

DEVICE MANUAL

Bio Molecular Systems

Mic qPCR Cycler

Designed for GeneProof diagnostic kits

See www.geneproof.com for the current kits list



CONTENTS

1. PURPOSE	3
1.1. PCR REACTION PREPARATION	3
1.2. DEVICE PROGRAMMING	3
1.3. PCR AMPLIFICATION START	4
1.4. RESULT QUALITATIVE ANALYSIS AND DETECTION EVALUATION	10
1.5. RESULT QUANTITATIVE ANALYSIS AND DETECTION EVALUATION	
1.6. TROUBLESHOOTING	14
2. GENETIC DIAGNOSTICS	17
2.1. DEVICE PROGRAMMING	17
2.2. PCR AMPLIFICATION START	
2.3. ANALYSIS OF THE RESULT AND EVALUATION OF DETECTION	23
3. CUSTOMER SERVICE	24
4. CONTACT INFORMATION	24

Mic qPCR Cycler



1. Purpose

This device manual describes in detail the process of using GeneProof PCR kits for microbiological DNA diagnostics with the device Mic qPCR Cycler.

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 assays, 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 assays on your local disc and open them in the software micPCR.

Click the arrow next to the **Save As** button and save the created Assay under the name **of used PCR kit** as the **micPCR Files** type.

💛 micPCR v2.6.5						- 0 ×
Help New Open Save Save As						No Instruments Found
* Untitled - 2 HSV ×				Information		
	mistry Type Hydrolysis Probes	×				
\land Analysis + +	Targets Name Description Forward Primer	HSV1	Name	Amplicon Length Seque		
	Reverse Primer Contains Alleles		← → ~ ↑	> micPCR > Assays > GeneProof	v O Prohledat: GeneProof P	
	Probes	Name	Uspořádat 👻 Nová složka Název	Datum změny Typ	Velikost	
	Probes	HSV IC	Polocha Polocha Construction State Construction Constructin Constructin Construction Con	рания ленту 13.1.12018 14.38 mic Assay File 19.11.2018 10.59 mic Assay File	11 18 11 18 12 18	
	Description		Název souboru: HSV		~	
	Forward Primer Reverse Primer Contains Alleles		Uložit jako typ: micPCR Assay Files (*.micassay)		<u>U</u> ložit Zrušit	
	Probes	Name Cy5	5' Madifier ■ Cy™ 5 V	Sequence	3' Modifier	

Fig. 1.1 Save Assay

Save the Assay into a folder called **GeneProof**, which should be created in the default **Assays** folder. Default path is Documents\Bio Molecular Systems \micPCR\Assays.



1.3. PCR Amplification Start

1.3.1 Saved Assay opening

1. Start the micPCR software and select New -> Run.

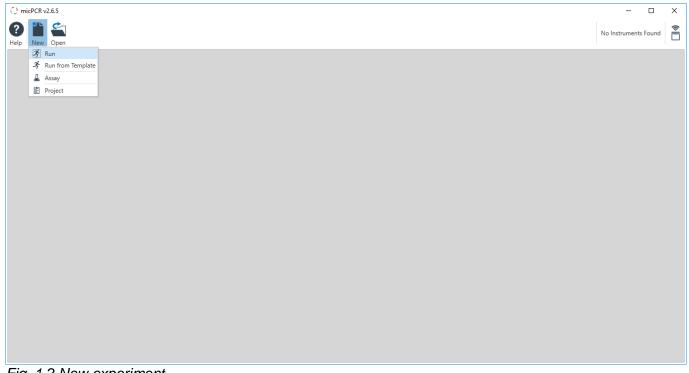


Fig. 1.2 New experiment



2. In the Assays field click on the + symbol and choose the assay of your choice from the **GeneProof** folder in the **My assays** tab.



Fig. 1.3 Loading of a saved Assay



1.3.2 Samples editing

- 1. In Samples tab, define the samples used in the experiment.
- 2. Add an assay to all of the samples in the Assay column.

In case of quantitative detection

3. For negative controls set Negative control in the Type column.

4. For calibrators set **Standard** in the **Type** column and enter the corresponding quantity in the **Standard Concentration** column: 10 000, 1 000, 100 a 10 (keep Copies/µl).

lp New Open Save Save) Intitled - 2 ×	- As								No Instruments Fou
🖁 Assays +					Samples			6 🕁 🖻 🖻	Available Assays
▶ HSV	Search						18 18	1: A1_ A7_ III III III / III HSV	
🛠 Run Setup	Col	iour Name	Туре	Groups	Assay	Standards Concentration			
Run Profile	1	Sample 1	Unknown		HSV	copica) pc		0	
	2		Unknown		HSV				
		Sample 3	Unknown		HSV				
Information		Sample 5	Unknown		HSV				
Data		Sample 5	Unknown		HSV				
		Sample 6	Unknown		HSV				
🗹 Analysis		Sample 7	Unknown		HSV				
	8		Unknown		HSV				
	9		Unknown		HSV				
Melt +		Sample 10	Unknown		HSV				
Absolute Quantification +	11		Positive Control		HSV				
Allelic Discrimination +	12		Unknown		HSV	10000			
	13		Unknown		HSV	1000			
Identifier +	14		Unknown		HSV	100			
Relative Quantification +	15		Unknown		HSV	10			
Reports +			Unknown						
reports +	17		Unknown						
	18		Unknown						Groups
	19		Unknown					•	+
	20		Unknown						
	21		Unknown						
	22		Unknown						
	23		Unknown						
			Unknown						
	25		Unknown						
	26		Unknown						
	27		Unknown						
	28		Unknown						
	29		Unknown						
	30		Unknown						
	31		Unknown						
			Unknown						
	33		Unknown						
	34		Unknown						
	35		Unknown						
	36		Unknown						
	37		Unknown						
	38		Unknown					~	

Fig. 1.4 Define samples in case of quantitative detection



In case of qualitative detection

- 3. For positive controls set **Positive control** in the **Type** column.
- 4. For negative controls set **Negative control** in the **Type** column.

