

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

GeneProof a.s.Vídeňská 101/119, 619 00 Brno – Dolní Heršpice, Czech Republic · info@geneproof.comGFX/CFX96/Dx Real-Time PCR Detection System1/25



CONTENTS

1. PURPOSE	3
1.1. PCR REACTION PREPARATION	3
1.2. DEVICE PROGRAMMING	3
1.3. PCR AMPLIFICATION START	4
1.4. QUALITATIVE ANALYSIS OF THE RESULT AND EVALUATION OF DETECTION	11
1.5. RESULT QUANTITATIVE ANALYSIS AND DETECTION EVALUATION	14
2. GENETIC DIAGNOSTICS	16
2.1. DEVICE PROGRAMMING	16
2.2. PCR AMPLIFICATION START	17
2.3. ANALYSIS OF THE RESULT AND EVALUATION OF DETECTION	23
3. CUSTOMER SERVICE	25
4. CONTACT INFORMATION	25

Bio-Rad CFX/CFX96/Dx Real-Time PCR Detection System



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 <u>www.geneproof.com</u>. Save the downloaded templates on your local disc to the ExpressLoad.

G 🔵 🗢 📕 « CFX	► U:	sers 🕨 admin 🕨 ExpressLoad 🛛 👻	Search ExpressLoad	۶
Organize 🔻 New	folde	r	:==	• 🕐
🧾 Desktop	*	Name	Date modified	Туре
〕 Downloads		CFX 2stepAmp	24.6.2010 11:16	Bio-Rad P
🔠 Recent Places		CFX_2StepAmp_Fast	24.6.2010 11:16	Bio-Rad P
E Libertin		🖾 CFX_2StepGradientAmp	24.6.2010 11:16	Bio-Rad Pro
De sum entr		CFX_3StepAmp	24.6.2010 11:16	Bio-Rad P
Documents Music	Ξ	GFX_3StepGradientAmp	24.6.2010 11:16	Bio-Rad P
Iviusic Dictures		🖾 CFX_RT_qPCR	24.6.2010 11:16	Bio-Rad P
Videos		🖾 PrimePCRMelt48	15.10.2012 10:46	Bio-Rad P
Videos		🖾 PrimePCRMelt96	15.10.2012 10:46	Bio-Rad P
Computer		🖾 PrimePCRMelt384	15.10.2012 10:46	Bio-Rad P
		🖾 Qualification_Plate_96	15.10.2012 10:46	Bio-Rad P
	F	🔄 Qualification_Plate_384	15.10.2012 10:46	Bio-Rad P
🕎 s (\\10.18.12.2) (s =	٠ III		
File name:	GeneP	roof DNA PCR		
Save as type:	rotoc	ol File (*.prcl)		
A Hide Folders			Save	ancel

Fig. 1.1 Save template

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

Bio-Rad CFX/CFX96/Dx Real-Time PCR Detection System



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

Bio-Rad CFX/CFX96/Dx Real-Time PCR Detection System



1.3.2 Using the saved plate

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

R	un Setu	up otocol) Plate 🕠	> Start Run									×
	(Create New							C	Express Load			
	GeneProof DNA PCR plate.pltd												
GE_96 wells_SYBR only.pltd Selected Plate GE_96 wells_All Channels.pltd												2	
	GeneProof DNA PCR plate.pltd GeneProof DNA PCR plate.pltd Qualification_Plate.std												
	Preview Quick Plate 384 wells All Channels.pltd												
	Fluorophores: FAM, HEX, Cy5 Plate Typ Quick Plate 384 wells SYBR Only.pltd												
		1	2	3	4	5	6	7	8	Quick Plate_4 Quick Plate_9	8 wells_FAM 6 wells_SYB	_MJWhite.plto R Only.pltd	4 []
	A	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	QuickPlate_96 Sample Plate_ Sample Plate	6 wells_All Ch 96 wells_All (96 wells_SYB	annels.pltd Channels.pltd BR.pltd	
	В	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk
	с	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk
	D	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk
	E	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk
	F	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk
	G	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk
	н	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk
											<< Prev	•	Next >>

Fig. 1.3 Using the saved plate

Bio-Rad CFX/CFX96/Dx Real-Time PCR Detection System



1.3.3 PCR plate editing

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

Run Setup	
Protocol III Plate III Start Run	
Create New Select Existing	Express Load GeneProof DNA PCR plate.pltd
Selected Plate GeneProof DNA PCR plate.pltd	Edit Selected

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.

