Medical–Biological
Research & Technologies

QUANT ASSAY SOFTWARE MANUAL FOR **MPP-96** PHOTOMETER

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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:

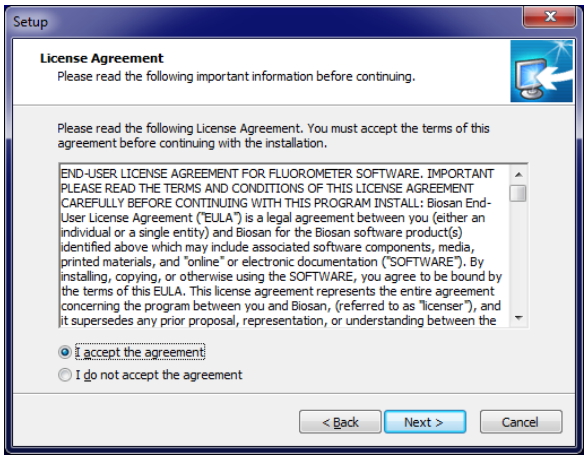
- Quantitative assays: the ability to install up 20 standards and choose fit model from 5/4 parameter logistic, linear and piecewise linear models
- BestFit function for the selection of the best calibration curve.
- Multiplex analysis - up to 7 different tests on the same plate
- Qualitative assays: 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.