ntitled - 2 * HSV * Untitled - 4	×				Sample	es		6 48 9		Available Assays	
► HCV	Search						↓ 於 ↓ 說 <u>1.</u> A1. A7. H6 H12		HCV		
						Standards Concentration	4 AA 4 A3 45 H6 H12				
Run Setup	Colour	Name	Туре	Groups	Assay	Copies/µL V					
Run Profile	1	Sample 1	Unknown		HCV			^			
Samples	2	Sample 2	Unknown		HCV						
Information	3	Sample 3	Unknown		HCV						
	4	Sample 4	Unknown		HCV						
Data	5	Sample 5	Unknown		HCV						
	6	Sample 6	Unknown		HCV						
Analysis	7	Sample 7	Unknown		HCV						
Cycling +	8	Sample 8	Unknown		HCV						
	9	Sample 9	Unknown		HCV						
Melt +	10	Sample 10	Unknown		HCV						
Absolute Quantification +	11	Negative control	Negative Control		HCV						
Allelic Discrimination +	12	Positive control	Positive Control		HCV						
Identifier +	13		Unknown								
Relative Quantification +	14		Unknown								
Relative Quantification +	15		Unknown								
Reports +	16		Unknown								
	17		Unknown								
	18		Unknown							Groups	
	19		Unknown						+		+
	20		Unknown								
	21		Unknown								
	22		Unknown								
	23		Unknown								
	24		Unknown								
	25		Unknown								
	26		Unknown								
	27		Unknown								
	28		Unknown								
	29		Unknown					_			
	30		Unknown								
	31		Unknown								
	32		Unknown								
	33		Unknown								
	34		Unknown								
	35		Unknown								
	36		Unknown								
	37										

Fig. 1.5 Define samples in case of qualitative detection



1.3.3 Experiment starting

Save the experiment before starting the device.

1. In the top bar, select **Save** and save the created experiment as the **micPCR Run Files** type. To make search easier it is recommended to create the **Experiments** folder.