Plate E File	ditor - Ger Setting	neProof DN s Editing	IA PCR plat Tools	te.pltd										
	100%	-	Scan Mode	All Chann	iels -	- 👌	Well Group	s 🕅 Ti	race Styles	💷 Spr	eadsheet V	ew/Importer		Plate Loading Guid
	1 Unk	2 Unk	3 Unk	4 Unk	5 Unk	6 Unk	7 Unk	8 Unk	9 Unk	10 Unk	11 Unk	12 Unk	Select Fl	luorophores
A	Target IS Target	Sample Type												
в	Unk Target IS Target	Load FAM	Target Name											
с													HEX	<pre></pre>
D													Load Sam	nple Name
Е													Load Rep	licate #
F													Replic	cate Series ent Settings
G													Clear I	Replicate # ar Wells
н														
									· · · · · ·					
Plate	Type: BR	White 📝	ew Sample	Well Grou	p 📃 Biol	ogical Set	Well No	te					ОК	Cancel

Fig. 1.5 Delete wells

Bio-Rad CFX/CFX96/Dx Real-Time PCR Detection System



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.

Plate E	Plate Editor - GeneProof DNA PCR plate.pltd													
File	Setting	s Editing	Tools											?
	100%	▼ 100 S	ican Mode	All Chann	iels 🔹	• 👶	Well Group	s 🕅 Tı	race Styles.	🗐 💷 Spr	eadsheet V	iew/Importer	😫 P	Plate Loading Guide
	1 2 3 4 5 6 7 8 9 10 11 12											12	Select Flu	uorophores
A	Target IS Target	Target IS Target	Target IS Target	Target IS Target	Target IS Target	Target IS Target	Target IS Target	Target IS Target	Target IS Target	Target IS Target	Target IS Target	Target IS Target	Sample Type	•
в	Unk Target IS Target	Unk Target IS Target	Unk Target IS Target	Unk Target IS Target	Unk Target IS Target	Unk Target IS Target	Unk Target IS Target	Unk Target IS Target	Pos Target IS Target	Pos Target IS Target	Neg Target IS Target	Neg Target IS Target	Load	Unknown Standard NTC Positive Control Negative Control
с													HEX	<pre>NRT </pre>
D													Load Samp	ple Name
E													Load Repli	icate #
F													Replica	ate Series
G													Clear F	Replicate #
н													Clea	ar Wells
Plate	Plate Type: BB White View OK Cancel													

Fig. 1.6 Qualitative detection plate

7/25

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.

Plate E	ditor - Ger	neProof DN	IA PCR plat	e.pltd									
File	Setting	s Editing	Tools										?
	100%	• 🔯	Scan Mode	All Chanr	iels -	- 🔒	Well Group	os 🗖 Ti	race Styles.	🔳 Spr	eadsheet V	ïew/Importer	🐏 Plate Loading Guide
	1	2	3	4	5	6	7	8	9	10	11	12	Select Fluorophores
A	Unk Target IS Target	Unk Target IS Target	Unk Target IS Target	Unk Target IS Target	Target IS Target	Unk Target IS Target	Sample Type Standard 🔻						
	Unk Target	Unk Target	Unk Target	Unk Target	Unk Target	Unk Target	Std 1,00E+04	Std 1,00E+03	Std 1,00E+02	Std 1,00E+01	Neg Target	Neg Target	Land Treat Name
В	IS Target	IS Target	IS Target	IS Target	IS Target	IS Target	1,00E+04	1,00E+03	1,00E+02	1,00E+01	IS Target	IS Target	FAM Target
<u> </u>													HEX <none> ▼</none>
с													Cv5 Target -
													Load Sample Name
ľ													Image: Another American Ame
													E Deslinete #
E													
<u> </u>													Parliante Series
F													
													Load Concentration:
													☑ 1,00E+04
G													<all></all>
													Dilution Series
н													Experiment Settings
													Clear Replicate #
			ew.			View							Clear Wells
Plate	e Type: BR \	White 🔘	Target Nar	me 💿 Con	centration	Sample	e 🔲 Well	Group	Biological S	Set 📃 W	ell Note		OK Cancel

Fig. 1.7 Quantitative detection plate

Bio-Rad CFX/CFX96/Dx Real-Time PCR Detection System



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.

