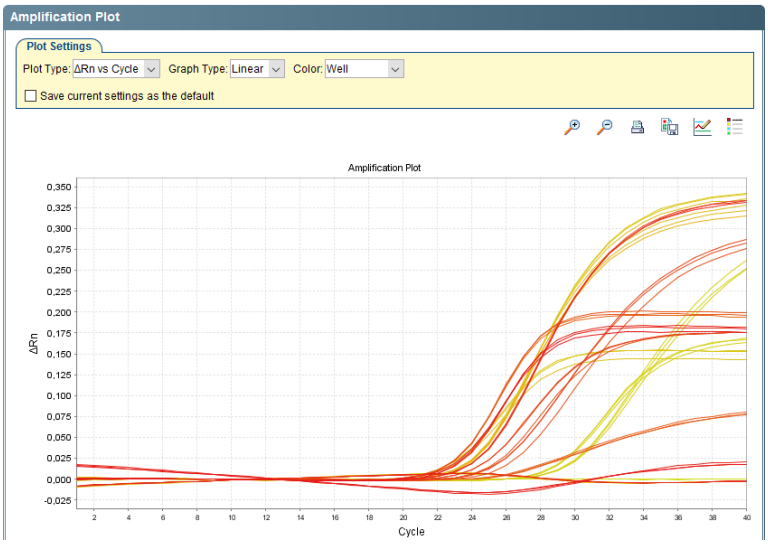
Fig. 2.6 Linear display setting

2.3.3 Setting the Baseline and Treshold parameters

1. In **Plot type,** select **ΔRn vs Cycle**.
2. In the table next to the graph, mark the samples required for evaluation.
3. Click to **Analysis Settings** to select all channels one by one and uncheck **Use Default Settings.** After that uncheck **Automatic Threshold** and **Automatic Baseline.**
4. Set **Start Cycle** and **End Cycle** values to cover as much of the curves as possible before the exponential phase and press the **Apply Analysis Settings** button. Incorrect baseline settings will result in lowering curves.



Right



Wrong

Fig. 2.7 Right and wrong Baseline Settings

5. In the table below the graph mark only the positive WT, MUT and HET controls of the given examination.
6. In the **Threshold** field, enter any value from the **ΔRn** axis and move the Threshold line above the non-specific background so that only the **FAM** amplification curve (eg FII WT) crosses the Threshold line in the case of a **positive WT control**, only the **HEX** amplification curve (FII MUT). In the case of a **positive HET control**, **FAM** and **HEX** amplification curves cross the Treshold line.
7. Push the button **Reanalyze**.