Assays + ► HCV Search Run Setup Colour Run Potitie 1 Sample 1 Sample 1 Sample 2 Sample 2 Information 3 Abolate Quantification + 5 Abolate Quantification + 10 Abolate Quantification + 11 Reports + 13 Cal 4 15 Cal 4 16 - 22 - 23 - 24 - 25 Sample 3 4 Sample 4 7 Sample 7 8 Sample 10 9 Sample 10 10 Sample 10 11 NC 12 Cal 4 15 Cal 4 16 - 17 - 18 - 23 - 24 - 25 - <th>Type Unknown Unknown Unknown Unknown Unknown Unknown Unknown Unknown Standard Standard Standard Standard Unknown Unknown Unknown</th> <th>known known known known known known known known whown ystife Control ystife Control ystife Control whown mdard whown known dard Souther So</th> <th>HI HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH</th> <th>CV CV nto počítač → De</th> <th>Standards Concentration</th> <th>Datum změny</th> <th></th> <th>u At. AZ. AT. AZ.</th> <th>ledat: Experiments</th> <th>HCV x p 2 x x x x x x x x x x x x x</th> <th></th> <th>able Assays</th> <th></th>	Type Unknown Unknown Unknown Unknown Unknown Unknown Unknown Unknown Standard Standard Standard Standard Unknown Unknown Unknown	known known known known known known known known whown ystife Control ystife Control ystife Control whown mdard whown known dard Souther So	HI HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH	CV CV nto počítač → De	Standards Concentration	Datum změny		u At. AZ. AT. AZ.	ledat: Experiments	HCV x p 2 x x x x x x x x x x x x x		able Assays	
Run Setup Colour Name Run Profile 1 Sample 1 Sample 1 2 Sample 1 Joha 3 Sample 2 Data 4 Sample 2 Analysis 7 Sample 2 Cycing 6 Sample 4 Absolute Quantification + Relative Quantification + Relative Quantification + Reports 6 Cal 2 1 Cal 2 Cal 4 16 Cal 2 16 Cal 2 17 Cal 4 18 Cal 2 20 Cal 4 19 Cal 2 10 Cal 2 11 Cal 2 12 Cal 4 13 Cal 3 14 Cal 3 15 Cal 4 16 Cal 2 17 Cal 2 18 Cal 3 20 Cal 4 21 Cal 4 22 Cal 4 23 Cal 2 24 Cal 3 25 Cal 4 26 Cal 2 27 Cal 3 28 Cal 3	Unknown Unknown Unknown Unknown Unknown Unknown Unknown Unknown Unknown Sandard Sandard Sandard Sandard Unknown Unknown Unknown	known known known known known known known known whown ystife Control ystife Control ystife Control whown mdard whown known dard Souther So	HI HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH	CV CV CV nto počítač > Dr zka	Copies/µL v	Datum změny	periments Typ	v Ö	ledat: Experiments	×	c		
Run Profile 1 Sample 1 Sample 1 2 Sample 2 Information 3 Sample 4 Data 3 Sample 5 Analysis 6 Sample 6 Sample 6 Sample 6 Sample 6 Analysis 7 Sample 6 Sample 6 Sample 6 Sample 6 Aboulde Countification + 11 Nc Aboulde Countification + 11 Sample 7 Reports + 12 Cal 4 18 Cal 2 2 20 Cal 4 12 Cal 2 19 Cal 2 2 2 20 Cal 4 12 2 21 2 2 2 22 2 2 2 23 2 2 2 24 2 2 2 24 2 2 2 24 2 2 2 24	Unknown Unknown Unknown Unknown Unknown Unknown Unknown Unknown Unknown Sandard Sandard Sandard Sandard Unknown Unknown Unknown	known known known known known known known known whown ystife Control ystife Control ystife Control whown mdard whown known dard Souther So	HI HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH	CV CV CV nto počítač > Dr zka	Copies/µL v	Datum změny	Тур		ledat: Experiments	م ر			
amplet 2 Sample 2 andysis 2 Sample 2 Analysis 3 Sample 2 Sycing + 4 Sample 2 Addustis 7 Sample 2 Sycing + 8 Sample 2 Addustis - 8 Sample 2 Mailysis - 8 Sample 2 Velic Discrimination + 12 Cal 1 Netroficer + 13 Cal 2 Velic Discrimination + 13 Cal 2 Velic Discrimination + 13 Cal 3 Neports + 14 Cal 3 18 - - - 20 - - 21 - - 22 - - 23 - - 24 - - 25 - - 26 - - 27 - - 28 - - 29 - - 20 - - 21 - - 22 - - 23 - - <th>Unknown Unknown Unknown Unknown Unknown Unknown Unknown Unknown Sandard Sandard Sandard Sandard Sandard Unknown Unknown Unknown</th> <th>known Ulożt known C Ulożt known C J Ulożt known C J Ulożt known Uspoład known W Ryw gatire Control W Sy gatire Control W Sy ndard W S U Skown G S St known S S St known S S S S S</th> <th>H H H H H H H H H H H H H H H H H H H</th> <th>CV CV nto počítač > Do zka</th> <th></th> <th>Datum změny</th> <th>Тур</th> <th></th> <th>ledat: Experiments</th> <th>م ر</th> <th></th> <th></th> <th></th>	Unknown Unknown Unknown Unknown Unknown Unknown Unknown Unknown Sandard Sandard Sandard Sandard Sandard Unknown Unknown Unknown	known Ulożt known C Ulożt known C J Ulożt known C J Ulożt known Uspoład known W Ryw gatire Control W Sy gatire Control W Sy ndard W S U Skown G S St known S S St known S S S S S	H H H H H H H H H H H H H H H H H H H	CV CV nto počítač > Do zka		Datum změny	Тур		ledat: Experiments	م ر			
angled 2 2 5 seruple 2 formation 4 6 Saruple 4 bata 4 5 Saruple 4 handysis 6 6 Saruple 4 yclog + 8 Saruple 4 beckute Countification + 10 Saruple 2 beckute Countification + 11 M reports + 13 Cal 3 elaber Countification + 11 Cal 3 elaber Countification +	Unknown Unknown Unknown Unknown Unknown Unknown Unknown Unknown Sandard Sandard Sandard Sandard Sandard Unknown Unknown Unknown	known Ulożt known C Ulożt known C J Ulożt known C J Ulożt known Uspoład known W Ryw gatire Control W Sy gatire Control W Sy ndard W S U Skown G S St known S S St known S S S S S	H H H H H H H H H H H H H H H H H H H	CV CV nto počítač > Do zka	okumenty > Bio Molecular Sy	Datum změny	Тур		ledat: Experiments	م ر	c		
formation 3 Serple 3 bata 5 Sample 4 sample 5 Sample 6 Sample 7 vialysis 7 Sample 7 ycling + 8 Sample 7 boolub Quantification + 10 Sample 7 leb: Docrimation + 12 Cal 1 leb: Docrimation + 12 Cal 2 leb: Docrimation + 12 Cal 2 leb: Docrimation + 12 Cal 3 leb: Docrimation + 12 Cal 4 leports + 12 Cal 2 leports - 12 Image: 12 leports - 12 Image: 12 <t< td=""><td>Unknown Unknown Unknown Unknown Unknown Unknown Unknown Sandard Standard Standard Standard Unknown Unknown Unknown Unknown Unknown</td><td>known C Uleith known C Uleith known Uspoldid known Uspold known C C C C C C C C C C C C C C C C C C C</td><td>H vižit jako → · · · · · · · · · · · · · · · · · · ·</td><td>CV nto počítač → Do zžka</td><td>okumenty > Bio Molecular Sy</td><td>Datum změny</td><td>Тур</td><td></td><td></td><td>م ر</td><td></td><td></td><td></td></t<>	Unknown Unknown Unknown Unknown Unknown Unknown Unknown Sandard Standard Standard Standard Unknown Unknown Unknown Unknown Unknown	known C Uleith known C Uleith known Uspoldid known Uspold known C C C C C C C C C C C C C C C C C C C	H vižit jako → · · · · · · · · · · · · · · · · · · ·	CV nto počítač → Do zžka	okumenty > Bio Molecular Sy	Datum změny	Тур			م ر			
