



QUANT ASSAY SOFTWARE MANUAL FOR MPP-96 PHOTOMETER

RIGA© 03.08.2021

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Disclaimer

Purpose

This program is designed to operate the photometer MPP-96 and analyse the results obtained from it.

Using QuantAssay it is possible to program the analysis of the following assays:

- Quantitative assays: the ability to install up 20 standards and choose fit model from 5/4 parameter logistic, linear and piecewise linear models
- BestFit function for the selection of the best calibration curve.
- Multiplex analysis up to 7 different tests on the same plate
- Qualitative assays: the ability to install up to 8 types of controls (weak positive, strong positive, negative, etc.)
- Avidity / affinity assay
- Save, load and export results
- Create visual reports

This manual describes how to install the program, control the device, create and edit assays, analyse the results and troubleshoot the program.

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Installation

Welcome window



License Agreement

Information Window





the path of the installation



Advanced Settings



process software installation

The installation of the drivers





License Agreement



Ready to Install

The installation of drivers



finished installing of the software



the installation of drivers is finished

The program is ready to start.

Account control: Administrators and common users rights

1. Administrator rights: now you are able to set to access levels for the ordinary users and master users.

Ordinary users can:

- Use software
- Browse Assays
- Save templates

Master users can:

- Use software
- Create/Edit Assays
- Save templates

So, the ordinary users cannot change or create assays. If you have a single user who is also a master user than this feature can get annoying when creating or editing assays, so to use the software without being asked each time do the following: Run the software as administrator.



But user would need to do that every time he uses software.

Or if user wants to set this forever: Go to Properties/Compatibility tick the "Run this program as an administrator" checkbox and apply the changes.





🯓 QuantAssay Prop	erties	×				
Security	Details	Previous Versions				
General	Shortcut	Compatibility				
If you have problem an earlier version of matches that earlier	ns with this program a Windows, select the version.	ind it worked correctly on e compatibility mode that				
Help me choose	the settings					
Compatibility mod	e					
Run this prog	gram in compatibility	mode for:				
Windows XP (S	Service Pack 3)	-				
Settings						
Run in 256 c	colors					
🔲 Run in 640 x	480 screen resolutio	n				
📃 Disable visua	al themes					
Disable desk	top composition					
Disable displ	ay scaling on high D	PI settings				
Privilege Level						
Run this prog	Run this program as an administrator					
Change settings for all users						
	ОК	Cancel Apply				

Note! Because of the that modification, we had to move all assays to common documents folder, and your user would need to do that manually. (If you were using versions below 0.7.x.x)

Here is an instruction how to move files:

2.1. Open new the software and close it. (This will create needed folders)

2.2. Copy all assays: In Program Files(x86)/QuantAssay find folder Methodics. Copy this folder

to: C:/Users/Public/Public Documents/QuantAssay/ then Replace conflict files if being asked.

Performing a Measurement



- 1. Open the program
- 2. Go to the tab "Available Devices"

Available Devices	

3. Select the wavelengths at which you want to measure

Wavelength	
ratelengen	
4 05 nm	Channel 1
🔲 450 nm	Channel 2
🔲 490 nm	Channel 3
🔲 620 nm	Channel 4

4. Optional: enter the reference channel and if you would like to mix the plate before the measurement:

Enable reference	Ref. filter, nm	
Mix before measure		
Mixing		
Mixing amplitude, mm	8	
Frequency, 1/s	12	
Time	4	

5. Click on the "Start" button



6. Then, in aprox. 5 to 15 sec., the program will automatically open the tab "Input Data", which will display the measurement results:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
	0.001	0.001	0.001	0.002	0.002	0.002	0.002	0.002	0.003	0.003	0.003	0.003
В												
	0.000	0.001	0.001	0.001	0.001	0.001	0.002	0.002	0.003	0.003	0.003	0.003
С												
	0.001	0.001	0.002	0.001	0.001	0.002	0.002	0.001	0.002	0.002	0.002	0.002
D												
	0.001	0.001	0.002	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
E												
	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.003	0.003	0.003	0.003	0.003
F												
	0.000	0.001	0.001	0.001	0.001	0.002	0.002	0.002	0.002	0.002	0.002	0.002
G												
	0.001	0.001	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
н												
	0.001	0.001	0.002	0.002	0.003	0.003	0.003	0.003	0.003	0.004	0.004	0.004

- 7. To save the experiment in the format of Quant Assay file, click on the "Save" button
- 8. To save the data in the format of the plate, click on XLS button, which is located next to "Assay editor" button
- 9. To save the data in .csv .xls .pdf formats, click on the corresponding icons



XLS 96 well

Running a test

1. Open the software



window after opening the program

QuantAssa	у											
ile <u>O</u> ption	s											
EW		XLS CSV		L 🎝								
xp_2001_1	004_0 🔯 Ex	0_2001_104	7_1 🛛									
Avai	ilable Devices		I	input Data			View Res	ults				
									10			Choose an assay
1	2	3	4	5	0	/	8	9	10	11	12	VIS 96 well
A												Choose a Template or Save as
												· · · · · · · · · · · · · · · · · · ·
B												1 What to show in a cell:
												A ///// Sample Name -
С												Туре
D										-		Cell Name Sample Name
												Main channel
F												Calculate
-												
F												
_												
G												
н												

2. Select an assay (here are listed predefined assays):

Choose an assay	
	-
Avididty	
Multiplex 4 Targets	
Qualitative	
Quantitative Reverse	
Quantitative	

- a. Avidity
- b. Multiplex 4 Targets
- c. Qualitative
- d. Quantitative reverse
- e. Quantitative
- 3. Each assay is described in more details in the section **Assay Editor.** Here we describe

the use of the simplest assay - Qualitative

4. Qualitative assay:

This assay adopted to put a specified threshold value of Optical Density (OD): A sample is regarded as positive if the corresponding OD value is equal to or greater than the threshold (OD critical), which in this example is calculated by the formula:

= Negative Control (N1) +0.2

where N1 - Is the mean OD value for the negative control samples.

Quality control is also taken into account by following conditions:

- the OD value of the Positive Control (P1) are at least 0.8 OD,

where P1 - Is the mean OD value the positive control

- the OD value of the Negative Control (N1) are less than 0.2 OD,

where N1 - is the average OD value for the negative control

5. Fill virtual plate with: Types of Group 1 Reset Reset Bkg P₁ - N₁ - X samples:

Test - Test sample

- Bkg Background (the average value of those samples will be deducted from the whole plate, the deducted values can only be observed in the Results tab, data input tab will remain the same)
 - Positive Control 1
 - Negative Control 1 (Threshold/OD critical is calculated based on OD value of those samples)

x

Name Smp

Group

1 🚍

 P_1

 N_1

- Remove the sample

1 🚍

Reset

- In this field are specified a name (constant), suffix (counted), and a group (counted). For example, if you add a test sample, it will

be referred to as Smp 1 and will apply to group 1, and the counter of suffix and groups will jump to 2, as shown in the following picture:

Name Group	Smp	2 🐑 Reset	× Test	•
	1	2	3	4
A	Smp1 T1			

- 6. The methods of filling the plate:
 - a. For a quick filling of the plate with test samples: fill in one of the wells (eg A1), with a test sample.

	1	2	3	4	5
A	Sample1 T1				
в					
С					
D					

b. In order to fill all the remaining wells with remaining samples, place the mouse cursor on the small square in the lower right corner of the cell, hold the left mouse button, and lead to the desired cell (as in Excel).

A



After									
	1	2	3						
A	Sample1								
	T1								
В									
С									

1 2 Sample1 Sample4 Sample7

Before

	T1	T4	Т7
B	Sample2	Sample5	Sample8
	Т2	Т5	т8
С	Sample3	Sample6	Sample9
	тз	Т6	Т9

3

c. To enter the name of the sample, click on the desired cell by double-clicking the mouse. The following window appears:

Set test sample name: Smp1 Position: A1 < Previous Next > Ok Cancel	Set Sample Name	×
Position: A1 <pre> <pre> <pre> <pre> <pre> <pre> </pre> <pre> <pre> <pre> <pre> <pre> </pre> </pre> <pre> <p< td=""><td>Set test sample name:</td><td>Smp1</td></p<></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre>	Set test sample name:	Smp1
< Previous Next > Ok Cancel		Position: A1
	< Previous Next >	Ok Cancel

To confirm the name: press the OK button. To cancel, press: Cancel. To move to the next / previous cell, click on the appropriate button.

d. To make sample repeats, select two adjacent cells in which the sample is and press on the "Sample" button. To fill the remaining part of the plate in this pattern -- hold down left mouse button on the little black square and drag the mouse to the desired cell. If the samples are filled in 3, 4, etc. repetitions, fill the appropriate number of adjacent cells.