Fig. 2.8 Treshold settings

2.3.4 Evaluation

1. In the table next to the graph, mark the samples required for evaluation.

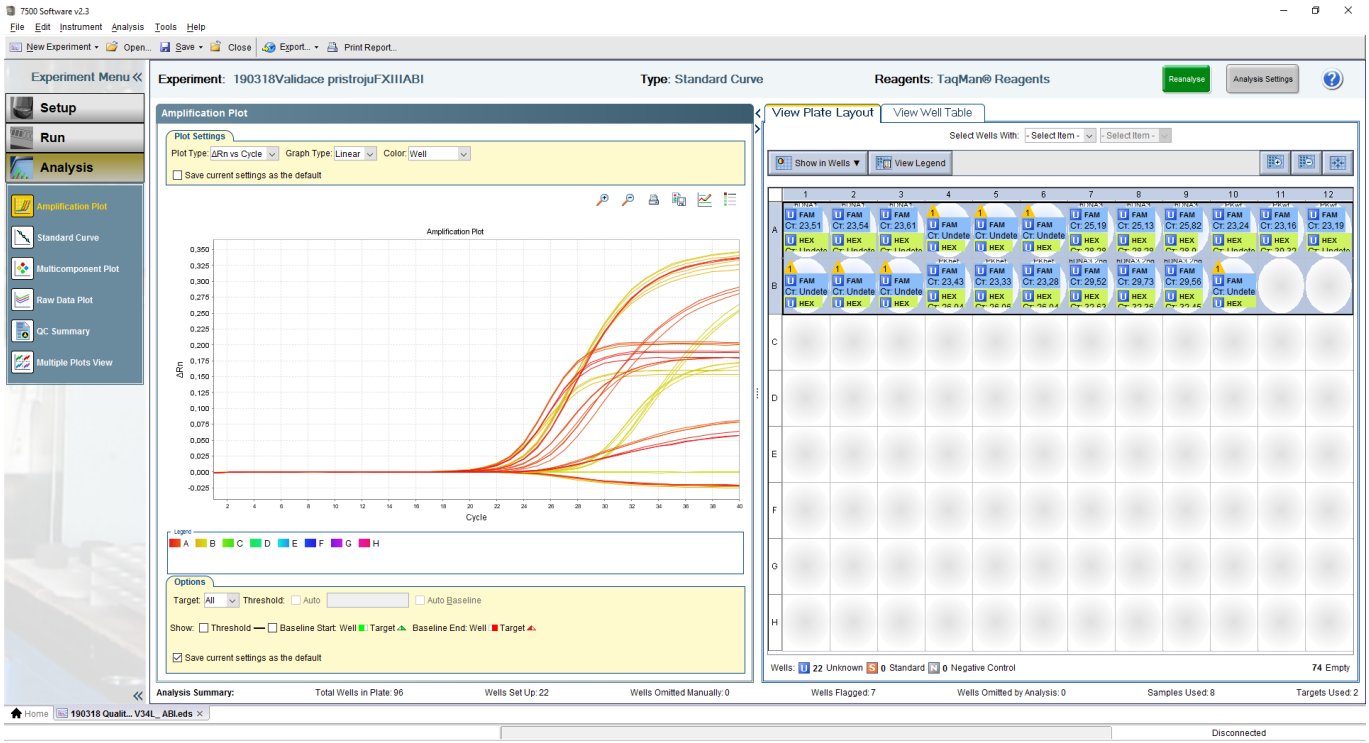
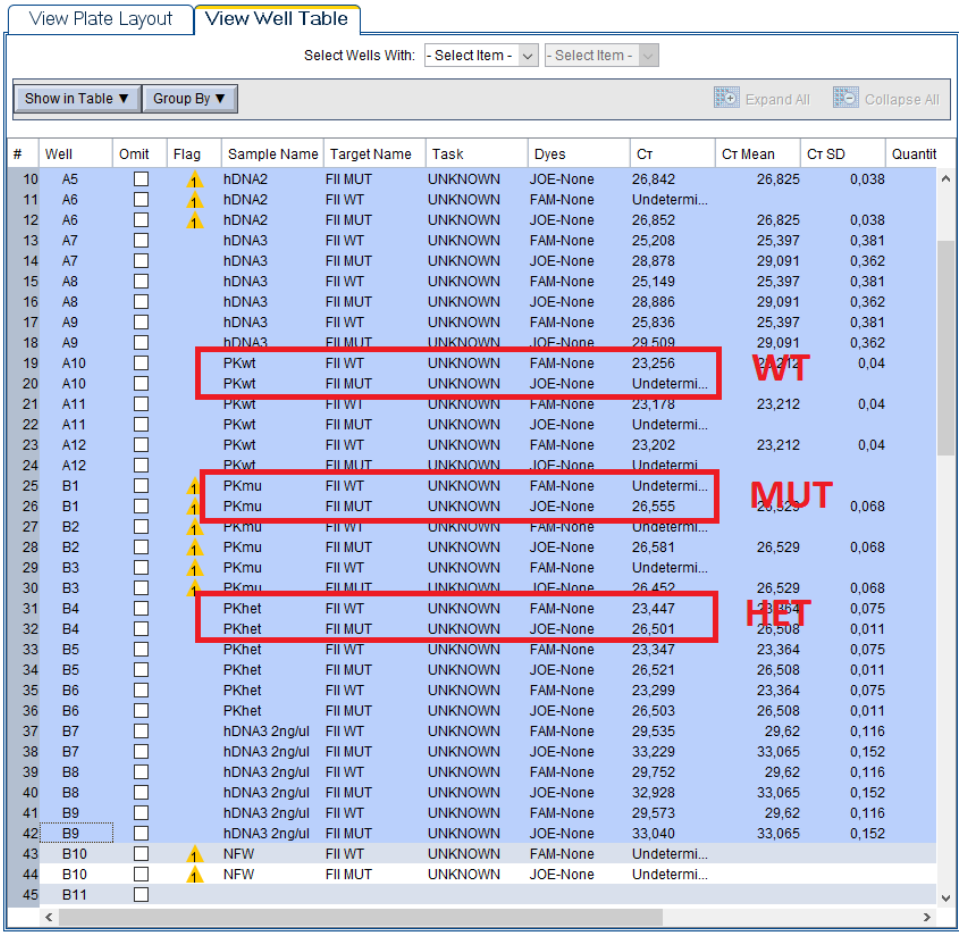