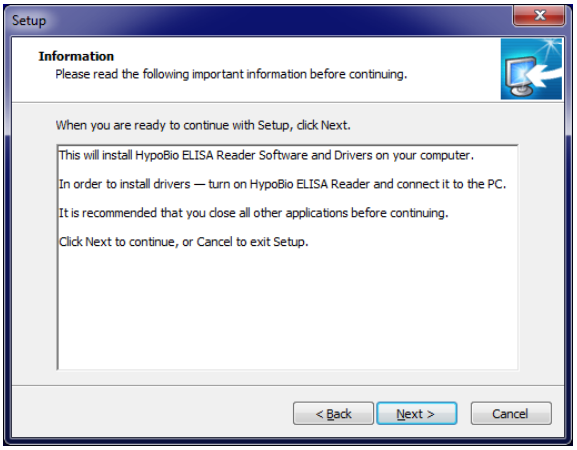
Installation

Welcome window

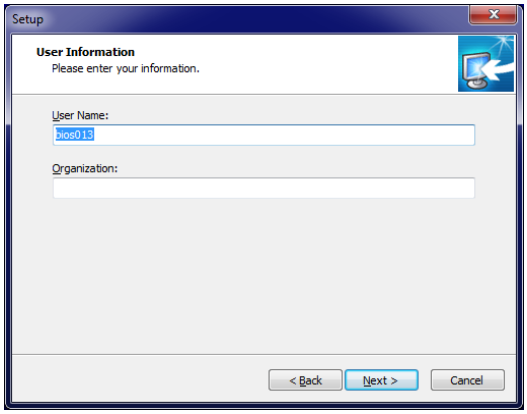
License Agreement



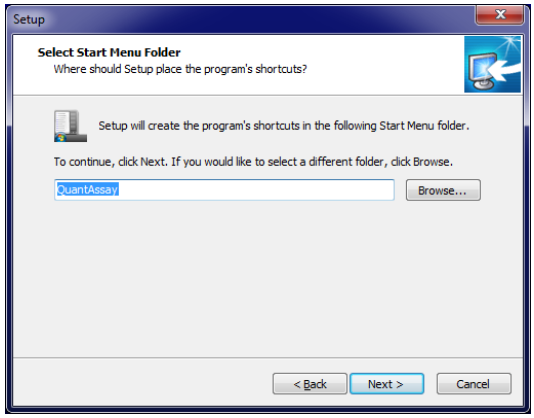
Information Window



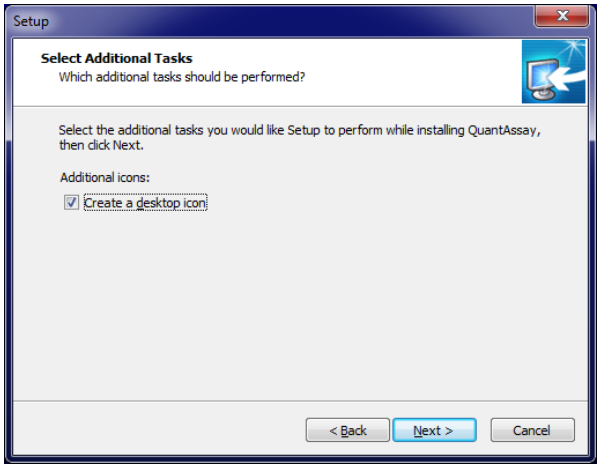
information of the user



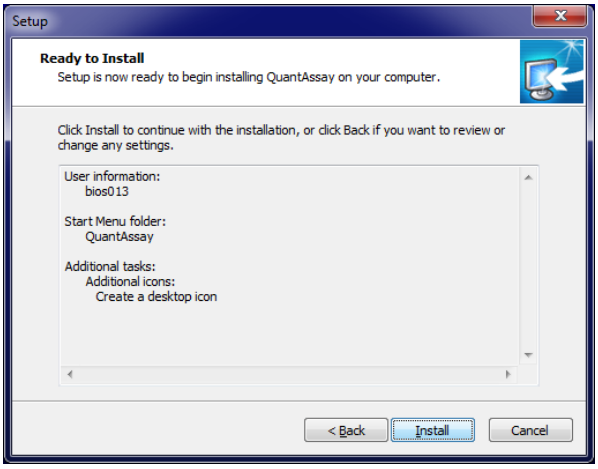
the path of the installation



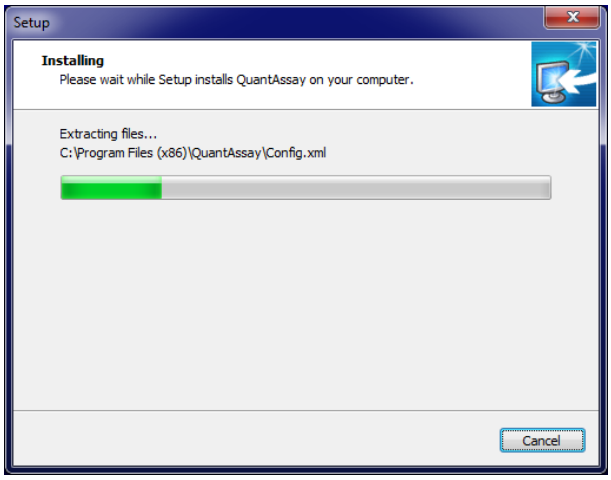
Advanced Settings



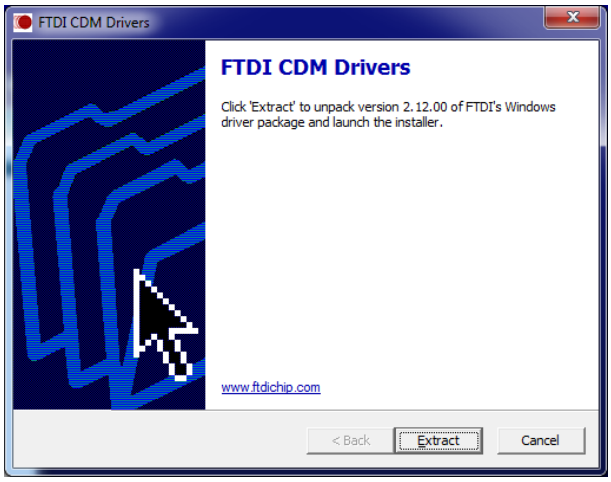
Ready to Install



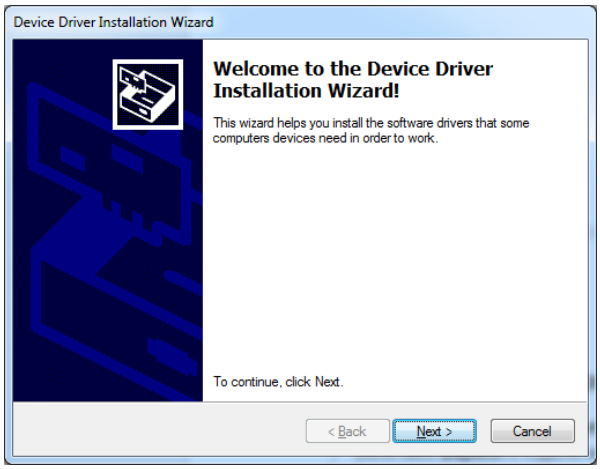
process software installation



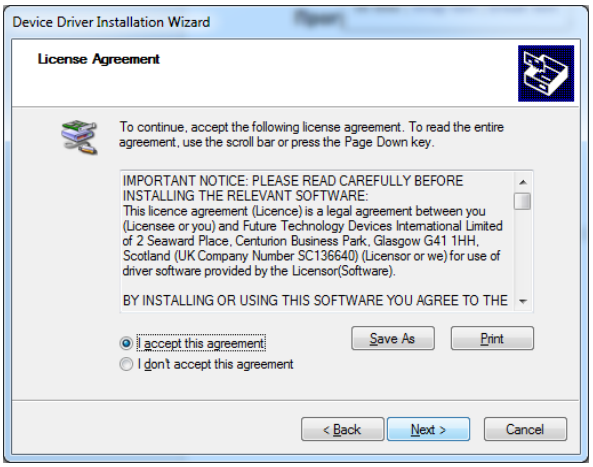
The installation of the drivers



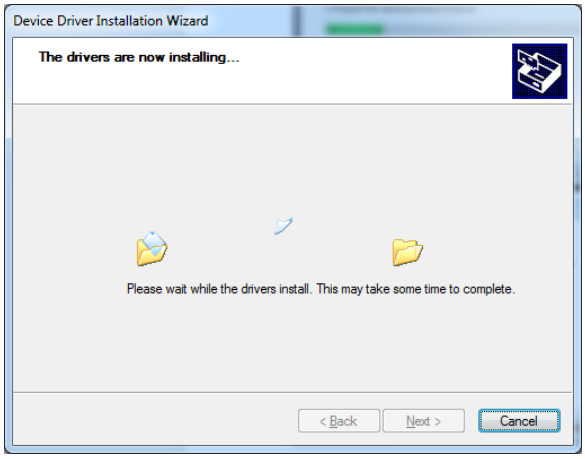
Drivers installation process



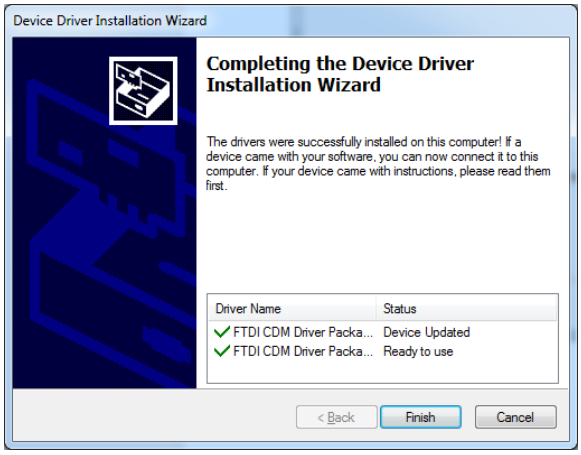
License Agreement



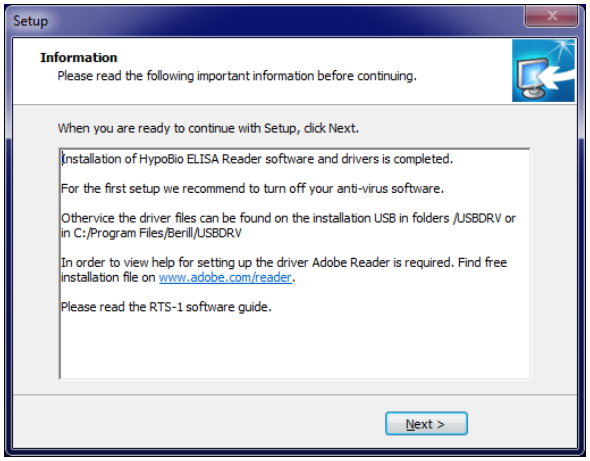
The installation of drivers



the installation of drivers is finished



finished installing of the software



The program is ready to start.