Be	efore						After				
		1	2	3	4		1	2	3	4	
	A	Sample1	Sample1			Α	Sample1	Sample1	Sample4	Sample4	
		Т1	T1				T1	T1	T4	T4	
I	B			-		В	Sample2	Sample2	Sample5	Sample5	
							T2	T2	Т5	Т5	
	C					С	Sample3	Sample3	Sample6	Sample6	
							тз	тз	Т6	Т6	

e. Fill in the controls: for positive controls select P1; for negative controls, select the



7. Example of a filled plate

J Qı	antAssay				1 1 2				1 20-	10.1	- 10	1.5						
File	<u>Options</u>																	
NEW	LOAD		xLS CS	*#	L 🎝												(bioSan
Exp_	2001_1634	0 🔯																
	Availabl	e Devices		Ŀ	nput Data			View Resu	ılts									
Nam	e Smp	81 🝧	x Test	Dha	D		т	v					Cho	ose a	n assa	У		
Grou	Group 81 🛞 Reset																	
	1 2 3 4 5 6 7 8 9 10 11 12 Choose a Template or Save as																	
A	Smp1	Smp9	Smp17	Smp25	Smp33	Smp41	Smp49	Smp57	Smp65	Smp73	Positive	Positive						
	т1	Т9	T17	T25	T33	T41	T49	T57	T65	173	P1	P1		1	Wha	t to show in a c	ell:	
В	Smp2	Smp10	Smp18	Smp26	Smp34	Smp42	Smp50	Smp58	Smp66	Smp74	Negativ	Negativ	A		Sam	ple Name	-	
	т2 Т2	т10	T18	T26	T34	T42	т50	T58	T66	174	N1	N1			Тур	2	•	
C	Smp3	Smp11	Smp19	Smp27	Smp35	Smp43	Smp51	Smp59	Smp67	Smp75	Negativ	Negativ	Cell	Nan	ne	Sample Name		Туре
	T3	T11	T19	T27	T35	T43	151	T59	T67	175	N1	N1	#1	A1	Mair	Smp1		T1
	Smp4	Smp12	Smp20	Smp28	Smp36	Smp44	Smp52	Smp60	Smp68	Smp76	Bka	Bka	Ca	lculate		▼		
	TA	T12	T20	T20	1726	TAA	150	T60	T69	176	D.	D						
-	Crue F	0	0	0.000	0	Const	132 Cmat2	0	0	0	Die	Die						
E	Smpo	Smp13	Smp21	Smp29	Smp37	Smp45	Smpos	Subor	Suboa	Smp77	вкд	вкд						
	т5	T13	T21	T29	T37	T45	T53	T61	т69	777	В	В						
F	Smp6	Smp14	Smp22	Smp30	Smp38	Smp46	Smp54	Smp62	Smp70	Smp78	Bkg	Bkg						
	T6	T14	T22	T30	T38	T46	T54	T62	T70	178	В	В						
G	Smp7	Smp15	Smp23	Smp31	Smp39	Smp47	Smp55	Smp63	Smp71	Smp79	Bkg	Bkg						
	Π	T15	T23	T31	T39	T47	T55	T63	771	T79	В	в						
H	Smp8	Smp16	Smp24	Smp32	Smp40	Smp48	Smp56	Smp64	Smp72	Smp80	Bkg	Bkg						
	т8	T16	T24	T32	T40	T48	T56	T64	T72	т80	в	в						

8. to save the template of the plate, enter its name in the "Save As" and press the save



icon

- 9. To start the measurement, click on the "Start"
 - a. If the selected method the wavelength is not set, then program will jump to the Available Devices tab, where you can set the wavelength and other parameters
 - b. Press "Start" when ready

		ID: Not connected	
	Wavelength		1
	🔲 405 nm	Channel 1	
	🔽 450 nm	Channel 2	
	🔲 490 nm	Channel 3	
	🔲 620 nm	Channel 4	
🔲 Enal	ole reference	Ref. filter, nm	~
Mix	before measure		
-Mixing) speed D strokes/min →		
Time	e, sec. 4		
Star	t		

🎩 Qi	antAssay													
<u>F</u> ile	jile Options													
NEW	LOAD		XLS CS		L 🎝								biolan	
Exp_	2001_1634	0 💌				1								
	Availabl	e Devices		Ir	nput Data			View Resu	lts					
Nam	e Smp	81 💌	x Test	- Bkg	D	- N	-	x					Choose an assay	
Grou	Group 81 Reset LSS V LS 66 VILS 66 VIL													
	1 2 3 4 5 6 7 8 9 10 11 12 Choose a Template or Save as													
	- Smn1	Smn9	Smn17	Smn25	Smn33	Smn41	Smn49	Smn57	Smn65	Smn73	Positive	Positive		
 ^	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1 What to show in a cell:	
	0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 Source A Sample Name -													
B	Smp2	Smp10	Smp18	Smp26	Smp34	Smp42	Smp50	Smp58	Smp66	Smp74	Negativ	Negativ	450 nm -	
	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001	0.000	0.000	0.000	0.000	0.000	Cell Name Sample Name Type	
C	Smp3	Smp11	Smp19	Smp27	Smp35	Smp43	Smp51	Smp59	Smp67	Smp75	Negativ	Negativ	#1 A1 Smp1 T1	
	0.000	0.001	0.001	0.001	0.002	0.002	0.002	0.002	0.002	0.003	0.002	0.003	Main channel	
D	Smp4	Smp12	Smp20	Smp28	Smp36	Smp44	Smp52	Smp60	Smp68	Smp76	Bkg	Bkg	Calculate 450 nm 👻	
	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001		
E	Smp5	Smp13	Smp21	Smp29	Smp37	Smp45	Smp53	Smp61	Smp69	Smp77	Bkg	Bkg		
	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.002		
F	Smp6	Smp14	Smp22	Smp30	Smp38	Smp46	Smp54	Smp62	Smp70	Smp78	Bka	Bka		
1	0.000	0.001	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002		
	Cmp7	Cmp1E	Cmp22	Cmp21	Cmp20	Cmp47	ConsEE	Cmp62	Com 71	Cmp70	Blea	Dieg		
G	Smb1	Subt2	Smp23	Smb31	Sub38	Smp47	Suib22	Sunbo3	Smb/1	Smp79	вку	вку		
	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001		
H	Smp8	Smp16	Smp24	Smp32	Smp40	Smp48	Smp56	Smp64	Smp72	Smp80	Bkg	Bkg		
l	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001		

10. Next, the program will take you back to the "Data Input" tab.

le 🤉	2ptions									
w	LOAD	xLS CSV								hin
20.20	01 1624		•• /							
xψ_2ι	01_1034_	<u></u>								
	Available	Devices	Inp	out Data		View Results				
Cell	Туре	Sample Name	Group	OD 450 nm	Result 1	Result 2	Mean (OD)	Standard Deviation (OD)	Coefficient of Variation (OD)	Sort by
A1	T1	Smp1	1	0.000	-	0.00	0.000	0.000	0.00%	 Cols
A2	т9	Smp9	9	0.000	-	0.00	0.000	0.000	0.00%	Rows
A3	T17	Smp17	17	0.000	-	0.00	0.000	0.000	0.00%	
A4	T25	Smp25	25	0.000	-	0.00	0.000	0.000	0.00%	
A5	Т33	Smp33	33	0.000	-	0.00	0.000	0.000	0.00%	
A6	T41	Smp41	41	0.000	-	0.00	0.000	0.000	0.00%	
A7	T49	Smp49	49	0.000	-	0.00	0.000	0.000	0.00%	
A8	T57	Smp57	57	0.000	-	0.00	0.000	0.000	0.00%	
A9	T65	Smp65	65	0.000	-	0.00	0.000	0.000	0.00%	
A10	T73	Smp73	73	0.000	-	0.00	0.000	0.000	0.00%	
A11	P1	Positive control P1		0.000	Error		0.000	0.000	5.29%	
A12	P1	Positive control P1		0.000	Error		0.000	0.000	5.29%	
B1	T2	Smp2	2	-0.001	-	-0.01	-0.001	0.000	0.00%	
B2	T10	Smp10	10	-0.001	-	-0.01	-0.001	0.000	0.00%	
B3	T18	Smp18	18	-0.001	-	-0.01	-0.001	0.000	0.00%	
B4	T26	Smp26	26	-0.001	-	-0.01	-0.001	0.000	0.00%	
B5	T34	Smp34	34	-0.001	-	-0.01	-0.001	0.000	0.00%	
B6	T42	Smp42	42	-0.001	-	-0.01	-0.001	0.000	0.00%	
B7	T50	Smp50	50	-0.001	-	-0.01	-0.001	0.000	0.00%	
B8	T58	Smp58	58	0.000	-	0.00	0.000	0.000	0.00%	
B9	T66	Smp66	66	0.000	-	0.00	0.000	0.000	0.00%	
B10	T74	Smp74	74	0.000	-	0.00	0.000	0.000	0.00%	
B11	N1	Negative control		0.000	ОК		0.001	0.001	112.87%	
B12	N1	Negative control		0.000	ОК		0.001	0.001	112.87%	
C1	Т3	Smn3	3	0.000	-	0.00	0.000	0.000	0.00%	*

11. To view the results in the table format, go to "View Results" tab.

12. To export data in PDF, Excel and CSV click on the corresponding icon



. .