Fig. 2.9 Evaluated curves after setting Baseline and Threshold

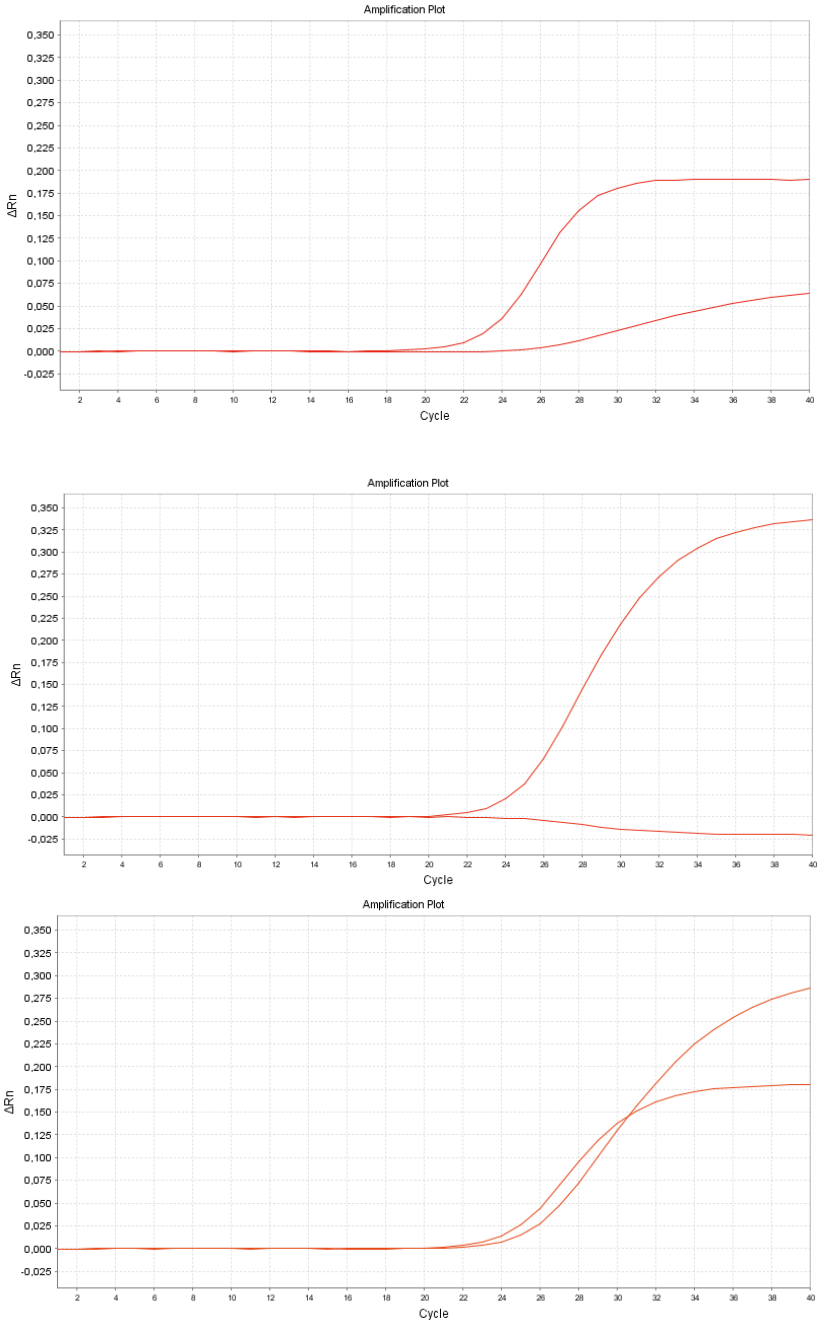
2. Switch to **View Well Table**.
3. If the sample in the **Ct** column has a numerical value for the **FAM** detector (**e.g. FII WT**) – it is a **standard genotype**. A numerical value for the **HEX** detector (FII MUT)- it is a **mutant genotype** and numerical values **for both detectors** means **heterozygous genotype**.