Save As	(Table	×
🔾 🗢 🖡 « Users	▶ admin ▶ RealTimeProtocols ✓ 4y Search RealTime	Protocols
Organize 🔻 New fo	older	:= • 🕡
Desktop	Name Date modified	Туре
🐞 Downloads 🗐 Recent Places	No items match your search.	
肩 Libraries		
Documents	=	
Pictures		
💾 Videos		
🖳 Computer		
🏭 OS (C:)		
→ HP_RECOVERY (E → s (\\10.18.12.2) (S	+	•
File name: GF	P PCR-YYMMDD	-
Save as type: Pla	ate File (*.pltd)	•
) Hide Folders	Save	Cancel

Fig. 1.8 Save edited plate

Bio-Rad CFX/CFX96/Dx Real-Time PCR Detection System



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.

Run Setup	— ×
Protocol III Plate III Start Run	
- Run Information	
Protocol: GeneProof DNA PCR.prcl	
Notes:	
	-
Scan Mode: All Channels	
Start Run on Selected Block(s)	
Block Name △ Type Run Status Sample Volume ID/Bar Code	
DX100142 "96FX" Idle 40	
Select All Blocks	
G Flash Block Indicator	
Start B	in and the second secon
<< Prev	Next >>

Fig. 1.9 Starting PCR test

Bio-Rad CFX/CFX96/Dx Real-Time PCR Detection System



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

Bio-Rad CFX/CFX96/Dx Real-Time PCR Detection System

11/25



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

Baseline Threshold		×	<u> </u>
Baseline Cycles			
Auto Calculated			
O User Defined	Во	d indicates a changed value.	
Well	⊾ Fluor 🛇	Baseline Begin ◇ Baseline End ◇	
1 A01	FAM	2 24	-
All Selected Ro	ws: Begin: 46	End: 1	h. V
	Reset All User Defir	ned Values	
Single Threshold			
Auto Calculated:	455,97		
O User Defined:	193,50]	
		OK Cancel	

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

Bio-Rad CFX/CFX96/Dx Real-Time PCR Detection System





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.

Bio-Rad CFX/CFX96/Dx Real-Time PCR Detection System

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

Bio-Rad CFX/CFX96/Dx Real-Time PCR Detection System



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.

ŀ.	File V	/iew Settir	igs Tools											
	-	3 🔍	🛐 🚷 Vie	w/Edit Plate	. 📸 Well Grou	p: All Wells		- ?						
	📶 Qua	antitation	Quantitation [Data 🔲 Ger	ne Expression	End Point	Allelic Discrimin	ation 🔮 QC	Run Information					
	Results Step Number: 4													
	Well	♦ Fluor	△ Content ♦	Target 🔇	ò 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		
4	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

Bio-Rad CFX/CFX96/Dx Real-Time PCR Detection System



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 <u>www.geneproof.com</u>.

Save the downloaded templates on your local disc to the ExpressLoad file.