13. To save the experiment in QuantAssay format, click Save icon

Kinetic Mode

To make measurements over time do the following:

Go to Input Data tab and find following panel in the bottom right corner:

🔒 Kine	Kinetic Mode Panel										
 ✓ 405 nm ✓ 450 nm ✓ 490 nm ✓ 620 nm 	Channel 1 Channel 2 Channel 3 Channel 4	Measurement freq. (sec) Number of measurements	3								
Start	Stop	Results	Cyde №:								

Here simply choose channels, set measurement frequency (in seconds) and number of measurements (in the example above software will do 12 measurements with 3 seconds intervals between).

You can stop the measurements any time by click stop, to get the results click on the Results and in the new tab press XLS button, which will export data to Excel.

If you want to make more measurement, simply put the maximum number of measurements (99999).

Quick conversion table. 1 min = 60 sec, 10 min = 360 sec, 1 hour = 21 600 sec, 2 hour = 23 200 sec.

Assay Editor

- 1. Assay Editor will allow you to program the following types of analysis:
 - Qualitative analysis
 - Quantitative analysis: linear and reverse
 - Analysis of avidity
 - Multiplex assay
- 2. for each type of the assay it is possible to define:
 - the number of types of positive controls (strong, weak, etc.)

- the number of types of negative controls (no 1'/ 2' antibody conjugate, water sample) Note: Each type of the Control can be analyzed separately from the rest of the positive or negative controls

- For multiplex analysis, you can select the number of targets (antigens)
- Primary wavelength channel

- Reference channel (OD values obtained on the reference channel will be subtracted from the OD values obtained on the primary wavelength channel)

- for quantitative methods: the choice of the calibration curve between the "Best Fit" and piecewise linear models. (Best Fit will automatically select the model with highest coefficient of determination (R²) among the: 5 parameter logistic, 4 parameter logistic, linear and various regression models.

- description of the assay

Creating Qualitative assay

1. For example we need to create a qualitative assay with following criteria: Measurement channel at 450 nm.

In this assay the sample will analysed as positive, if the corresponding OD value is equal or greater than the Critical (Threshold) OD, which is calculated by the formula:

= NC1 + 0.2 OD, where NC1 is the Average OD of Negative Control 1.

Quality control of Negative and positive controls should meet following criteria:

- OD value of the positive control must be greater than 1 OD
- OD value of the negative control must be less than 0.1 OD

The following steps show how to create this assay:

2. Click on "Create" button. Following window will appear:

3 Create Assa	ву		-				
Create	Save	Load	Delete				
			Assay Create 🛛	7			
			Metodic name:				
			Qualitative				
			Assay type		Wavelength		
			Quantitative	1	🛄 405 nm	Channel 1	Enable reference
			Qualitative	1 ▼ Neg. control count	450 nm	Channel 2	👻 Ref. filter, nm
			Avidity	1 T Group Count	620 nm	Channel 4	Mix before measure, s
			Multiplex	▼ Standards count	Description		
			Form			•	

3. Enter the name of the assay e.g. "Qualitative", select the type of assay: "Qualitative", leave the number of the positive/negative Controls: e.g. 1, set the wavelength to: 450 nm. Give a short description of the assay. Click **Form**

Assay Create 📧		
Measurement option Metodic name:	15	
Qualitative		
Assay type		Wavelength
Quantitative	Pos. control count	405 nm Channel 1 Enable reference
Qualitative	1 🔻 Neg. control count	V 450 nm Channel 2 Ref. filter, nm
Avidity	1 💌 Group Count	620 nm Channel 3 Mix before measure, s
Multiplex	▼ Standards count	Description
Form		

4. In the next window you can select the types of results for this assay:

Qualita	tive								
	Choose Results types for Qualitative Assay O Positive / Negative								
Ħ	Positive / Gray Zone / Negative								
	Type in gray zone margin: Symmetric Non Symmetric 0.10 OD from Critical OD (Treshold) 0.10 0.10 0.10 0.10 0.10 0.10 0.10 0.10 0.10 0.10 0.10 0.10 0.10 0.10 0.10 0.10 0.10 0.10 0.10								
3x 2x $1x$ $\boxed{2x}$ Show Coefficient of Positivity in Results									
Cont	inue Cancel								

1) "Positive / Negative" -- according, if the sample OD is greater or equal to the Critical

(Threshold) OD, the result will be marked as "Positive", else the sample will be marked as "Negative"

2) "Positive / Gray Zone / Negative" -- according, if the sample OD is greater or equal to the threshold OD plus the value indicated in the box "Gray zone = +/–", the result will be marked as "Positive", else if the sample will be between the threshold OD plus/minus OD value indicated in the box below the result will be marked as "Gray Zone", else the sample will be marked "Negative"



If you leave the tick in the check box "Show Coefficient of Positivity in Results" that would output the ratio of test sample divided by threshold OD. Click "Continue".

- As we see, the assay editor automatically filled most of the fields in order to analyze the results and to perform quality control. Here is what is being filled automatically and what it means:
 - 6. Tab "Variables and formula"

Variables and formulas											
Variable	Description	Formula									
[C]	Critical OD	[N1]+0.1									
[F]	Coefficient of positivity	[T_0]/[C]									

Two variables were created: [C] and [F], where [C] - is the Critical (Threshold) OD, and [F] - is the ratio of test sample divided by Critical (Threshold) OD or, how we call it — Coefficient of Positivity.

Critical (Threshold) OD is calculated by the formula [N1] +0.1, where [N1] - is the average value of negative control 1. So if N1 is 0.1, than Critical OD will be equal to 0.2 OD

7. Next, we need to perform quality control and analyze our test samples: Tab "Results Interpretation".

As we see, the assay editor automatically fills most of the fields. Here is what is being filled automatically and what it means:

Result interpretation										
For variable	Conditional	Result 1	Result 2							
		True	False	True	False					
ពា	[T]>[C]	+	-	[F]	[F]					
[P1]	[P1]>1	ОК	Error							
[N1]	[N1]<0.2	ОК	Error							

Columns:

- In the column "For variable" you can set for which variable following conditional will be used, e.g. variable [T] means that the conditional and results filled in the next fields will be used for the test samples, to chose another variable, right-click on the field under the column and select an appropriate variable.

- In the column "Conditional" are specified conditional formula by which the "Results 1 and 2" are interpreted, the condition is being interpreted by logical operation "IF, THAN", and outputs the result in "Result 1 and 2" sub-columns "True" or "False".

In our example:

Condition [T]> [C] means that if the test sample OD ([T]) is greater than the critical OD ([C]), then the "Result" 1 will be "+".

In the column "Result 2", regardless of the condition, positivity coefficient [F] will always be outputted.

Further, quality control:

For the negative control ([N1_0] is the same as [N1]) is written condition [N1] <0.2, which means that if the OD of neg. control is less than 0.20, then "Result 1" outputs "Ok", if not, then "Error".

For the positive control ([P1_0] is the same as [P1]) the condition is [P1]> 1.0 that is, if the OD of Pos. control is more than 1.0, then "Result 1" outputs "Ok", else "Error".

- 8. Save the assay and close the window of "Assay Editor""
- 9. Choose your newly created assay and run it:

Choose an assay

	-
Qualitative	

Create a Qualitative Reverse assay with Negative/Suspect/Positive results

This example will feature IDEXX® Pseudorabies Virus gpl Antibody Test Kit®. Go to the calculations chapter of their manual.

First of all, create the assay

 Create As.
Create
Andrey test Avidity Multiplex 4 New test PPC Qualitative Qualitative Quantitative Semi Quant Test for quantitative test for quantitative

Type in the name: Pseudorabies Virus gpl Antibody Test Kit Assay type select Qualitative Wavelenght set 620nm

Controls

$$NC\overline{x} = \frac{A1 A(650) + A2 A(650) + A3 A(650)}{3}* PC\overline{x} = \frac{A4 A(650) + A5 A(650)}{2}$$

*Example shows Negative Control run in Triplicate.

We see that there is used 1 PC and 1 NC, since our software is always calculating the mean of each of the controls (no matter the number of replicates), we don't need to do anything additional here.

Leave the Pos./Neg. control group count as 1 each. Click Form.

Assay type	_		
○ Quantitative	1	~	Pos. control count
Qualitative	1	\sim	Neg. control count
○ Avidity	1	\sim	Group Count
OMultiplex		\sim	Standards count
Form			

In the next window, select Results type as Positive/Gray Zone/ Negative

Leave Symmetric gray zone

Deselect Show coefficient of Positivity in Results, or you can leave it, if you wish. Press Continue

Qualita	itive		×
	-Choose	e Results types for Qualitative Assay	
	Posi	itive / Gray Zone / Negative	
[Type in Sym	gray zone margin: imetric 0.10 OD from Critical OD (Treshold) Symmetric 0.10 and + 0.10)
	33		-
	fi ∐Sh	ow Coefficient of Positivity in Results	
Cont	tinue	Cancel	

Now we need to enter the S/N ratio:

Samples

$$S/N = \frac{\text{Sample A}(650)}{NCX}$$

In the Variables and formulas change Description for the [F] variable to S/N, and change it's corresponding formula to

[T_0]/[N1]

You can use right click for selecting OD Sample > 0,

type in "/",

use right click for selecting K (Negative control) 1 > 0

Variables and formulas							
Variable	Description	Formula					^ -
[C]	Critical OD	[N1]+0.1					
[F]	Coefficient of positivity	T 01/[N1]					
	,	C-1-370-1-1	Backgi	round			
			K (Pos	itive control) 1	>		×
			K (Neg	gative control) 1	>	0	
			Group	average	>	Standard	Deviation (OD)
		1	S OD Sai	mple	>	2	
			Critica	I OD	>		
			Wild C	Card	>		

Go to Result interpretation table, clear all Conditional fields and type in the new conditionas from the Calculations chapter of the kit's manual.