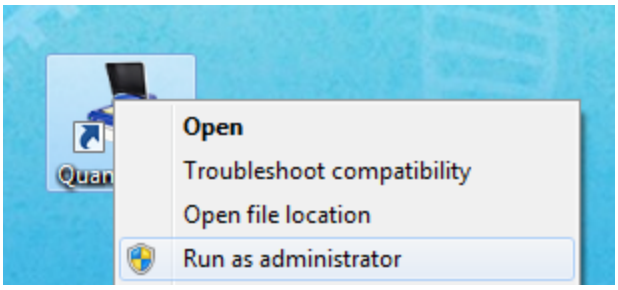
Account control: Administrators and common users rights

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:

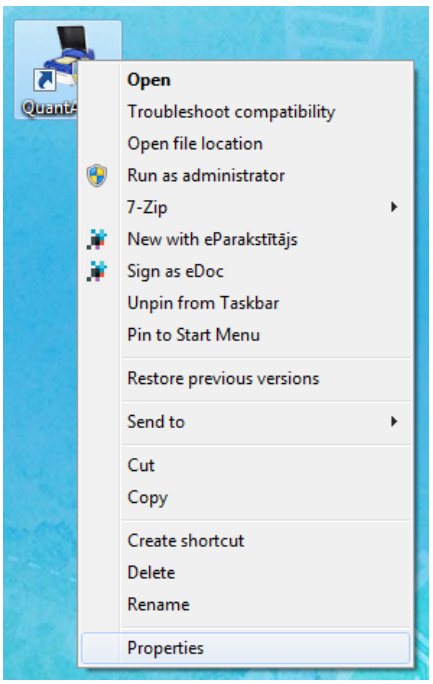
- Use software
- Create/Edit Assays
- Save templates

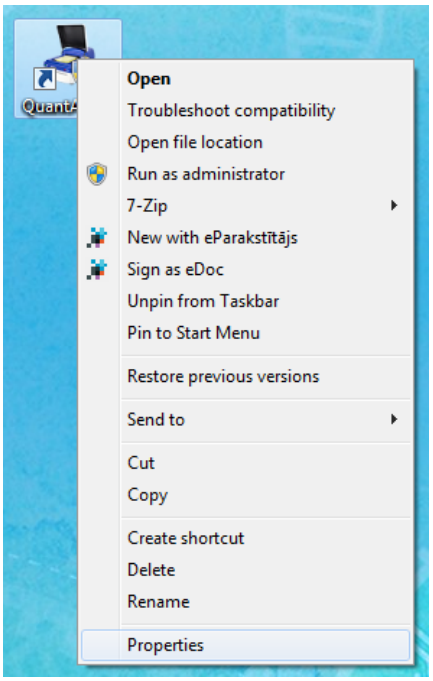


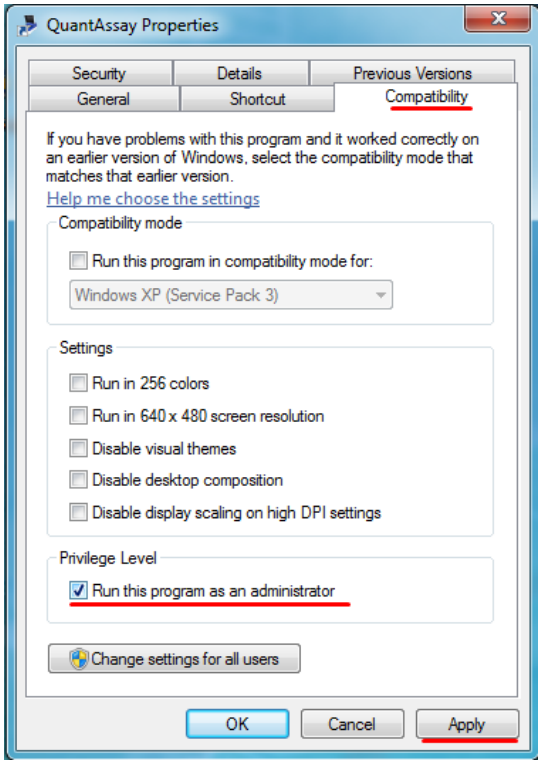
So, the ordinary users cannot change or create assays. If you have a single user who is also a master user than this feature can get annoying when creating or editing assays, so to use the software without being asked each time do the following: Run the software as administrator.

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.







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)

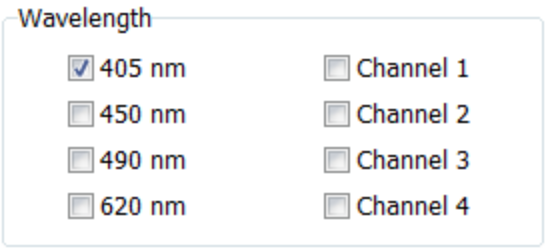
Here is an instruction how to move files:
2.1. Open new the software and close it. (This will create needed folders)
2.2. Copy all assays: In Program Files(x86)/QuantAssay find folder Methodics. Copy this folder to: C:/Users/Public/Public Documents/QuantAssay/ then Replace conflict files if being asked.