Save As					×
CFX V CFX	▶ Use	ers 🕨 admin 🕨 ExpressLoad	- - 4	Search ExpressLoad	Q
Organize 🔻 New f	older				• 🔞
📃 Desktop	*	Name		Date modified	Туре
Downloads		🖾 CFX_2stepAmp		24.6.2010 11:16	Bio-Rad Prot
Recent Places		CFX_2StepAmp_Fast		24.6.2010 11:16	Bio-Rad Prot
E 11		CFX_2StepGradientAmp		24.6.2010 11:16	Bio-Rad Prof
Cibraries		CFX_3StepAmp	24.6.2010 11:16	Bio-Rad Prot	
Music	=	CFX_3StepGradientAmp		24.6.2010 11:16	Bio-Rad Prot
		CFX_RT_qPCR		24.6.2010 11:16	Bio-Rad Prot
Videos		PrimePCRMelt48		15.10.2012 10:46	Bio-Rad Prot
La viacos		PrimePCRMelt96		15.10.2012 10:46	Bio-Rad Prot
Computer		PrimePCRMelt384		15.10.2012 10:46	Bio-Rad Prot
A OS (C:)		Qualification_Plate_96		15.10.2012 10:46	Bio-Rad Prot
HP RECOVERY (E		Qualification_Plate_384		15.10.2012 10:46	Bio-Rad Prot
⊊ s (\\10.18.12.2) (S	+ 4		III		Þ
File name: G	enePro	oof DNA PCR			•
Save as type:	otoco	File (* prcl)			-
Save as type.	01000	rine (spici)			•
Hide Folders			(Save	Cancel

Fig. 2.1 Save template

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

Bio-Rad CFX/CFX96/Dx Real-Time PCR Detection System



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

Bio-Rad CFX/CFX96/Dx Real-Time PCR Detection System



2.2.2 Using the saved plate

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

Run Setu	Run Setup Protocol Image: Plate Image: Start Run											
	Create New											
	GeneProof DNA PCR plate.pltd											
Select	GE_96 wells_SYBR only.pltd											
Gana	GeneProof DNA PCR plate pltd GeneProof DNA PCR pltd GeneProof DNA											
Gener		n plate.pitu							Qualification_F	Plate_384.pito Plate_96.pltd	1	
Previe	w								Quick Plate_3 Quick Plate 3	84 wells_All (84 wells_SYE	Channels.pltd 3R Only.pltd	
Huorop	phores:	FAM, H	EX, Cy5			1		Plate Type	uick Plate_4	8 wells_FAM	pltd MIW/bite plt/	.
	1	2	3	4	5	6	7	8	uick Plate_9	6 wells_SYB	R Only.pltd	·
A	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Quick Plate_96 Sample Plate_ Sample Plate	5 wells_All Ch 96 wells_All (96 wells_SYI	annels.pltd Channels.pltd BR.pltd	
В	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk
с	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk
D	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk
E	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk
F	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk
G	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk
н	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk
	<pre></pre>											

Fig. 2.3 Using the saved plate

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

Run Setup	×
Protocol III Plate III Start Run	
Create New Select Existing	Express Load GeneProof DNA PCR plate.pltd
Selected Plate GeneProof DNA PCR plate.pltd	Edit Selected

Fig. 2.4 Edit plate

Bio-Rad CFX/CFX96/Dx Real-Time PCR Detection System



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.

Plate E	Plate Editor - GeneProof PCR GENETIKA Plate.pltd														
<u>F</u> ile	Settings	s Editing	<u>T</u> ools												?
	👖 100% 👻 🔯 Scan Mode 📶 Channels 👻 🤀 Well Groups 🖾 Trace Styles 💷 Spreadsheet View/Importer 😫 Plate Loading Guide														
	1 Unk	2	3 Unk	4 Unk	5 Unk	6 Unk	7	8	9	10	11	12	Select	Fluorophores	
A	WT MUT	WT MUT	WT MUT	WT MUT	WT MUT	WT MUT	WT MUT								
	FII Unk WT	FII Unk WT	FII Unk WT	FII Unk WT	FII Unk WT	FII Unk WT	FII Unk WT						Sample Type		-
В	MUT FV	MUT FV	MUT FV	MUT FV	MUT FV	MUT FV	MUT FV						Load	Target Name	
с	WT MUT	WT MUT	WT MUT	WT MUT	WT MUT	WT MUT	WT MUT							<none></none>	
	AC Unk	AC Unk	AC Unk	AC Unk	AC Unk	AC Unk	AC Unk							CIONEZ	
	MUT CT	MUT CT	MUT CT	MUT CT	MUT CT	MUT CT	MUT CT						Load Sa	mple Name	
E														one>	
													Load Re	plicate #	
F													Rep	licate Series	×
G													Experin	nent Settings	
													Clear	Replicate #	
														ear Wells	
Diete	Time: PD 1	Vie	ew												
Plate	e type: BR \	white 🔽	Sample	Well Grou	p 📃 Biol	ogical Set	Well No	ote							ei

Fig. 2.5 Delete wells

Bio-Rad CFX/CFX96/Dx Real-Time PCR Detection System



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.