First of all we need to enter the Validation criteria, which is:

Validity criteria

 $NC\overline{x} - PC\overline{x} \ge 0.300$

In the Results interpretation table, for variables P1 and N1, write following conditionals:

[N1]-[P1]>=0.3 , if True = Ok if False = Error,

you can use Right click for selecting the controls. See below for finished example screenshot.

retation			
Conditional	Result 1		F
	True	False	1
[N1]-[P1]>=0.3	ОК	Error	
[N1]-[P1]>=0.3	OK	Error	
	Conditional [N1]-[P1]>=0.3 [N1]-[P1]>=0.3	Conditional Result 1 [N1]-[P1]>=0.3 OK [N1]-[P1]>=0.3 OK	Conditional Result 1 Image: N1]-[P1]>=0.3 OK False [N1]-[P1]>=0.3 OK Error

Now go to interpretation chapter of the kit's manual, which is:

15 Interpretation:

Negative	Suspect	Positive*
S/N > 0.70	$0.60 < S/N \le 0.70$	$S/N \le 0.60$

*Confirm all positives in duplicate.

Note: IDEXX has instrument and software systems available which calculate results and provide data summaries.

For Variables T (test samples) , write following conditionals:

[F]>0.7 ; True = Negative

([F]>0.6) && ([F]<=0.7); True = Suspect

[F]<=0.6 ; True = Positive

you can use Right click for selecting the samples and logical operators.

See below for finished example screenshot.

Result interp	retation			
For variable	Conditional	Result 1		Re
		True	False	Tru
[ד]	[F]>0.7	Negative		
נדן	([F]>0.6) && ([F]<=0.7)	Suspect		
[1]	[F]<=0.6	Positive		
[P1]	[N1]-[P1]>=0.3	ОК	Error	
[N1]	[N1]-[P1]>=0.3	ок	Error	

Your assay is good to go!

Create a Quantitative Assay

We want to create a quantitative assay with following criteria:

Measurement channel is 450 nm., with reference channel at 620 nm and mix before measuring. 6 standards with concentrations of: 0, 5, 10, 25, 100, 500 International Units (IU)are being used. Calibration curve should be fitted automatically by choosing the best fitting curve (based on R² value) and test samples concentrations will be calculated by using that curve.

We want the test samples OD value of which are greater than OD value of Standard 1 to be marked as positive samples.

We want to exclude extrapolation.

Quality control of Standards and of Negative and Positive controls should meet following criteria:

- Each standard of a higher concentration should have OD greater than the lower standard

 $(OD_{standard_0} < OD_{standard_1}, OD_{standard_1} < OD_{standard_2}, etc)$

- OD value of the positive control should be greater than 1 OD

- OD value of the negative control should be less than 0.1 OD

The following steps show procedure of creation of this assay

1. Click on "Create" button. Following window will appear:

Measurement option	5	
Assay name:		
Assay Name (05.04	12:39:35)	
Assay type		Wavelength
Quantitative	1 Pos. control count	405 nm Channel 1 Enable reference
Qualitative	1 Neg. control count	450 nm Channel 2 Ref. filter, nm
Avidity	1 👻 Group Count	620 nm Channel 4 Mix before measure, s
Multiplex	2 Standards count	Description Curve fit method
Form		Best fit (Recommended)

Enter the name of the assay eg "Qualitative", select the type of assay: "Qualitative", set the number of standards to 6, leave the number of the "Positive"/"Negative Controls": eg 1, set the "Wavelength": 450 nm, set enable reference, select 620 nm and set "Mix before measure, s". Give a short "Description" of the assay. Leave selecetion on the "Best fit (Recommended)" in the "Curve fit method" or select the default curve that will be used for calculation. Click "Form"

		Choose a type of Quantitative Assay Quantitative Standard (OD directly proportional to the conc.) Quantitative Reverse (OD inversely proportional to the conc.) Continue Cancel	
Measurement options			
Assay name:			
Quantitative			
Assay type		Wavelength	
Quantitative	1 💌 Pos. control count	405 nm Channel 1	
Qualitative	1 🔻 Neg. control count	√ 450 nm Channel 2 620 nm √ Ref. filter, nm	
Avidity	Group count	□ 490 nm □ Channel 3 ✓ 620 nm(Ref) □ Channel 4 □ Mix before measure, s	
Multiplex	6 👻 Standards count	Description Curve fit method	
	Constants list	▼ Best fit (Recommended) ▼	

Quantitative

- 3. In the next window you can select the type of the quantitative assay: "Quantitative Standard" or "Quantitative Reverse" ("Reverse" means that with the increase of concentration the OD is decreasing, "Standard" means that with the increase of concentration the OD is also increasing)
- 4. As we see, the assay editor automatically fills most of the fields to analyze the results and to perform quality control. Here is what is being filled automatically and what it means:
- 5. Tab "Variables and formulas"

Variables and formulas Stand	ards	
Variable	Description	Formula
[C]	Critical OD	[N1]+0.1
[F]	Coefficient of positivity	[T_0]/[C]

Two variables were created: [C] and [F], where [C] - is the "Critical (Threshold) OD", and [F] - is the ratio of test sample divided by threshold OD or "coefficient of positivity". In our example both formulas are irrelevant as we will quantify the results by fitting plots via standards. What we need to set is the concentration of standards, to do that click on the Standards tab

Variables an	d formulas Standa
Variable	Concentration
[50]	
[51]	
[52]	
[53]	
[54]	
[55]	

In column "Variable" [S0], [S1], etc. stands for Standard 0, Standard 1, etc.

In column "Concentration" fill in the concentration values. In field "Units" choose "IU" (international units).

Variables an	d formulas Standa	rds		
Variable	Concentration	_	Units	I U •
[50]	0		IU	
[51]	5			
[52]	10			
[53]	25			
[54]	100			
[55]	500			
		•		

6. Next, we need to perform quality control and analyze our test samples: Tab "Results Interpretation".

As we see, the assay editor automatically filled most of the fields. Here is what is being filled automatically and what it means:

Result interpretation					
For variable	Conditional	Result 1	Result 1		
		True	False		
[S0]	[S0]<[S1]	ОК	Error		
[S1]	[S1]<[S2]	ок	Error		
[S2]	[52]<[53]	ок	Error		
[S3]	[53]<[54]	ок	Error		
[S4]	[S4]<[S5]	ок	Error		
[S5]	[S4]<[S5]	ок	Error		
[1]	([SMin]<[T])&&([T]<[SMax])	In Range	Out of Range		
[P1]	[P1]>1	ОК	Error		
[N1]	[N1]<0.2	ок	Error		

Columns:

- In the column "For variable" you can set for which variable following conditional will be used, eg variable [S0] means that the conditional and results filled in the next fields will be used for the Standard 0. To chose other variable, right-click on the field under the column.

- In the column "Conditional" are specified conditional formula by which the "Results 1 and 2" are interpreted, the condition is being interpreted by logical operation "IF, THAN", and outputs the result written in "Result 1" sub-columns "True" or "False".

In our example:

Condition [S0]< [S1] means that if the Standard 0 ([S0]) is less than the Standard 1 ([S1]), then the "Result 1" will be "Ok", else it will be "Error".

Further:

Analysis of test samples:

[1]	([SMin]<[T])&&([T]<[SMax])	In Range	Out of Range

The conditional ([Smin]<[T])&&([T]<=[Smax]) means that any test sample ([T]) that is

greater than Standard minimum ([S1]) and less or equal to Standard maximum ([S5]) will be outputted as "In range" in "Result 1", else it will be outputted as "Out of range" That is how we can exclude extrapolation. However the calculated concentration value will be outputted in both cases.

For the negative control ([N1_0] is the same as [N1]) is written a condition [N1] <0.2,

which means that if the OD of neg. control is less than 0.2 OD, then "Result 1" outputs "Ok", else outputs "Error".

For the positive control ([P1_0] is the same as [P1]) the condition is [P1]>1.0 that is, if the OD of Positive control is more than 1.0, then "Result 1" outputs "Ok", else "Error".

- 7. Save the assay and close the window of "Assay Editor""
- 8. Choose your newly created assay and run it:
- 9. In order to add Standards do the following:

in Qualitative assay

a. Select the well with Standard 0 (if in duplicate select 2 wells)



b. Drag the black square till the well with the last standard



from drop box c. Setting samples and controls, and obtaining results is the same as

•	sta	ı - X	
	Std	Standard 0	
_	Std	Standard 1	Ľ
	Std	Standard 2	
	Std	Standard 3	⊢
	Std	Standard 4	
	Std	Standard 5	ŀ
	\mathbb{C}	Reverse	

Choose an assay

Qualitative

Ouantitative

Ŧ

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Create a quantitative assay with concentration based interpretation

There is a posibility to base your interpretation of results not based on OD, but on calculated cocentration, to do that, do the following: when setting conditional, use concentration type of value (e.g. [O])

[52]	3	Concentration	
[53]	4	Critical OD	+
Result interp	retation	Coefficient of positivity	•
For variable	Conditiona	Standards	•
[S4]	[S4]<[S5]	Wild Card	•
[S5]	[S4]<[S5]	Logical operators	+
Π		Formula (Sample OD within standard range)	

In the following example, all test samples with calculated concentration greater than 3 units, will result as a positive (+) result, other will output as negative (-). Standards and Controls can be interpretated in the same way.