A Sample 5 B Sample 6 Call Sample 7 B Call 1 B Cal 2 B Cal 4 B Cal 2 B Cal	Unknown Unknown Unknown Unknown Unknown Unknown Negative Cc Sandard Sandard Sandard Sandard Unknown Unknown Unknown	known Cupofad known Cupofad known Uspofad known Warown Server Server mdard Server Server Server Server mdard Server Server Server Server mdard Server	ziti jako →	nto počítač → Di ižka	okumenty > Bio Molecular Sy	Datum změny	Тур			م ر	C		
ata 5 5 Sample 7 nalysis 6 Sample 7 cling + 8 Sample 7 eth 9 Sample 7 Sample 7 rolute Quantification + 10 Sample 7 ekb Pcrimation + 12 Cal 1 stave Quantification + 13 Gal 3 eports + 16 - 22 0 2 23 0 - 24 0 - 25 0 - 26 0 - 28 0 -	Unknown Unknown Unknown Unknown Unknown Standard Standard Standard Standard Unknown Unknown Unknown Unknown	known C Usori known C Usori known Uspolia known S Ryw known S Rym gafw Control P gafw Control P		žka	okumenty > Bio Molecular Sy	Datum změny	Тур			م ر			
nalysis cling + el - solute Quantification + entifier + entifier +	Unknown Unknown Unknown Unknown Negative CC Standard Standard Standard Unknown Unknown Unknown Unknown	known Uspoldi Known W Spoldi Known & Byn Known gatier Control IP P gatier Control IP P Indard IIP D Known IIP H Known IIP H Known IIP State Known IIP State	řádat • Nová slo Rychlý přístup Plocha • Stažené soubory • Dokumenty • Obrázky • GeneProof HCV Validačné Listy	žka	okumenty > Bio Molecular Sy	Datum změny	Тур						
nalysis 7 Semple 7 cling + 9 Sample 9 volute Quantification + 10 - Sample 9 volute Quantification + 12 - Cal 2 taitwe Quantification + 13 - Cal 2 taitwe Quantification + 16 - - 10 - Cal 2 - Cal 4 taitwe Quantification + 16 - - 10 - Cal 2 - - - 20 S -<	Unknown Unknown Unknown Negatre C Sandard Sandard Sandard Unknown Unknown Unknown	Anovn Uspořád knovn knovn knovn knovn knovn P gative Control gative Control Madard B knovn G knovn G knovn W knovn knovn	řádat • Nová slo Rychlý přístup Plocha • Stažené soubory • Dokumenty • Obrázky • GeneProof HCV Validačné Listy	žka		Datum změny	Тур						
cling + 8 Sample 9 tit + 10 Sample 9 total Countification + 11 NC extifice + 12 Cal 1 extification + 15 Cal 2 abase Countification + 15 Cal 2 eports + 17 - 18 - - - 20 - - - 21 - - - 22 - - - 23 - - - 24 - - - 25 - - - 26 - - - 28 - - -	Unknown Unknown Negative CC Standard Standard Standard Unknown Unknown Unknown Unknown	known Uspołał known known w Ryn gative Control P S Indard S D Control P S Indard D D Indard D D Indard D D Known G Known W Known S Str Known	Rychlý přístup Plocha * Stažené soubory * Dokumenty * Obrázky * Dokumenty GeneProof HCV Validačné Listy		^			Velikost		= ▼ (2)	6		
chrg • • • Sample 0 soluite Quantification • 11 • N.C. els. Discrimitation • 12 • Call teitre Quantification • 13 • Call teitre Quantification • 15 • Call eports + 16 • • 19 •	Unknown Unknown Negative CC Sandard Sandard Unknown Unknown Unknown Unknown	known known gative Control indard P indard B known k	Plocha * Stažené soubory * Dokumenty * Obrázky * Dokumenty * Dokumenty * GeneProof HCV Validačné Listy	Název	^			Velikost					
volute Quantification + 10 - Stampent 0 fill - Cal 1 - Cal 2 table Carinination + 12 Cal 2 table Cal 2 - Cal 2 table Cal 3 - Cal 4 table Cal 3 - - table Cal 3 - - 10 - - - - 11 - - - - - 12 Cal 4 -	Negative Co Standard Standard Standard Unknown Unknown Unknown Unknown Unknown	known gatte Control gatte Control gatte Control indard indard i D Indard i D Indard known i Known i V Known i Sit	Plocha * Stažené soubory * Dokumenty * Obrázky * Dokumenty * Dokumenty * GeneProof HCV Validačné Listy			Hledání neodpovíc	ídají žádné položky.						
elsc Discrimination + 12 Cal 1 entifier + 13 Cal 2 lative Quantification + 15 Cal 4 eports + 16 Cal 2 17 0 - - 18 0 0 - 20 0 - - 21 0 - - 22 0 - - 23 0 - - 24 0 - - 28 0 - - 28 0 - - 28 0 - -	Standard Standard Standard Unknown Unknown Unknown Unknown Unknown Unknown	yative control indard J S D indard D D known G known H known W known W known S Sit Known M	r Stažené soubory # Dokumenty # Obrázky # Dokumenty GeneProof HCV Validačné Listy			Hiedani neodpović	idaji zadne polozky.						
entilier + 13 Cal 2 lstive Quantification + 15 Cal 3 eports + 16 - 10 - Cal 3 - 11 - - - 15 - Cal 4 - 16 - - - 17 - - - 18 - - - 19 - - - 20 - - - 21 - - - 22 - - - 23 - - - 24 - - - 25 - - - 26 - - - 27 - - - 28 - - -	Standard Standard Unknown Unknown Unknown Unknown Unknown Unknown	Indurd Indurd I I I I I I I I I I I I I I I I I I I	Dokumenty * Obrázky * Dokumenty GeneProof HCV Validačné Listy										
httier Quantification + 11 Cal 3 atrive Quantification + 15 Cal 4 pports + 16 Cal 4 16 Cal 4 19 Cal 4 19 Cal 4 19 Cal 4 19 Cal 4 20 Cal 3 21 Cal 4 22 Cal 4 23 Cal 4 24 Cal 4 25 Cal 4 26 Cal 4 27 Cal 4 28 Cal 4 29 Cal 4 29 Cal 4 29 Cal 4 29 Cal 4 20 Cal 4	Standard Standard Unknown Unknown Unknown Unknown Unknown	Indard E 0 Indard D 0 Known G 6 Known H 4 Known W 5 Known S 5tř	Obrázky 📌 Dokumenty GeneProof HCV Validačné Listy										
atore Quantification + 14 (Gal 3 eports + 16 Gal 4 19 Gal 4 19 Gal 4 19 Gal 2 20 S (Gal 4 20 S (Gal 4	Standard Unknown Unknown Unknown Unknown Unknown	Indard D L L L L L L L L L L L L L L L L L L	Dokumenty GeneProof HCV Validačné Listy										
eports + 15 Cal 4 16 17 0 19 0 20 0 21 0 22 0 23 0 24 0 23 0 24 0 23 0 24 0 25 0 26 0 28	Unknown Unknown Unknown Unknown Unknown	known G known G known W known W known M known M	GeneProof HCV Validačné Listy										
17 18 19 20 21 22 23 24 25 26 27 28	Unknown Unknown Unknown Unknown	known G known G known W known W known Ø known M	GeneProof HCV Validačné Listy										
17 18 19 20 21 22 23 24 25 26 27 28	Unknown Unknown Unknown	known H known V known known M known known known	HCV Validačné Listy								G		
19 20 21 22 23 24 25 26 27 28	Unknown Unknown	known V known de Sitr known known	Validačné Listy								6		
20 21 22 23 24 25 26 26 26 27 28	Unknown	known 💣 Siłč known known										Groups	
21 22 23 24 25 26 26 27 28 28		known known	Sit										+
22 23 24 25 26 27 28	Unknown	known											
23 24 25 26 27 28 28 2	Unknown												
24 25 26 26 27 28 27 28 28 20 20 20 20 20 20 20 20 20 20 20 20 20	Unknown												
25 26 27 28	Unknown												
26 2 7 2 8 3	Unknown		lázev souboru: YYM	MDD Experiment						~			
27 28	Unknown	known	Uložit jako typ: micPO							×			
28	Unknown	known	mer o	an num rates									
	Unknown		- (4 - 1 - 2) - ·						Uložit	Zrušit			
	Unknown		yt siożky						oroalt	LI USIL			
	Unknown										1		
30	Unknown								- 1				
31	Unknown												
32	Unknown												
33	Unknown												
34	Unknown												
35		known											
36	Unknown												