Plate E	Plate Editor - GeneProof PCR GENETIKA Plate.pltd												
<u>F</u> ile	File Settings Editing Tools ? Image: Setting Setting Tools ? ? Image: Setting Setting Tools ? ? Image: Setting Setting Tools ? Image: Tools												
	1	2	3	4	5	6	7	8	9	10	11	12	Select Fluorophores
A	Pos WT MUT FII PK WT	Pos WT MUT FII PK MUT	Pos WT MUT FII PK HET	Unk WT MUT FII	Unk WT MUT FII	Unk WT MUT FII	Neg WT MUT FII						Sample Type
в	Pos WT MUT FV	Pos WT MUT FV	Pos WT MUT FV	Unk WT MUT FV	Unk WT MUT FV	Unk WT MUT FV	Neg WT MUT FV						Unknown Standard NTC
с	Pos WT MUT AC	Pos WT MUT AC	Pos WT MUT AC	Unk WT MUT AC	Unk WT MUT AC	Unk WT MUT AC	Neg WT MUT AC						FAM Positive Control Negative Control HEX NRT
D	Pos WT MUT CT	Pos WT MUT CT	Pos WT MUT CT	Unk WT MUT CT	Unk WT MUT CT	Unk WT MUT CT	Neg WT MUT CT						Load Sample Name
E													
F													
G													Experiment Settings
н													Clear Replicate #
													Clear Wells
Plate	• Type: BR V	Vie Vhite	ew Sample 📃] Well Grou	ıp 📃 Biolo	ogical Set	🔲 Well No	ote					QK <u>C</u> ancel



Bio-Rad CFX/CFX96/Dx Real-Time PCR Detection System



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.

Save As	rep Tank		x
😋 🔵 🗢 📗 « Us	ers 🕨 admin 🕨 RealTimeProtocols 💿 👻 🍫 Search RealTime	Protocols	Q
Organize 🔻 Ne	w folder	== -	?
🧮 Desktop	Name Date modified	Туре	
🗼 Downloads 📃 Recent Places	No items match your search.		
📜 Libraries			
Documents	=		
J Music			
Pictures			
Videos			
🖳 Computer			
🏭 OS (C:)			
	/ (E		
🖵 s (\\10.18.12.2) (5		Þ
File name:	GP PCR-YYMMDD		•
Save as type:	Plate File (*.pltd)		-
Alide Folders	Save	Cancel	

Fig. 2.7 Save edited plate

Bio-Rad CFX/CFX96/Dx Real-Time PCR Detection System



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.

Due Catur						ſ	
Kun Setup		et Due				l	_
Protocol	Plate V Sta						_
- Run Informati	ion						
Protocol:	GeneProof PCR GE	NETIKA.prci					
Notes:		ж					
							Â
Core Made	All Channels						× .
Start Run on	Selected Block(s)						
			-				
	Block Name	Δ	Туре	Run Status	Sample Volume	ID/Bar Code	
Select All	Blocks						
🔵 <u>F</u> lash B	Block Indicator	Open Lid	<u> C</u> lose Lid				
						Start Run	
						<< Prev Next >	<u> </u>
						Hog /	

Fig. 2.8 Starting PCR test

Bio-Rad CFX/CFX96/Dx Real-Time PCR Detection System



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.

Bio-Rad CFX/CFX96/Dx Real-Time PCR Detection System

23/25



2.3.7 Examples of typical curves



Obr. 2.1 Typical WT curve







Obr. 2.3 Typical HET curve

Bio-Rad CFX/CFX96/Dx Real-Time PCR Detection System



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: <u>support@geneproof.com</u> Orders

Phone: +420 543 211 679 e-mail: <u>sales@geneproof.com</u>

Bio-Rad CFX/CFX96/Dx Real-Time PCR Detection System

25/25