Performing a Measurement

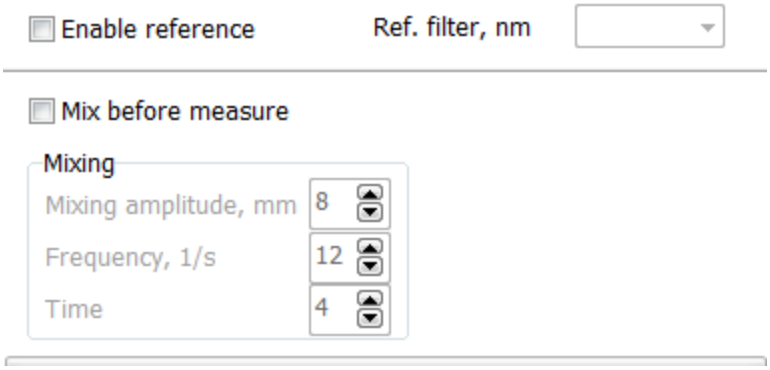
1. Open the program 

2. Go to the tab "Available Devices"

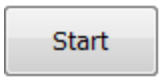
3. Select the wavelengths at which you want to measure



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



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:

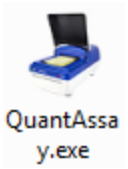
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.001	0.001	0.001	0.002	0.002	0.002	0.002	0.002	0.003	0.003	0.003	0.003
B	0.000	0.001	0.001	0.001	0.001	0.001	0.002	0.002	0.003	0.003	0.003	0.003
C	0.001	0.001	0.002	0.001	0.001	0.002	0.002	0.001	0.002	0.002	0.002	0.002
D	0.001	0.001	0.002	0.003	0.003	0.003	0.003	0.003	0.003	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
H	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 

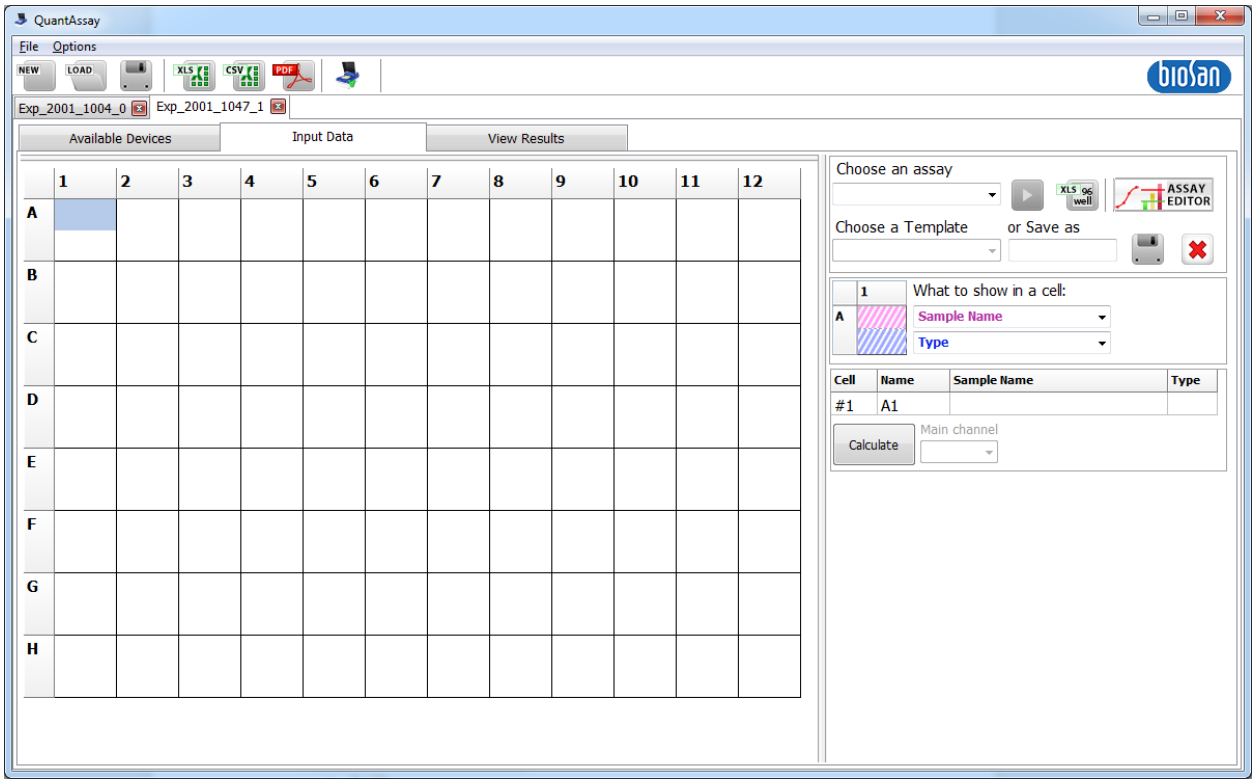
9. To save the data in .csv .xls .pdf formats, click on the corresponding icons

Running a test

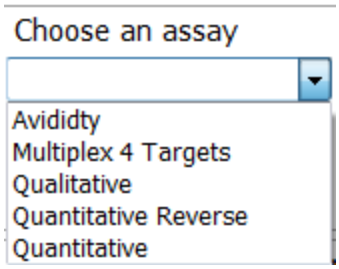
1. Open the software



window after opening the program



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



   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 samples:

- Test sample

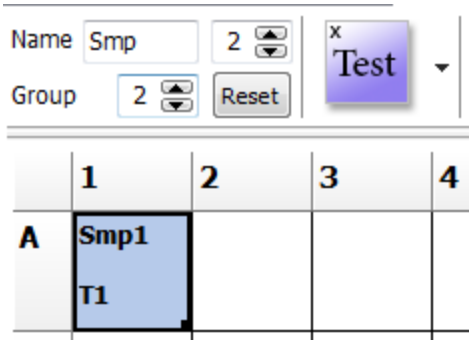
- Background (the average value of those samples will be deducted from the whole plate, the deducted values can only be observed in the Results tab, data input tab will remain the same)

- Positive Control 1

- Negative Control 1 (Threshold/OD critical is calculated based on OD value of those samples)

- Remove the sample

- 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:

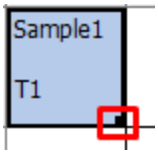


6. The methods of filling the plate:

   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				
B					
C					
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).