Fig. 1.6 Save experiment



2. In the upper right corner of the window, select the device and click the Start Run button.

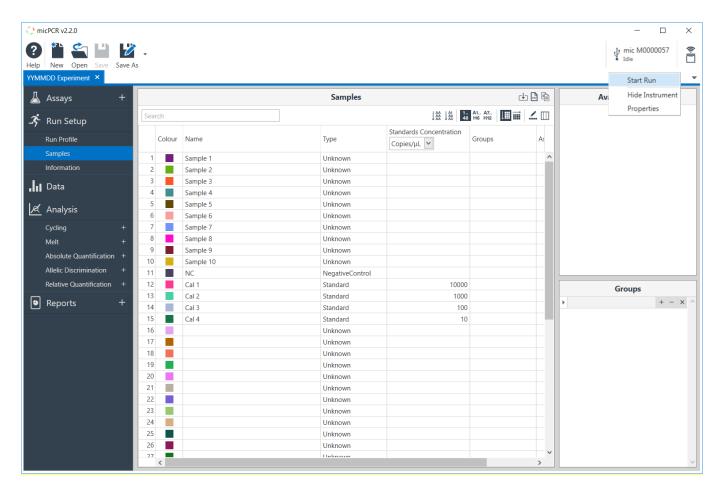


Fig. 1.7 Start experiment

1.4. Result qualitative analysis and detection evaluation

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.

1.4.4 Detection analysis in linear scale

The manufacturer recommends using this method for evaluation of the detection result as a first choice and also in the presence of clearly positive samples with a Ct value lower than 40. For the evaluation of samples with a Ct value greater than 40, it is recommended to use logarithmic evaluation (see 1.4.5 **Detection analysis in logarithmic scale**).

In Analysis tab select Cycling -> Name of the target of your detection to evaluate studied microorganism, or Cycling -> Name of your detection with IS sufix to evaluate internal standard.
 Select linear scale with the button in the upper right corner of Cycling Analysis window.

3. In **Parameters** window uncheck **Auto Set Threshold** and move the threshold line just above the reaction basal noise.

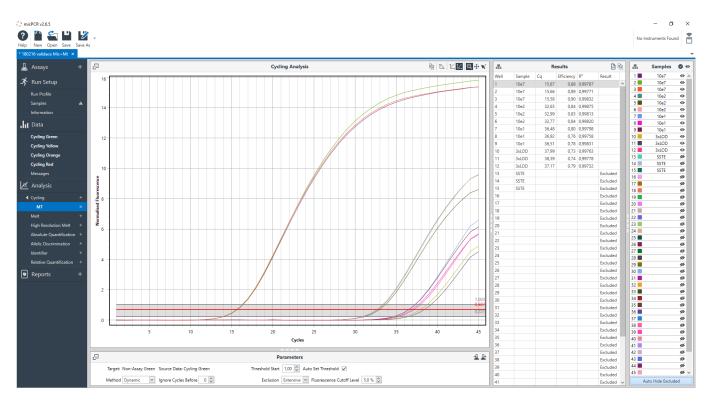


Fig. 1.8 Linear scale analysis

Cq values are displayed in the Results window.

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

Mic qPCR Cycler

Version: DOC_0279_A02_1.0 Effective date: 14. 9. 2020 Annex EN_2.0_25. 9. 2019



1.4.5 Detection analysis in logarithmic scale

In this method for evaluation, it is possible to detect weakly positive samples which, when evaluated using linear scale, could escape detection. Any sample with a numerical Cq value greater than 40 is considered to be a weakly positive sample, requiring this method of evaluation. Only one sample should be viewed at the same time for this evaluation method.

In Analysis tab select Cycling -> Name of the target of your detection to evaluate studied microorganism, or Cycling -> Name of your detection with IS sufix to evaluate internal standard.
 Select logarithmic scale with the button in the upper right corner of Cycling Analysis window.
 In Parameters window uncheck Auto Set Threshold and move the threshold line just above the reaction basal noise.

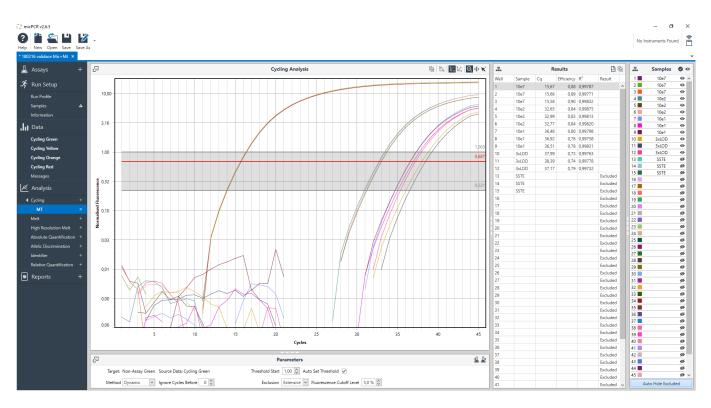


Fig. 1.9 Logarithmic scale analysis

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

1.5. Result quantitative analysis and detection evaluation

1. In Analysis tab select Absolute Quantification -> Name of the target of your detection -> Cycling.

2. On the bottom of the Samples tab uncheck the Auto Hide Excluded button, so that it turns grey.3. In Parameters window uncheck Auto Set Threshold and move the threshold line just above the

reaction basal noise.