#	Well	Omit	Flag	Sample Name	Target Name	Task	Dyes	Ct	Ct Mean	Ct SD	Quantit
10	A5		1	hDNA2	FII MUT	UNKNOWN	JOE-None	26,842	26,825	0,038	
11	A6		1	hDNA2	FII WT	UNKNOWN	FAM-None	Undetermi...			
12	A6		1	hDNA2	FII MUT	UNKNOWN	JOE-None	26,852	26,825	0,038	
13	A7			hDNA3	FII WT	UNKNOWN	FAM-None	25,208	25,397	0,381	
14	A7			hDNA3	FII MUT	UNKNOWN	JOE-None	28,878	29,091	0,362	
15	A8			hDNA3	FII WT	UNKNOWN	FAM-None	25,149	25,397	0,381	
16	A8			hDNA3	FII MUT	UNKNOWN	JOE-None	28,886	29,091	0,362	
17	A9			hDNA3	FII WT	UNKNOWN	FAM-None	25,836	25,397	0,381	
18	A9			hDNA3	FII MUT	UNKNOWN	JOE-None	29,509	29,091	0,362	
19	A10			PKwt	FII WT	UNKNOWN	FAM-None	23,256	23,212	0,04	
20	A10			PKwt	FII MUT	UNKNOWN	JOE-None	Undetermi...			
21	A11			PKwt	FII WT	UNKNOWN	FAM-None	23,178	23,212	0,04	
22	A11			PKwt	FII MUT	UNKNOWN	JOE-None	Undetermi...			
23	A12			PKwt	FII WT	UNKNOWN	FAM-None	23,202	23,212	0,04	
24	A12			PKwt	FII MUT	UNKNOWN	JOE-None	Undetermi...			
25	B1		1	PKmu	FII WT	UNKNOWN	FAM-None	Undetermi...			
26	B1		1	PKmu	FII MUT	UNKNOWN	JOE-None	26,555	26,529	0,068	
27	B2		1	PKmu	FII WT	UNKNOWN	FAM-None	Undetermi...			
28	B2		1	PKmu	FII MUT	UNKNOWN	JOE-None	26,581	26,529	0,068	
29	B3		1	PKmu	FII WT	UNKNOWN	FAM-None	Undetermi...			
30	B3		1	PKmu	FII MUT	UNKNOWN	JOE-None	26,452	26,529	0,068	
31	B4			PKhet	FII WT	UNKNOWN	FAM-None	23,447	23,364	0,075	
32	B4			PKhet	FII MUT	UNKNOWN	JOE-None	26,501	26,508	0,011	
33	B5			PKhet	FII WT	UNKNOWN	FAM-None	23,347	23,364	0,075	
34	B5			PKhet	FII MUT	UNKNOWN	JOE-None	26,521	26,508	0,011	
35	B6			PKhet	FII WT	UNKNOWN	FAM-None	23,299	23,364	0,075	
36	B6			PKhet	FII MUT	UNKNOWN	JOE-None	26,503	26,508	0,011	
37	B7			hDNA3 2ng/ul	FII WT	UNKNOWN	FAM-None	29,535	29,62	0,116	
38	B7			hDNA3 2ng/ul	FII MUT	UNKNOWN	JOE-None	33,229	33,065	0,152	
39	B8			hDNA3 2ng/ul	FII WT	UNKNOWN	FAM-None	29,752	29,62	0,116	
40	B8			hDNA3 2ng/ul	FII MUT	UNKNOWN	JOE-None	32,928	33,065	0,152	
41	B9			hDNA3 2ng/ul	FII WT	UNKNOWN	FAM-None	29,573	29,62	0,116	
42	B9			hDNA3 2ng/ul	FII MUT	UNKNOWN	JOE-None	33,040	33,065	0,152	
43	B10		1	NFW	FII WT	UNKNOWN	FAM-None	Undetermi...			
44	B10		1	NFW	FII MUT	UNKNOWN	JOE-None	Undetermi...			
45	B11										

Fig. 2.10 Evaluation

2.3.5 *Examples of typical curves*



Typical curve WT

Typical curve MUT

Typical curve HET

Fig. 2.11 Typical curves

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