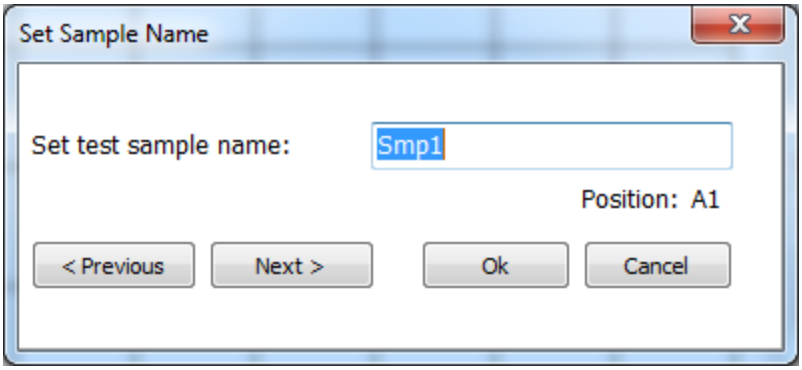
After

	1	2	3
A	Sample1 T1		
B			
C			

Before

	1	2	3
A	Sample1 T1	Sample4 T4	Sample7 T7
B	Sample2 T2	Sample5 T5	Sample8 T8
C	Sample3 T3	Sample6 T6	Sample9 T9

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



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.

Before

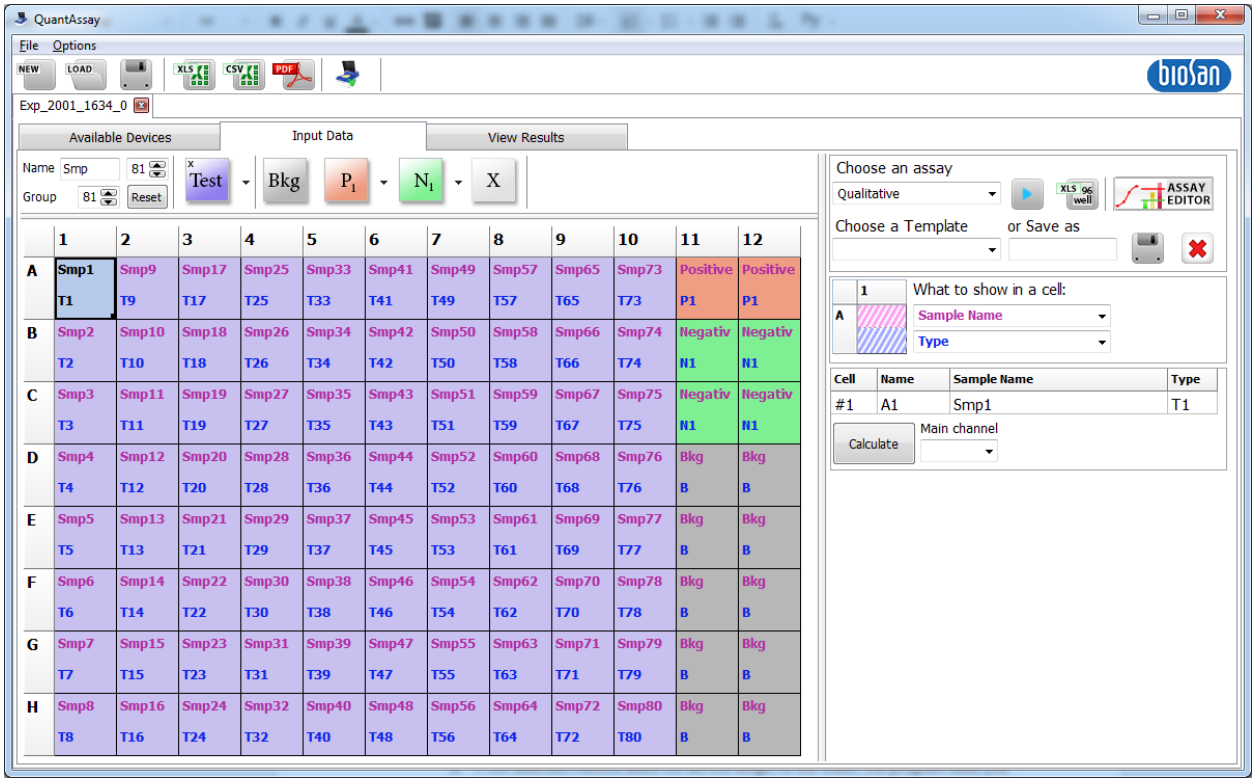
	1	2	3	4
A	Sample1 T1	Sample1 T1		
B				
C				

After

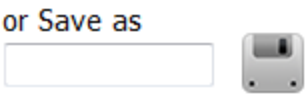
	1	2	3	4
A	Sample1 T1	Sample1 T1	Sample4 T4	Sample4 T4
B	Sample2 T2	Sample2 T2	Sample5 T5	Sample5 T5
C	Sample3 T3	Sample3 T3	Sample6 T6	Sample6 T6

e. Fill in the controls: for positive controls select P1; for negative controls, select the N1. 