[1]	[O]>3	+	-	
		1	I	-

Create a quantitative assay with qualitative interpretation

In the following example: all test samples with calculated concentration less than 1 unit, will result as a negative (-) result, samples with calc. concentration value from 1 (including) to 3, will output as a gray zone (+/-) result, samples with calc. concentration greater or equal to 3, will output as positive (+) result.

[1]	[0]<1	-	
[1]	[O]>=1&&[O]<3	+/-	
Ш	[O]>=3	+	

Create an avidity assay

Assay Create Ass Measurement option Metodic name:	say Create 📧			
Assay Name (27.01 Assay type Quantitative	17:30:40)	Wavelength	Channel 1	Enable reference
 Qualitative Avidity Multiplex 	1 Veg. control count 1 Group count I Standards count	490 nm 490 nm 620 nm	Channel 2 Channel 3	Ref. filter, nm
Form				

 Avidity assays analyses the samples by calculating the Index of Avidity (IA) of positive test samples. Index of Avidity is the ratio of optical density of a sample in the presence of a dissociating agent (dissociation ELISA) to an optical density of the same sample without dissociating agent (direct ELISA).

We want to create an avidity assay with following criteria:

So we begin by selecting a type of assay: Avidity.

Measurement channel is set to e.g. 450 nm.

Analyzed sample is regarded as positive if sample's $OD_{of direct ELISA}$ is greater or equal to the Critical (Threshold) OD which is calculated by the formula:

= NC1 + 0.2 OD, where NC1 is the Average OD of Negative Controls 1.

Index of avidity shall be calculated for all positive test samples by the formula:

= OD dissociation ELISA/ OD direct ELISA

All positive samples should be divided into 3 groups:

-Samples with avidity index less than 0.30 (or 30%), those samples will be marked as "+" (e.g. low avidity antibodies)

-Samples with avidity index greater than 0.30 (or 30%) and less or equal to 0.50 (or 50%), those samples will be marked as "++" (e.g. normal avidity antibodies)

-Samples with avidity index greater than 0.50 (or 50%), those samples will be marked as "+++" (high avidity antibodies)

Quality control of negative and positive controls should meet following criteria:

- OD $_{direct ELISA}$ of the positive control must be greater than 1 OD and the index of avidity greater than 0.30 (30%);

- OD value of the negative control must be less than 0.1 OD

The following steps show procedure of creation of this assay

- 2. Create a new assay, click "New"
- 3. Enter the name of the assay, eg Avidity, select the type of assay: Avidity, set the wavelength channel. Number of controls:1 Negative Control, 1 Positive Control. Give a short description of the assay. Click "**Form**"

Metodic name:				
Avididty				
Assay type		Wavelength		
Quantitative	1 🔹 Pos. control count	🔲 405 nm	Channel 1	Enable reference
Qualitative	1 🔹 Neg. control count	V 450 nm	Channel 2	- Ref. filter, nm
Avidity	2 💌 Avidity count	620 nm	Channel 3	Mix before measure, s
Multiplex		Description		
Form		Description	•]

4. In the following window you can set the analysis of the results:

Avidity	×			
Choose Results types for Avidity Assay Or a structure of the structure				
Positive / Gray Zone / Negative				
Gray zone margin:				
by to higher than OD crit	ical (treshold) value			
1.00 💭 2.00 🐑				
Type in avidity index margins for positive samp	es and it's result			
Margin Re	sult			
If AI < 0.30 🗭 +				
If AI >= 0.30 \textcircled{m} and 0.50 \textcircled{m} < +	F			
If AI >= 0.50 🖛 +-	++			
50% 30% ▼ Show Avidity index in Results				
Continue Cancel				

1) Positive / Negative, e.g. according if the OD of the sample is greater than or less than the threshold OD, program will output results "Positive" or "Negative", Avidity index will be calculated only for positive test samples

2) Positive / Gray Zone / Negative -- according, if the sample OD is greater or equal than the threshold OD multiplied by the value indicated in the field "to" (e.g. 2), the result will

be marked as "positive", else if the sample will be between the threshold OD multiplied by the value in the value indicated in the field "by" (e.g. 1) and "to" (e.g. 2) the result will be marked as Gray Zone, else the sample will be marked as "Negative". Avidity index will be calculated only for positive test samples

5. Next, you need to type in avidity index' margins for positive test samples and corresponding result:

Type in avidity index margins for positive samples and it's result				
Margin		Result		
If AI < 0.30 🐑		+		
If AI >= 0.30 🕋	and 0.50 🚔 <	++		
If AI	>= 0.50 💭	+++		
50% 🔽 Show Avidi	ity index in Results			

In this example avidity index (AI) below or equal to 0.30, will output "+" in results If the avidity index (AI) is between 0.30 and 0.50, will output "++" in results, and if the avidity index (AI) is equal to or greater than 0.50, will output "+++" in results Leave the checkbox "Show avidity index in results" that would output the AI in the results.

Click "Continue."

6. As we see, the assay editor automatically fills most of the fields in order to analyze the results and perform quality control.

Here is what is being filled automatically and what it means:

7. Tab "Variables and formulas"

Variables and formulas	
------------------------	--

Variable	Description	Formula
[C]	Critical OD	[N1]+0.1
[R]	Sample Ratio	[T_1]/[T_0]

Two variables were created: [C] and [R], where [C] - is the Critical (Threshold) OD, and [R] - is the Avidity Index.

Critical (Threshold) OD is calculated by the formula [N1] +0.1, where [N1] - is the average value of negative control 1. So if N1=0.1, than Critical OD = 0.2 OD

[R] is calculated by the formula $[T_1] / [T_0]$, where $[T_1]$ is sample with a dissociating agent (dissociation ELISA) - and $[T_0]$ - sample without dissociating agent (direct ELISA)

8. Next, we need to perform quality control and analyze our test samples:

Result interpretation									
Conditional	Result 1		Result 2						
	True	False	True	False					
[T_0]<[C]	-								
[R]<0.3 && [T_0]>=[C]	+		[R]						
[R]>=0.3 && [R]<0.5 && [T_0]>=[C]	++		[R]						
[R]>=0.5 && [T_0]>=[C]	+++		[R]						
	Conditional [T_0]<[C]	Conditional Result 1 True True [T0]<[C]	Conditional Result 1 True False [T0]<[C]	Fetation Result 1 Result 2 Conditional True False True [T_0]<[C]					

As we see, the assay editor automatically fills most of the fields. Here is what is being filled automatically and what it means:

Columns:

- In the column "For variable" you can set for which variable following conditional will be used, e.g. variable [T] means that the conditional and results filled in the next fields will be used for the test samples, to chose another variable, right-click on the field under the column and choose a needed variable.

- In the column "Conditional" are specified conditional formula by which the "Results 1 and 2" are interpreted, the condition is being interpreted by logical operation "IF, THAN", and outputs the result written in "Result 1" sub-columns "True" or "False".

In our example:

Conditional $[T_0] < [C]$ means that if the test sample's OD _{of direct ELISA} ($[T_0]$) is less than the critical OD ([C]), then the "Result" 1 will be "–".

Conditional [R]<0.3 && [T_0]>=[C] means, that if the Avidity Index is less than 0.3 **AND** the OD _{of direct ELISA} is greater or equal to Threshold OD, then the "Result 1" will be "+" and Avidity Index will be written in "Result 2"

Conditional [R]=>0.3 && [R]<0.5 && [T_0]>=[C] means, that if the Avidity Index is greater or equal to 0.3 **AND** is less than 0.5 **AND** the OD _{of direct ELISA} is greater or equal to Threshold OD, then the "Result 1" will be "++" and Avidity Index will be written in "Result 2"

Conditional [R]>=0.5 && [T_0]>=[C] means, that if the Avidity Index is greater or equal to 0.5 **AND** the OD _{of direct ELISA} is greater or equal to Threshold OD, then the "Result 1" will be "+++" and Avidity Index (Avidity Index) will be written in "Result 2"

Further, quality control

Result interpretation								
For variable	Conditional	Result 1	Result 1					
		True	False	1				
[P1_0]	[P1_0]>=[C] && ([P1_1]/[P1_0]>=0.3)	ОК	Error					
[P1_1]	[P1_0]>=[C] && ([P1_1]/[P1_0]>=0.3)	ОК	Error					
[N1_0]	[N1_0]<[C]	ОК	Error					
[N1_1]	[N1_1]<[C]	ок	Error					

For the positive controls ([P1_0] and [P1_1]) we need to check if OD $_{of direct ELISA}$ is greater than the threshold OD ([P1_0]>=[C]) **AND** the avidity index should be greater or equal to 0.3, since we do not have a variable for positive controls' avidity indexes, we need to specify it separately either in Tab "Variables and Formula" or specify it here: ([P1_1]/[P1_0]>=0.3), the "Result 1" outputs "Ok", else "Error".