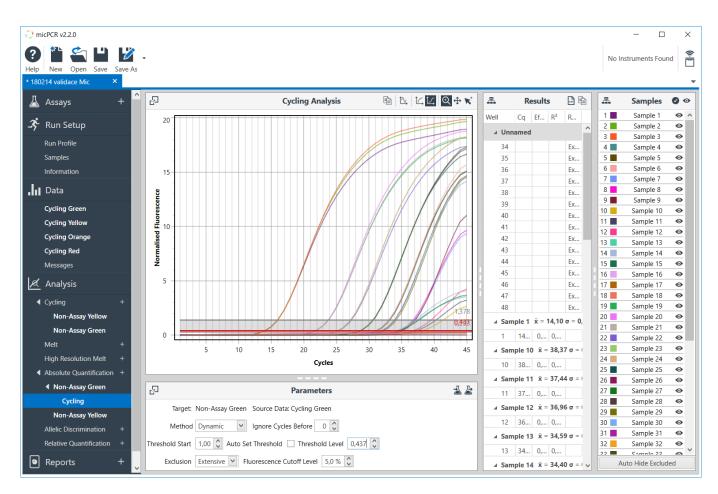
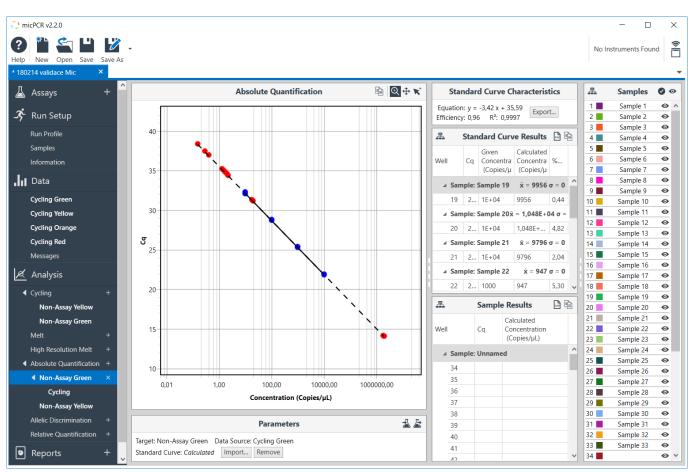


Fig. 1.10 Threshold settings

4. Switch up to Absolute Quantification -> Name of the target of your detection and evaluate the calibration quality in Standard Curve Characteristics window. The R² parameter in a well-performed calibration achieves a minimum value of 0.98 or higher. If the R² parameter is lower than 0.98, move the Threshold and repeat the analysis.





5. Cq and Calculated Concentration values are displayed in the Sample Results window.

Fig. 1.11 Standard Curve and Sample Results

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



1.6. Troubleshooting

1.6.6 Invalid positive curves

Some positive curves have an high initial fluorescence and are therefore tilted. In the **Parameters** window for **Method Dynamic**, increase the value in the **Ignore Cycles Before** field so that the curves are aligned.

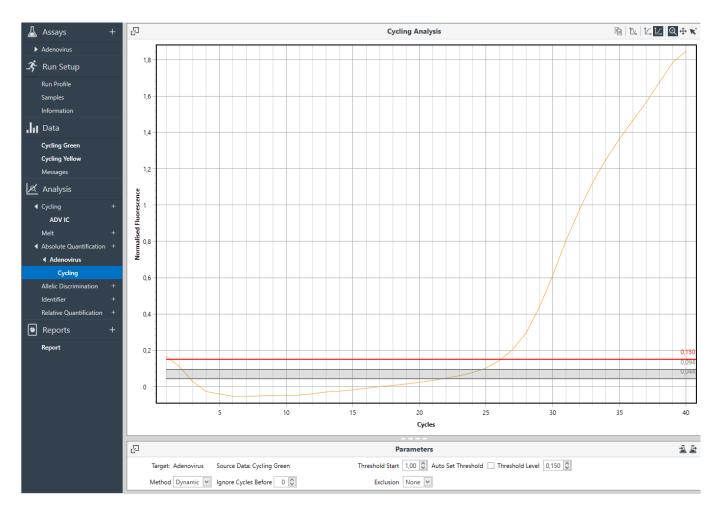


Fig. 1.12 Tilted curve in linear scale before correction

Mic qPCR Cycler

GeneProof

Molecular diagnostics for your routine

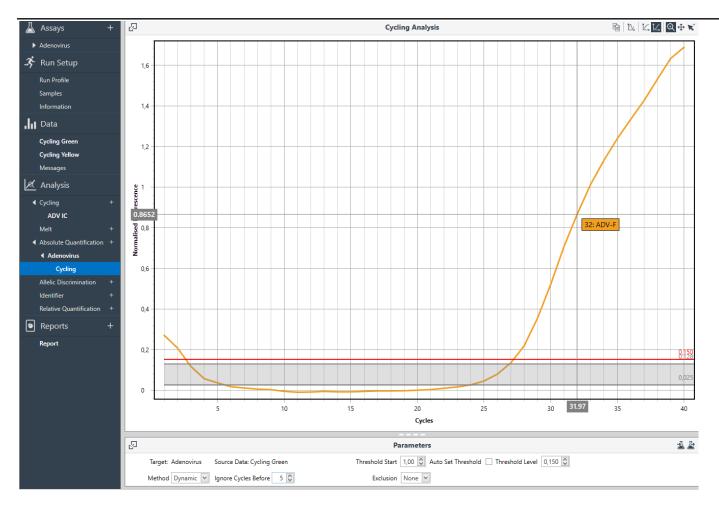


Fig. 1.13 Tilted curve after correction



1.6.7 Invalid negative curves

Due to lower fluorescence the negative samples may feature curves with linear growth of fluorescence and without the logarithmic shape that is so typical for amplification curves:

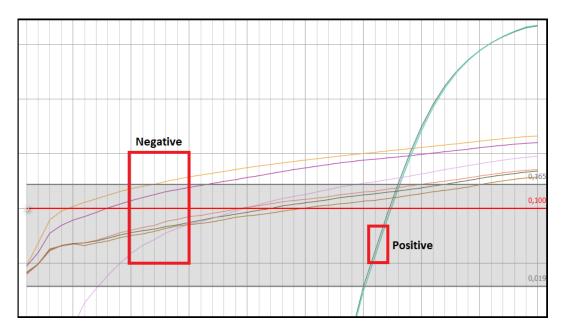
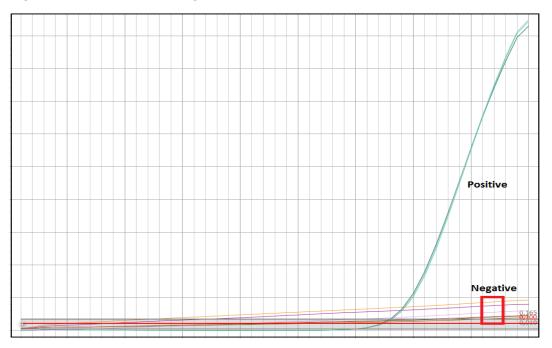
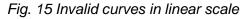


Fig. 1.14 Invalid curves in logarithmic scale





These curves are considered negative. Turn them off before setting the threshold.

Mic qPCR Cycler

16/24



2. GENETIC DIAGNOSTICS

This chapter describes in detail the process of using GeneProof PCR kits for genetic diagnostics using the instruments Mic qPCR Cycler.

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 and open them in the micPCR software.

tup				Assa	y Information						
n	Chemistry Type Hydrolysis Probes	×									
is +	Targets			Oligonucleotides							
scrimination ×	Name	Reporter Dye		Name		5' Label	Sequence	3' Label	✓ Include		
iscrimination A	▶ PAI	Allelic Target	~ ×	PAI Forward Primer		5 60001	bequence	5 6000	E mende	V	
	* Type here to add a new target		~	PAI Reverse Primer						v	
MIC				DALIMIT		FAM [™]	-		~	v	
2	😲 Uložit jako				;	 ► FAM™ ► HEX™ 	•		~		
	← → ~ ↑ 📴 > Tento počitač >	Dokumenty > Bio Molecular Systems > n	NICE Account > Ge	enetika 8. Dro	hledat: Genetika 🔎		-				
		bokumenty > bio molecular systems > m	ileren / Assays / or								
	Uspořádat 👻 Nová složka				l== • (·		Annelinen	0		
	Název	Datum	změny Typ	Velikost				Amplicon Le	ength U		
	R Rychlý přístup	12.02.2	018 16:05 mic Assi	ay File 12 kB							
	Plocha 🖈 🔔 CT		018 16:05 mic Assi						Vol	ume (μL)	
	- 🕹 Stažené soubory 🖈 🚊 Fil		018 16:02 mic Assi						10	orric (pic)	
	🗄 Dokumenty 🖈 📕 FV		018 16:05 mic Assi								
5	📰 Obrázky 🛷 📕 FXIII		018 16:04 mic Assi								
MYRA	ABI7500		018 16:01 mic Assi								
	Mikra										
	navody										
	🛄 Tento počítač								otal Volume (µL)		20
	💣 Siti							le	otal Volume (µL)		20
	JIC SIC										
	Název souboru: PAI					~					
	Uložit jako typ: micPCR Assay Files (*	.micassay)				~					
	-										
	 Skrýt složky 				Uložit Zrušit						

Fig. 2.1 Save template

Save the **Assay** to **GeneProof** file. Create an Assay folder, pathway as usual: Documents\Bio Molecular Systems\micPCR\Assays.



2.2. PCR amplification start

- 2.2.1 Opening of the saved template
 - 1. Start the micPCR software, select New -> Run.



Fig. 2.2 New experiment



2. In the Assays field click on the + symbol and choose the assay of your choice from the **GeneProof** folder in the **My assays** tab.

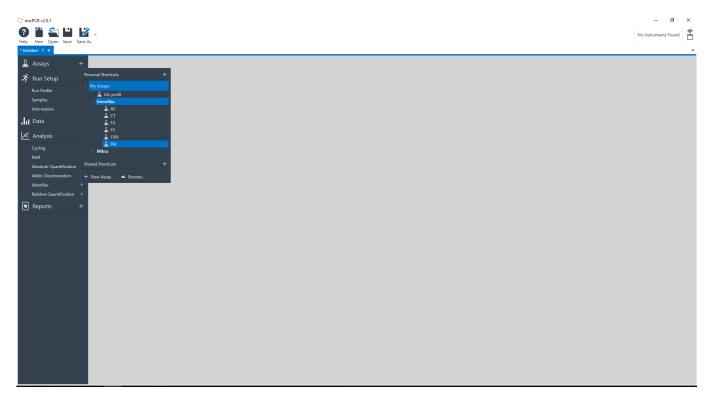


Fig. 2.3 Loading of a saved Assay



2.2.2 Sample editing

- 1. On the Samples tab, define the samples used in the experiment.
- 2. Assign an Assay to the Assay column for each sample.
- 3. For Negative controls in columnt Type set Negative Control.
- 4. For Positive controls in columnt Type set Positive Control.