7. Example of a filled plate

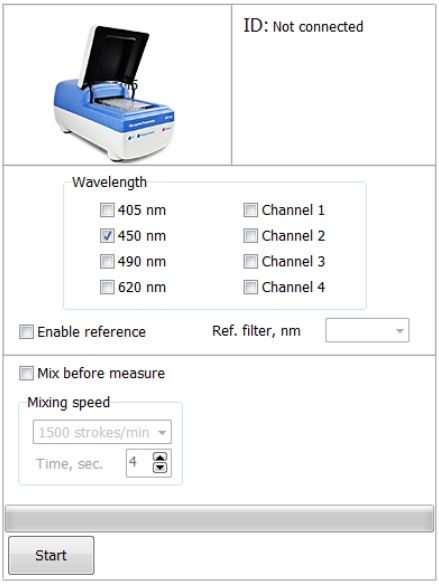


8. to save the template of the plate, enter its name in the "Save As" and press the save



icon

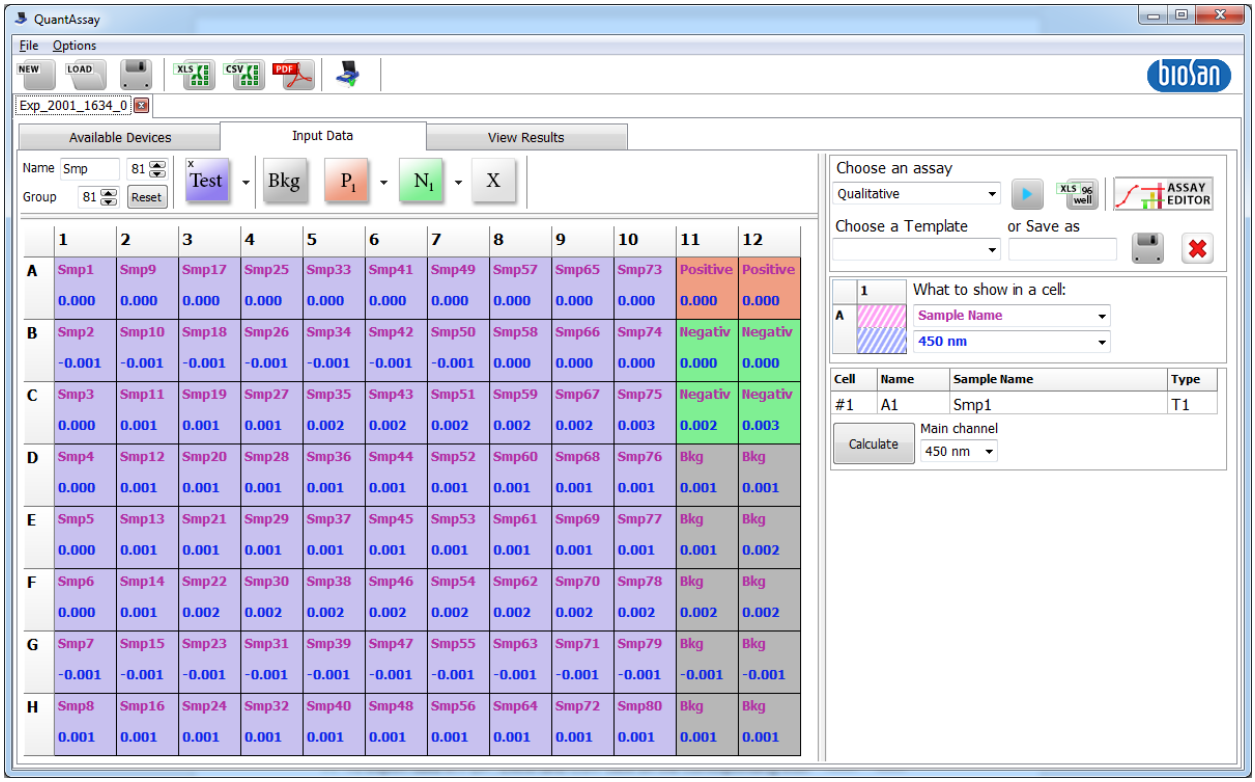
9. To start the measurement, click on the "Start"



   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

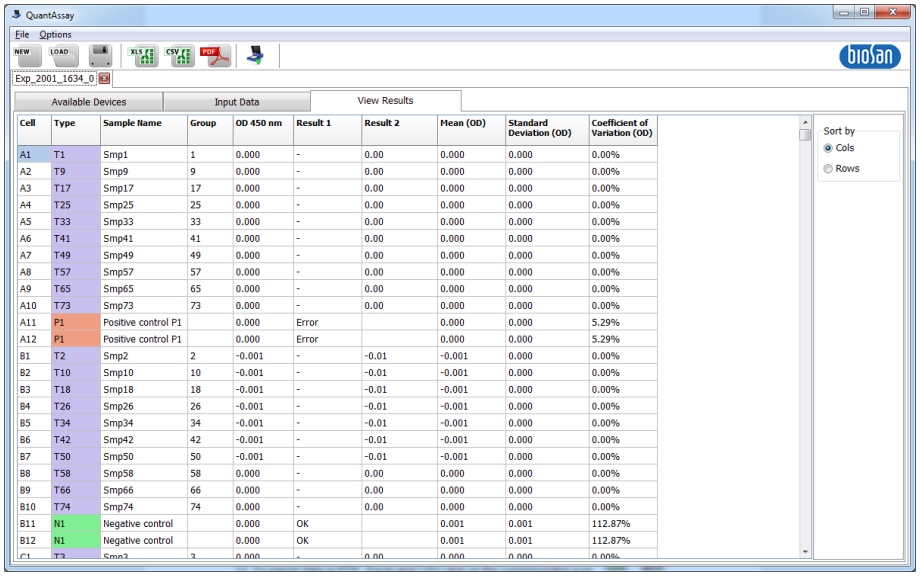
   b. Press “Start” when ready

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



	1	2	3	4	5	6	7	8	9	10	11	12
A	Smp1	Smp9	Smp17	Smp25	Smp33	Smp41	Smp49	Smp57	Smp65	Smp73	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
B	Smp2	Smp10	Smp18	Smp26	Smp34	Smp42	Smp50	Smp58	Smp66	Smp74	Negativ	Negativ
	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001	0.000	0.000	0.000	0.000	0.000
C	Smp3	Smp11	Smp19	Smp27	Smp35	Smp43	Smp51	Smp59	Smp67	Smp75	Negativ	Negativ
	0.000	0.001	0.001	0.001	0.002	0.002	0.002	0.002	0.002	0.003	0.002	0.003
D	Smp4	Smp12	Smp20	Smp28	Smp36	Smp44	Smp52	Smp60	Smp68	Smp76	Bkg	Bkg
	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
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	Bkg	Bkg
	0.000	0.001	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
G	Smp7	Smp15	Smp23	Smp31	Smp39	Smp47	Smp55	Smp63	Smp71	Smp79	Bkg	Bkg
	-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
	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