For the negative control ([N1_0] and [N1_1]) we need to check if OD_{of direct ELISA} is lower than the Critical (Threshold) OD, so the expression [N1_0]<[C] means that if the OD of negative control is less than Critical (Threshold) OD, then "Result 1" outputs "Ok", if not, then "Error".

- 9. Save the assay and close the "Assay Editor"
- 10. Choose your newly created assay and run it:
- 11. Choose the assay from the list and run it:



 In order to fill the samples do the following: Set 2 samples in two adjacent wells (or controls) and select the sample with protein dissociating agent (e.g. urea).

Click on the droplist near the "--- 0 " button, A/M 1 button

	Available Devices								
Nam Grou	Name Smp 2 2 Test								
	1	2	3						
A	Smp1	Smp1							
	0	0							

will appear, click on it.

Name Grou	e Smp p 2	2 💭 Reset	× Test	- Bkg	P ₁	- 1	√ 1 -	⁰ - X
	1	2	3	4	5	6	7	A/M1
A	Smp1	Smp1						
	0	0						

13. Now the sample in A2 is with urea, the bottom part of the well will now show "Control Reagent" type. **Note:** The well with urea will now appear with a slightly different color. Select any other well in order to see it. Controls will not appear with different color.

		1	2
1	A	Smp1	Smp1
		0	1
	A	o O	5mp1 1

14. To fill the plate with the same pattern, select both wells and drag the mouse till the end.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Smp1	Smp1	Smp9	Smp9	Smp17	Smp17	Smp25	Smp25	Smp33	Smp33	Smp41	Smp41
	0	1	0	1	0	1	0	1	0	1	0	1
В	Smp2	Smp2	Smp10	Smp10	Smp18	Smp18	Smp26	Smp26	Smp34	Smp34	Smp42	Smp42
	0	1	0	1	0	1	0	1	0	1	0	1
С	Smp3	Smp3	Smp11	Smp11	Smp19	Smp19	Smp27	Smp27	Smp35	Smp35	Smp43	Smp43
	0	1	0	1	0	1	0	1	0	1	0	1
D	Smp4	Smp4	Smp12	Smp12	Smp20	Smp20	Smp28	Smp28	Smp36	Smp36	Smp44	Smp44
	0	1	0	1	0	1	0	1	0	1	0	1
E	Smp5	Smp5	Smp13	Smp13	Smp21	Smp21	Smp29	Smp29	Smp37	Smp37	Smp45	Smp45
	0	1	0	1	0	1	0	1	0	1	0	1
F	Smp6	Smp6	Smp14	Smp14	Smp22	Smp22	Smp30	Smp30	Smp38	Smp38	Smp46	Smp46
	0	1	0	1	0	1	0	1	0	1	0	1
G	Smp7	Smp7	Smp15	Smp15	Smp23	Smp23	Smp31	Smp31	Smp39	Smp39	Smp47	Smp47
	0	1	0	1	0	1	0	1	0	1	0	1
Н	Smp8	Smp8	Smp16	Smp16	Smp24	Smp24	Smp32	Smp32	Smp40	Smp40	Smp48	Smp48
	0	1	0	1	0	1	0	1	0	1	0	1

15. When setting controls, be sure to put "A/M 1" again. **Note** Controls with urea will not appear with different color, to check if you have entered control with urea, choose "Control Reagent" in "What to show in a cell", controls with urea will have "1" in the bottom part of the cell

			Positive	Positive
	1	What to show in a cell:	0	1
A	•	Sample Name -	Negativ	Negativ
		Control Reagent 🗸	0	1

16. Obtaining results is the same as in other assays.

Tools for assay editing

Note, that only master users (administrator account in windows) can create or edit assays.

Create - Creates a new assay								
Save - Saves the assay								
Save as - Saves a copy of an assay (including all referenced templates) Load - Loads an assay Delete - Deletes a selected assay from the list of assays.								
Load Delete								
Measurement options Assay name: 5pl Assay type Image: I								

Using new variables: Wildcards

While creating new assays, users can use other than preset variables like Critical OD [C] or Coefficient of positivity [F]. Those variables are called Wildcards [W], and user can use 7 new variables per assay.

One of the examples of use:

User needs to see the ratio for positive and negative controls, for that he needs to do the following: add new variables by right clicking on the Variable empty cell and selecting a Wildcard, add a sutible descrition, under the formula column input following: Pos. control divided by critical OD: [P1]/[C], **input of variables can only done by right clicking on that cell,** mathematical operators (+ , - , * , /) can only be inputed from keyboard.



same for negative and other pos. control.

Variables an	nd formulas				
Variable			Description	Formula	
[W1]			ratio for Pos control	[P1]/[C]	
[W2]			ratio for neg control	[N1]/[C]	
	Wild Card	•	1		
	Delete		2		
Result inter	pretation				

For variable	Conditional	Result 1	Result 2		
		True	False	True	False
[N1]	[N1]<0.4	ок	Error	[W2]	[W2]
[P1_0]	[P1]>1	Ok	Error	[W1]	[W1]

Logical operations in the interpretation of results

Logical expressions can take one of two values - "true" or "false". Logic operations are used for complex logical expressions. We use logical operations as conditions for determining the results of the program.

For example, Condition:

For variable	Conditional	Result 1			
		True	False		
Μ	[T_0]>=1 && [T_1] >=2	Positive	Negative		

Here we have two conditions: OD sample type T_0 and OD sample type T_1, if the OD of both samples is greater than or equal to 1, then the conditions of "Yes" and as a result in front of the sample It will be written the result of "laid." If not, the result will be written "Neg."

to set other logical operators, choose from the menu by clicking the right button of the mouse

Logical operators	►	And
Formula (Sample OD within standard range)		Or
		NOT

Using Standard Deviation

If you are using replicates, It is also possible to use Standard Deviation value for the calculations. Below is the example where the Critical OD is being calculated by OD value of Negative Control plus 3 Standard deviations.

Variables and formulas						
Variable	Description	Formula				
[C]	Critical OD	[N1]+3*[N1_S]				

Models for quantitative analysis

For building calibration curves we

- 1. 5-parameter logistic model
- 2. 4-parameter logistic model
- 3. linear model
- 4. Piecewise linear model

5-parameter logistic model (5PL)

5-parameter logistic or 5PL nonlinear regression model that is used to analyze data in biological or immunological samples, such as ELISA or curves dose / response. It differs from the 4PL or 4-parameter logistic model in that it is asymmetric function and is better suited for immunological or biological data.

We use 2 5PL formulas:

$$F(x) = A + \frac{D}{\left(1 + \left(\frac{x}{c}\right)^{B}\right)^{E}} \qquad \qquad F(x) = \frac{A - D}{\left(1 + \left(\frac{x}{c}\right)^{B}\right)^{E}} + D$$

 $F(x) = A + (D/(1+(x/C)^{B})^{E})$ or $F(x)=(A-D)/(1+(x/C)^{B})^{E+D}$ where:

A — the OD value for the minimum asymptote

B — the Hill slope

C — the concentration at the inflection point

- D the OD value for the maximum asymptote
- E is the asymmetry factor



4-parameter logistic model (4PL)

4-parameter logistic or 4PL nonlinear regression model is used to analyze data in a biological or immunological samples, such as ELISA or curve dose / response. in 4PL 4 Formula:

$$F(x) = \frac{A - D}{1 + \left(\frac{x}{C}\right)^{B}} + D$$

$$F(x) = (A - D)/(1+((x/C)^{B}))+D$$

where:

- A the OD value for the minimum asymptote
- B the Hill slope
- C the concentration at the inflection point
- D the OD value for the maximum asymptote



Linear model

linear function - the function of the form

y = kx + b

basic functions: increment of the function is proportional to the increment of the argument (concentration).



The piecewise linear model

A piecewise linear function is a function defined on the set of points and is linear between each interval.



The index regression model



The logarith regression model



The exponent regression model



The cubic spline model





Available Devices		Inp	ut Data	V	/iew Results	Char	Chart			
Standards Given Concentrati		Calculated Concentration	OD 450 nm	Residuals	%Recovery	Sample Name	Cell	Show		Use Best Fit feature
S0	0	*1.412 IU	0.0001	1.411	NA	Std S0	A1	I		5 Parameter Logistics 1
S0	0	1.981 IU	0.0014	1.980	NA	Std S0	A2			
S1	5	5.062 IU	0.4005	0.062	101.244%	Std S1	B1			Set X-axis to log scale
S1	5	4.826 IU	0.3748	-0.174	96.527%	Std S1	B2			Set Y-axis to log scale
S2	10	9.986 IU	0.7626	-0.014	99.860%	Std S2	C1			Show samples
S2	10	9.918 IU	0.7590	-0.082	99.176%	Std S2	C2			Allow Extrapolate
S3	20	22.074 IU	1.1766	2.074	110.372%	Std S3	D1			
S3	20	20.849 IU	1.1471	0.849	104.247%	Std S3	D2			Recalculate HIT XLS
S4	50	45.665 IU	1.5478	-4.335	91.330%	Std S4	E1			
C1	50	45 007 11	1 5415	4.002	00.1020/	CH4 C4	50			-
1.8		A	= 19.1644, D = -1	9.1637, C = 2.4277 R-Square = 0.9	7, B = 18.6169, E = 0.00 991	15		4		
1.6										
1.4										
E ^{1.2}										
\$ 0.8										
0.6										
0.4										
	<u></u>									
	5 10 1	5 20 25	30 35	40 45 50 Concentra	55 60 65 tion IU	70 75 80	85 90	95 100		

Here you can select a needed model, by removing the tick from the field "Use the best fit feature" Next: select a model from the list below.