New Open Save Save A	ls							No Instruments Found
Assays +					Samples		6 + 8	Available Assays
PAI	Search						然 道 福 船 船 🔳 🖬 💪 📖	ΡΑΙ
FXIII						Standards Concentration		, FXIII
Run Setup	Colour	Name	Туре	Groups	Assay	Copies/µL 🖌		
Kun Setup	1	Sample 1	Unknown		PAI		^	
Run Profile	2	Sample 2	Unknown		PAI			
Samples	3	Sample 3	Unknown		PAI			
Information	4	Sample 4	Unknown		PAI			
information	5	Sample 5	Unknown		PAI			
Data	6	PC PAI WT	Positive Control		PAI			
	7	PC PAI MUT	Positive Control		PAI			
Analysis	8	PC PAI HET	Positive Control		PAI			
	9	NC PAI	Negative Control		PAI			
Cycling +	10	Sample 1	Unknown		FXIII			
Melt +	11	Sample 2	Unknown		FXIII			
Absolute Quantification +	12	Sample 3	Unknown		EXIII			
	13	Sample 4	Unknown		EXIII			
Allelic Discrimination +	14	Sample 5	Unknown		EXIII			
Identifier +	15	PC FXIII WT	Positive Control		FXIII			
Relative Quantification +	16	PC FXIII MUT	Positive Control		FXIII			
	17	PC FXIII HET	Positive Control		FXIII			
Reports +	18	NC FXIII	Negative Control		FXIII			Groups
	19	ite i i iii	Unknown		1700			+ + -
	20		Unknown					
	21		Unknown					
	22		Unknown					
	23		Unknown					
	23		Unknown					
	24		Unknown					
	26		Unknown					
	26		Unknown					
			Unknown Unknown					
			Unknown					
			Unknown					
	31		Unknown					
	32		Unknown					
	33		Unknown					
	34		Unknown					
	35		Unknown					
	36		Unknown					
	37		Unknown					

Fig. 2.4 Sample editing



2.2.3 Starting the experiment

Save the experiment before starting the device.

1. Select Save in the main menu and save the created experiment as **micPCR Run Files** type. To make search easier it is recommended to create the **Experiments folder**.

Assays +			Sample	s		6 🕁 🖻 🗎		vailable Assays
PAI	Search					兹 翁 4a Ale Ale III 🌆 🖬 🏒 💷	PAI	
FXIII				Standards Concentration			FXIII	
un Setup	Colour Name	Type Groups	Assay	Copies/µL 👻				
un setup	1 Sample 1	Unknown	PAI			^		
n Profile	2 Sample 2	Unknown	PAI					
mples	3 Sample 3	Unknown	PAI					
formation	4 Sample 4	Unknown	PAI					
	5 Camala 6	Unknown	DAL					
ata	6 Uložit jako				×			
	7 ← → × ↑ 📑 > Tento počítač >	Dokumenty > Bio Molecular Systems	> micPCR > Experiments	v Ö Prohler	lat: Experiments 🔎			
nalysis	8				8			
vcling +	9 Uspořádat 🔻 Nová složka				BE • 🕐			
elt +	10 Název	D	atum zmēny Typ	Velikost	_			
	11 🖈 Rychlý přístup		10.000000000000000000000000000000000000	- 1-11-	_			
bsolute Quantification +	12 Plocha x		Hledání neodpovídají žádné	położky.				
Ilelic Discrimination +	13 Stažené soubory 🖈				_			
lentifier +					_			
elative Quantification +	15 Dbrázky #							
	17 ABI7500				_		(<u> </u>	
leports +	17 18 Mikra				_			Groups
	19 navody							
	20							
	21 Tento počítač							
	22 💣 Sit							
	23							
	24							
	25							
	26 Název souboru: YYMMDD Experimen	Nt.			~			
	27 Uložit jako typ: micPCR Run Files				~			
	28							
	29 A Skrýt složky			U	ožit Zrušit			
	30						(
	31	Unknown					1	
	32	Unknown					1	
	33	Unknown					1	
	34	Unknown					1	
	34	Unknown Unknown						

Fig. 2.5 Save experiment



2. Click Start Run button in upper right corner of select device window.

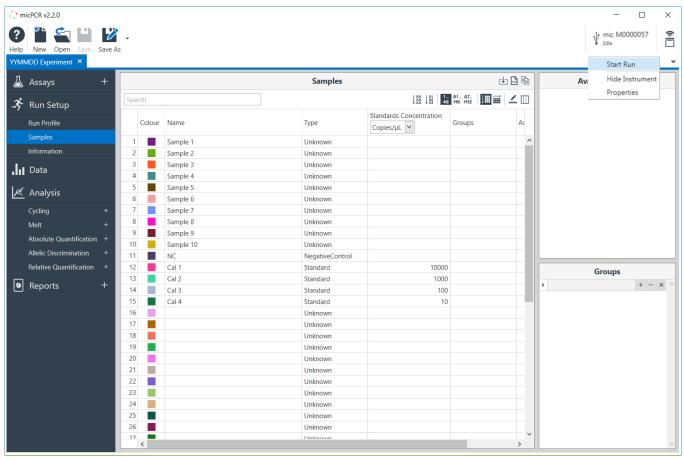


Fig. 2.6 Start experiment

2.3. Analysis of the result and evaluation of detection

2.3.4 Detection Analysis

- 1. In the Analysis tab select Allelic Discrimination-> mutation name for Genotype evaluation.
- 2. Set the threshold line manually so that in the **Results table** be: positive control WT evaluated as WT genotype, positive control MUT evaluated as MUT genotype and positive control HET evaluated as HET genotype.

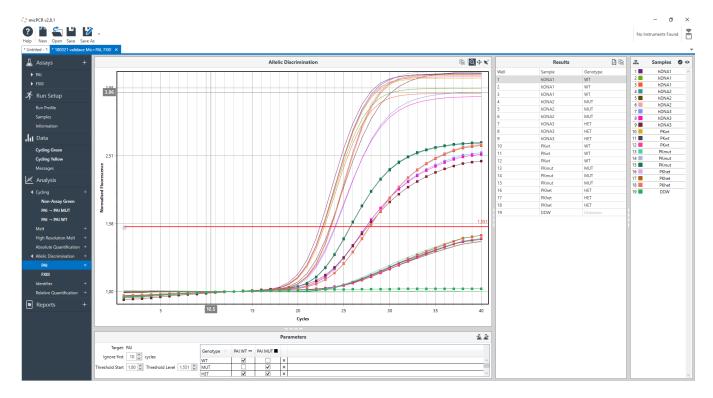


Fig. 2.7 Genotype Analysis

The genotype results are displayed in the **Results window**.



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

Orders

Phone: +420 730 176 222 e-mail: <u>support@geneproof.com</u> Phone: +420 543 211 679 e-mail: <u>sales@geneproof.com</u>