Use Best Fit feature	
5 Parameter Logistics 1	
4 Parameter Logistics	
5 Parameter Logistics 1	
5 Parameter Logistics 2	
Linear	
Piecewice linear	
Then click on the "Decalculate" hutton	Recalculate
THEIT CHER OF THE TRECAICUIALE DULLOF.	

User can switch X, Y axis to log scale, as well as to show samples on the curve and enable/disable extrapolation (for last feature Recalculate button should be pressed).

User can export calibration data to .xls file.

Loading a standards curve

First you need to create your curve. Open the program and load a quantitative experiment, like below:

3 0	■ QuantAssay v0.7.1.2 ×																
<u>F</u> ile	Ele Options																
NEW																	
Exp_	xp_1805_1034_0 🛛 test for quality 🔟 test for quant 🗵																
	Available Devices Input Data View Results Chart																
Name Smp 1 $\overrightarrow{\bullet}$ X Test \bullet Bkg P ₁ \bullet N ₁ \bullet Std \bullet X										Load			Choose an assay				
	1	2	3	4	5	6	7	8	9	10	11	12	Choose a Template or Save as				
Α	Std S0	Std S0	Smp2	Smp2	Smp11	Smp14					Positive	Positive	Plate_23.01.2018 16:31: ~				
	0.0080	0.0090	0.0080	0.0090	0.3407	0.4770	0.4293	1.6330	2.5920	4.3000	1.0002	1.0000	1 What to show in a cell:				
В	Std S1	Std S1	Smp3	Smp3	Smp12	Smp15					Positive	Positive	A Sample Name v				
	0.0600	0.0650	0.0600	0.0650	0.2839	0.3975	0.3577	1.3608	2.1600	3.6000	1.0003	1.0000	450 nm ~				
С	Std S2	Std S2	Smp4	Smp4	Smp13	Smp16					Negativ	Negativ	Cell Name Sample Name Type				
	0.1600	0.1690	0.1600	0.1690	0.2366	0.3312	0.2981	2.0000	1.8000	3.0000	0.2002	0.2000					
D	Std S3	Std S3	Smp5	Smp5							Negativ	Negativ	Main channel				
	0.6900	0.7200	0.6900	0.7200	0.1183	0.6300	0.1491	0.5670	0.9000	1.5000	0.2001	0.1999	Calculate 450 nm ~				
E	Std S4	Std S4	Smp6	Smp6							Bkg	Bkg	Kinetic Mode Panel				
	2.0000	2,1000	2.0000	2,1000	0.0592	0.3150	0.0745	0.2835	0.4500	0.7500	0.0001	0.0001					
-	Std 65	etd es	Smn7	Smn7							Bka	Pka					
F	5tu 35	500 35	Siller	Siller							Ding	bing					
	4.0001	4.0000	4.0001	4.0000	0.0296	0.1575	0.0373	U.1418	0.2250	0.3750	0.0001	0.0001					
G											Bkg	Bkg					
	2.0001	2.0000	2.0001	2.0000	0.0148	0.0788	0.0186	0.0709	0.1125	0.1875	0.0001	0.0001					
Н	Smp1										Bkg	Bkg					
	4.2000	0.2990	1.0000	1.0000	0.0074	0.0394	0.0093	0.0354	0.0563	0.0938	0.0001	0.0001					

Press on load curve icon:



In this windows click on the Export from plate, Set the name of the curve and press Ok. The curve is now saved for the later use.

Standards		- 0	×
Export from plate Save S	ave as Dele	te 🗹 Use loaded standards	
	S Parama	Loaded standards and optical density X Set name Sparameters IgG OK Cancel	alyte
			Close





Here you can now set other type of curve and Save it as new curve.

Now you need to load it in the new experiment.

Set you samples and measure the plate.

3 (- 🗆 X													
<u>F</u> ile	Eile <u>O</u> ptions													
NEW														
Exp_	xp_1805_1034_0 🛛 test for quality 🔟 test for quant 🔟 test for quant 🔟 Exp_1805_1126_5 🔟 Exp_1805_1127_6 🔟													
	Available Devices Input Data View Results Chart													
Name Smp 1 Kaset Group 1 Reset X Test Reset X Bkg P1 V N1 X Std X X Load Choose an assa test for quant											Choose an assay test for quant			
	1	2	3	4	5	6	7	8	9	10	11	12	Choose a Template or Save as	
Α	Smp1	Smp9	Smp17	Smp25	Smp33	Smp41	Smp49	Smp57	Smp65	Smp73	Smp81	Positive		
	т1	т9	T17	T25	тзз	T41	T49	T57	T65	T73	T81	P1	1 What to show in a cell:	
В	Smp2	Smp10	Smp18	Smp26	Smp34	Smp42	Smp50	Smp58	Smp66	Smp74	Smp82	Positive	A Sample Name ~	
	т2	т10	T18	T26	T34	T42	T50	T58	T66	174	T82	P1	Туре ~	
С	Smp3	Smp11	Smp19	Smp27	Smp35	Smp43	Smp51	Smp59	Smp67	Smp75	Smp83	Negativ	Cell Name Sample Name Type	
	тз	т11	Т19	T27	T35	T43	T51	T59	T67	175	T83	N1	#36 C12 Smp91 T91	
D	Smp4	Smp12	Smp20	Smp28	Smp36	Smp44	Smp52	Smp60	Smp68	Smp76	Smp84	Negativ	Main channel	
	T4	T12	T20	T28	T36	T44	T52	T60	T68	T76	T84	N1	Calculate 450 nm V	
E	Smp5	Smp13	Smp21	Smp29	Smp37	Smp45	Smp53	Smp61	Smp69	Smp77	Smp85	Smp93	Kinetic Mode Panel	
	т5	т13	T21	T29	T37	T45	T53	T61	T69	777	T85	т93		
F	Smp6	Smp14	Smp22	Smp30	Smp38	Smp46	Smp54	Smp62	Smp70	Smp78	Smp86	Smp94		
	т6	T14	т22	T30	T38	T46	T54	T62	T70	T78	T86	т94		
G	Smp7	Smp15	Smp23	Smp31	Smp39	Smp47	Smp55	Smp63	Smp71	Smp79	Smp87	Smp95		
	77	T15	T23	T31	T39	T47	T55	T63	771	T79	T87	т95		
н	Smp8	Smp16	Smp24	Smp32	Smp40	Smp48	Smp56	Smp64	Smp72	Smp80	Smp88	Smp96		
	T8	T16	T24	T32	T40	T48	156	T64	172	T80	T88	T96		
		.10	.21	.52	. 10	. 10					.00			

You will get following message:

Quantassay 0	×
Not enough standards	
	ОК

Press ok, go to Input Data tab.

Press on load curve icon:



Select the curve you need and close the windows, your results will be calculated:



If you do not want to use loaded curve, then go to Input Data: Load Curve, and disable "Use

loaded standards" box.

Results tab

	Available [Devices	out Data		View Results			hart				
Cell	Туре	Sample Name	Group	OD 450 nm	Result 1	Given Concentration	Mean	Concentration	Calculated Concentration	Mean (OD)	Standard Deviation (OD)	Coefficient Variation (
A1	SO	Std S0		0.0001	ОК	0 IU	*1.4.	12 IU	*1.412 IU	0.0008	0.0007	86.768%
A2	S0	Std S0		0.0014	ОК	0 IU	1.412	IU	1.981 IU	0.0008	0.0007	86.768%
A3	T1	Smp1	1	1.9557	Out of Range		*103	.001 IU	*103.350 IU	1.9541	0.0017	0.086%
A4	T1	Smp1	1	1.9524	Out of Range		*103	.001 IU	*102.653 IU	1.9541	0.0017	0.086%
A5	Т9	Smp9	9	0.0001	Out of Range		*1.4	12 IU	*1.412 IU	0.0001	0.0000	0.000%
A6	Т9	Smp9	9	0.0001	Out of Range		*1.4.	12 IU	*1.412 IU	0.0001	0.0000	0.000%
A7	T17	Smp17	17	0.0011	In Range		2.030	U IU	1.922 IU	0.0018	0.0007	36.541%
A8	T17	Smp17	17	0.0025	In Range		2.030	U IU	2.086 IU	0.0018	0.0007	36.541%
A9	T25	Smp25	25	4.1524	Out of Range		*143	38.244 IU	*12074.361 IU	4.2262	0.0738	1.747%
A10	T25	Smp25	25	4.3000	Out of Range		*143	38.244 IU	*17041.094 IU	4.2262	0.0738	1.747%
A11	P1	Positive control P1		1.9738	ОК		*104	.028 IU	*107.207 IU	1.9590	0.0148	0.758%
A12	P1	Positive control P1		1.9441	ОК		104.0	28 IU	100.946 IU	1.9590	0.0148	0.758%
B1	S1	Std S1		0.4005	ОК	5 IU	4.943	IU	5.062 IU	0.3876	0.0128	3.315%
B2	S1	Std S1		0.3748	ОК	5 IU	4.943	IU	4.826 IU	0.3876	0.0128	3.315%
B3	Т2	Smp2	2	1.9268	In Range		100.0	198 IU	97.461 IU	1.9399	0.0132	0.680%
B4	Т2	Smp2	2	1.9531	In Range		*100	.098 IU	*102.808 IU	1.9399	0.0132	0.680%
B5	T10	Smp10	10	0.0016	In Range		2.123	IU	2.002 IU	0.0031	0.0015	49.231%
B6	T10	Smp10	10	0.0046	In Range		2.123	IU	2.184 IU	0.0031	0.0015	49.231%
B7	T18	Smp18	18	0.0029	In Range		2.095	i IU	2.113 IU	0.0026	0.0003	11.518%
B 8	T18	Smp18	18	0.0023	In Range		2.095	i IU	2.074 IU	0.0026	0.0003	11.518%
B9	T26	Smp26	26	0.0040	In Range		2.115	i IU	2.162 IU	0.0029	0.0011	35.684%
B10	T26	Smp26	26	0.0019	In Range		2.115	i IU	2.040 IU	0.0029	0.0011	35.684%
B11	Т34	Smp34	34	0.0028	In Range		2.133	IU	2.109 IU	0.0033	0.0005	13.847%
B12 ∢	T34	Smp34	34	0.0038	In Range		2.133	IU	2.153 IU	0.0033	0.0005	13.847%

This tab displays results in the following columns:

Cell #

Туре

Sample name

Group

OD *** nm

Result 1 and 2

Give concentration (for quantitative assays, the blue font and * marked are extrapolated values) Mean concentration (for quantitative assays, the blue font and * marked are extrapolated values) Calculated concentration (for quantitative assays)

Mean OD

Standard deviation of OD (for samples repeats)

Coefficient of Variation of OD (for samples repeats)

For multiplex, avidity and qualitative assays columns relating to the concentration are not displayed.

For avidity methods column A/M indicates what sample was diluted with a dissociating agent (0 -- not diluted, 1 -- diluted)

For multiplex methods column A/M displays the group of the Also the results table can be sorted by column or rows.

In order to output results in PDF, Excel and CSV click on a corresponding icon



LIS export

	A	, II		-		•											
1	· ·	υ υ		Availab	le Device	s	1	nput Data				View	/ Resu	ilts			Chart
1	© ⊎ •	U U	Name	Smp	1 1	x				_	1	х		1		110	
1	ຂ⊍• ວຽ0•	0	Group	1	Reset	Test	➡ Bkg	P ₁	•	N ₁	•	Std	•	Х	Load	export	:
Expor	t builder																 X
Sa	ve	Cancel															
Expor	t settings	1	_														
														Exte	ension:		
Filer	name:	Exp_2	301_1612_	0_0								(ISV		sv		
		Separ	ator type											Ot	xt		
		◉;			Ο,		⊖ тав	() Other	r 🗌							
l –	Identifier t	VDec															
		ypes		ļ	Standard	Deviation (C)D)						Ch	eck all		Uncheck al	I
	Sample N	lame			Assay na	nt of Variatio me	n (OD)						Include	a haadars			
	A/M Group			~	🛽 Conc. uni	ts							Dewrite	a fila with	, same nan		
	OD 450 n	nm											Rewind	e nie wiu	i saine nan		
	Result 2																
II E	Given Co Mean Co	ncentrat ncentrat	ion ion (g/l)														
	Calculate	d Conce	ntration (g	/1)													
		-7 (31-7															
Exp	ort cont	tent															
	-																
1	l ype					_											
2	OD 450 r	vame															
4	Calculate	ed Conce	ntration (c	1/1)		_											
5	Conc. un	nits		<i></i>		_											
6	Cell																

When the experiment is finished, click on the LIS export button to start.

Select the file name extension for your data. You can choose either .csv or .txt format.

Choose the needed separator type:

Separator type			
	Ο,	⊖ TAB	() Other

Select the identifiers (headers) you want to export.

Identifier types

🗹 Cell	Standard Deviation (OD)
✓ Type	Coefficient of Variation (OD)
Sample Name	Assay name
□ A/M	Conc. units
Group	
✓ OD 450 nm	
Result 1	
Result 2	
Given Concentration	
Mean Concentration (g/l)	
Calculated Concentration (g/l)	
Mean (OD) (g/l)	

Select if you want to export the header names

Include headers

Export content panel is visualizing the the exportable headers.

Export content			
1	Cell		
2	Туре		
3	Sample Name		
4	OD 450 nm		
5	Calculated Concentration (g/l)		
6	Conc. units		

The Rewrite file with same name checkbox will rewrite the file with same name without prompting confirmation from you.

Rewrite file with same name

When finished press on Save button and select the path for exporting.

Temporary saves

By default software autosaves each measurement. Measurements can be found in "Documents/QuantAssay/Temporary saves"

This feature autosaves up to 50 measurements, if you have 50 measurements already, then the earliest measurements from the list will be overwritten.

Troubleshooting

World practice shows that software vendors in the case of software malfunctioning indicate that user have accepted of the license agreement by which the software was provided as is or/and the shortcomings of operating system compatibility with the PC hardware, which leads to errors of or reduced productivity of the program. Unfortunately, we state that this practice model is the best for us and we have to stick to it. But, we would be grateful if you send captured errors to <u>software@biosan.lv</u>, so that we can identify the cause and, possibly, make the program better.

1. - The device can not connect to the computer.

1.1 Check that the USB cable is firmly connected to the PC and to the instrument, try to eject and inster both ends.

1.2 Try restarting your devices/software/computer, if it does not help, then reinstall the software.

1.3 If the problem persists, go to the point 4 of this troubleshooting

- 2. The program can not close, says that the experiment is still going, but I stopped it.
 Try pressing the Play button on the toolbar (to start the experiment), and click on the Stop button, wait 5 seconds and then try to close it. If this does not work, open Task Manager (Ctrl + Shift + Esc) and close all processes "quantassay.exe"
- 3. Device does not respond to the program
 - Try to turn on or off the device, if necessary, try to plug it off and then on.
- 4. Drivers can not be installed
 - 1. Try to give administrator rights to the user who installs the program
 - 2. If previous step did not help, try the following:

Go to Control Panel/Device Manager

Expand the Other devices line:

Other devices

USB Serial Port

Other devices				
🔚 🔤 USB Serial Port				
Portable Devi Ports (COM 8 Commun Processors	Update Driver Software Disable Uninstall			
Smart card re Sound, video	Scan for hardware changes			
Storage contr	Properties			

Click on Properties, Select Driver tab, click on the Update Driver:

USB Serial Port Properties	×
General Driver Details	
USB Serial Port	
Driver Provider:	Unknown
Driver Date:	Not available
Driver Version:	Not available
Digital Signer:	Not digitally signed
Driver Details	To view details about the driver files.
Update Driver	To update the driver software for this device.
Roll Back Driver	If the device fails after updating the driver, roll back to the previously installed driver.
<u>D</u> isable	Disables the selected device.
<u>U</u> ninstall	To uninstall the driver (Advanced).
	OK Cancel

Select Search automatically for updated driver software (you should have internet connection on)

Search automatically for updated driver software Windows will search your computer and the Internet for the latest driver software for your device, unless you've disabled this feature in your device installation settings.

After installing the driver, following message should appear: Windows has successfully updated your driver software

Windows has finished installing the driver software for this device:



If you do not have internet connection, please use computer with internet to download latest drivers from: <u>http://www.ftdichip.com/Drivers/VCP.htm</u> under "Available as setup executable". Then transfer and install the driver on the computer where the units are connected.

If it did not help, please try to find a solution here: <u>https://support.microsoft.com/en-us/help/2654149/error-usb-device-not-recognized-when</u> <u>-you-try-to-access-a-usb-external-hard-drive</u>.

If that did not help, try to connect the instrument to the different computer, to check if the problem is on the computer or instrument end.

Also try to change the USB cable.

- My problem is not described here.
 -While working with the program a problem that is not described here may arise. There
 is an universal solution: reboot the computer and / or reinstall the program. Check the
 website for the latest software updates
- 6. I have connected the devices to USB 2.0 SS (super speed) terminals and my computer shuts down constantly, indicating that there is some sort of an error with FTDI driver.
 Please avoid connecting to USB 2.0 SS, connect the devices to the standard USB 2.0. Data for connecting to USB 3.0 terminals is yet not available.

Disclaimer

- 1. Program is provided "as is", as it was stated in the license agreement.
- 2. This documentation may not coincide with the latest version of the program. In this case, we are sorry and hope for your understanding, and we would be grateful if you could point us the inconsistencies, also we hope that the interface is really intuitive and does not require thorough explanation. In any case, write to <u>software@biosan.lv</u>, we will be happy to guide you!