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



Cell	Type	Sample Name	Group	OD 450 nm	Result 1	Result 2	Mean (OD)	Standard Deviation (OD)	Coefficient of Variation (OD)
A1	T1	Smp1	1	0.000	-	0.00	0.000	0.000	0.00%
A2	T9	Smp9	9	0.000	-	0.00	0.000	0.000	0.00%
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	T33	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	OK		0.001	0.001	112.87%
B12	N1	Negative control		0.000	OK		0.001	0.001	112.87%

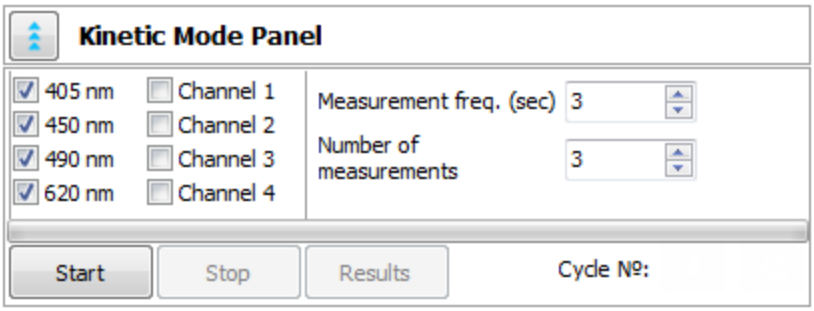
12. To export data in PDF, Excel and CSV click on the corresponding icon 

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:



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

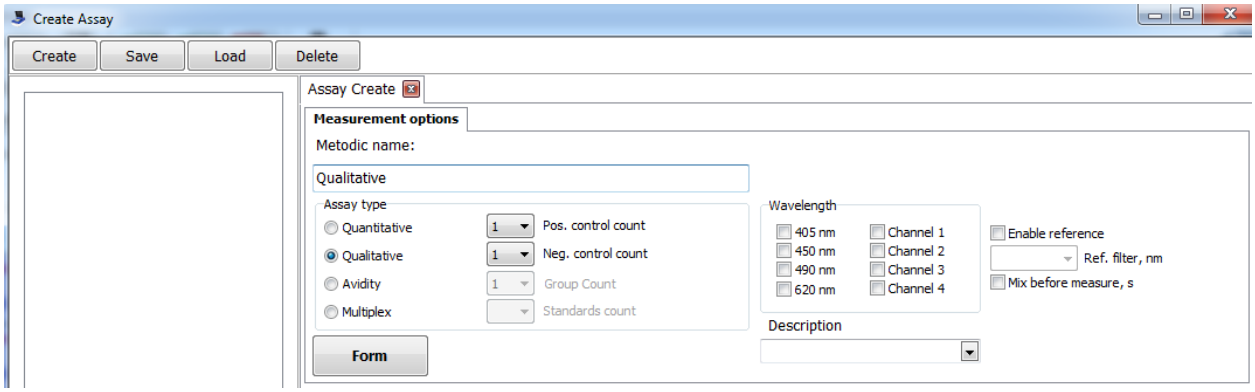
2. for each type of the assay it is possible to define:
   - the number of types of positive controls (strong, weak, etc.)
   - the number of types of negative controls (no 1'/ 2' antibody conjugate, water sample)
   Note: Each type of the Control can be analyzed separately from the rest of the positive 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 (R^2) among the: 5 parameter logistic, 4 parameter logistic, linear and various regression models.
   - description of the assay

Creating Qualitative assay

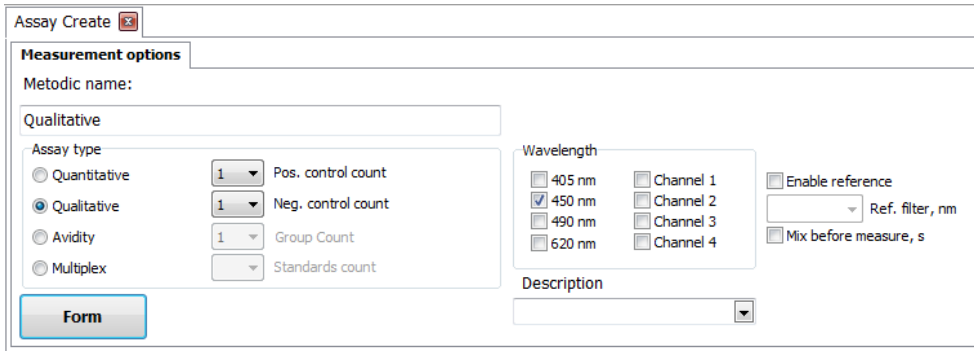
1. For example we need to create a qualitative assay with following criteria:
   Measurement channel at 450 nm.
   In this assay the sample will analysed as positive, if the corresponding OD value is equal or greater than the Critical (Threshold) OD, which is calculated by the formula:
   = NC1 + 0.2 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:

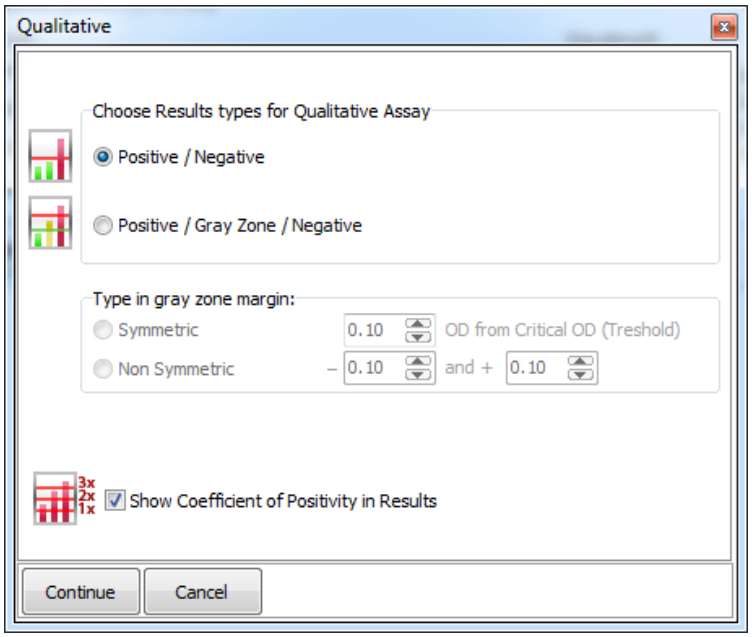
2. Click on “Create” button. Following window will appear:



3. 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**

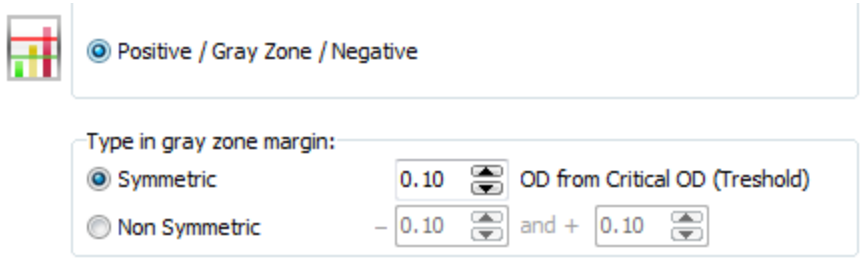


4. In the next window you can select the types of results for this assay:



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” .

5. 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
[T]	[T]>[C]	+	-	[F]	[F]
[P1]	[P1]>1	OK	Error		
[N1]	[N1]<0.2	OK	Error		

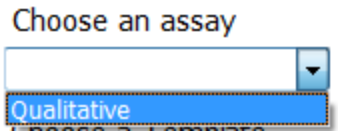
Columns:
- In the column “For variable” you can set for which variable following conditional will be used, e.g. variable [T] means that the conditional and results filled in the next fields will be used for the test samples, to chose another variable, right-click on the field under the column and select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

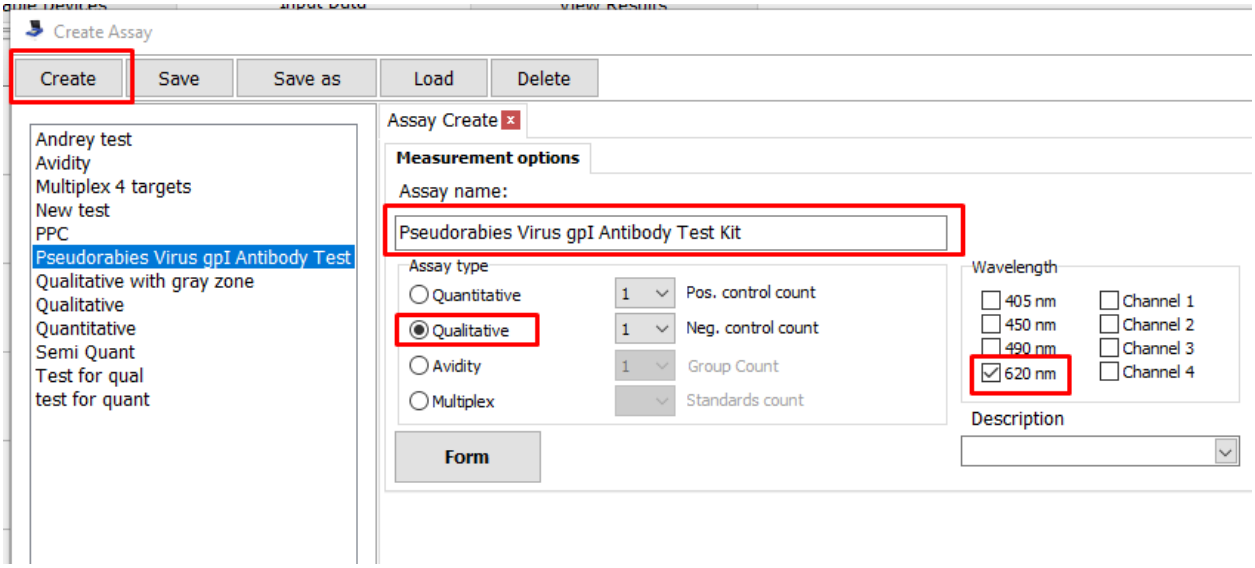
8. Save the assay and close the window of “Assay Editor”"
9. Choose your newly created assay and run it:



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

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

First of all, create the assay



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

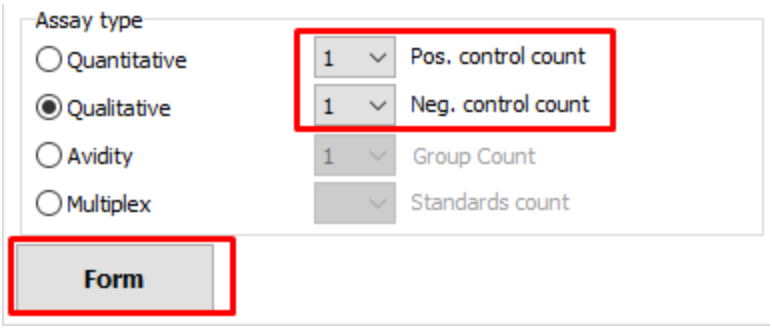
Controls

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

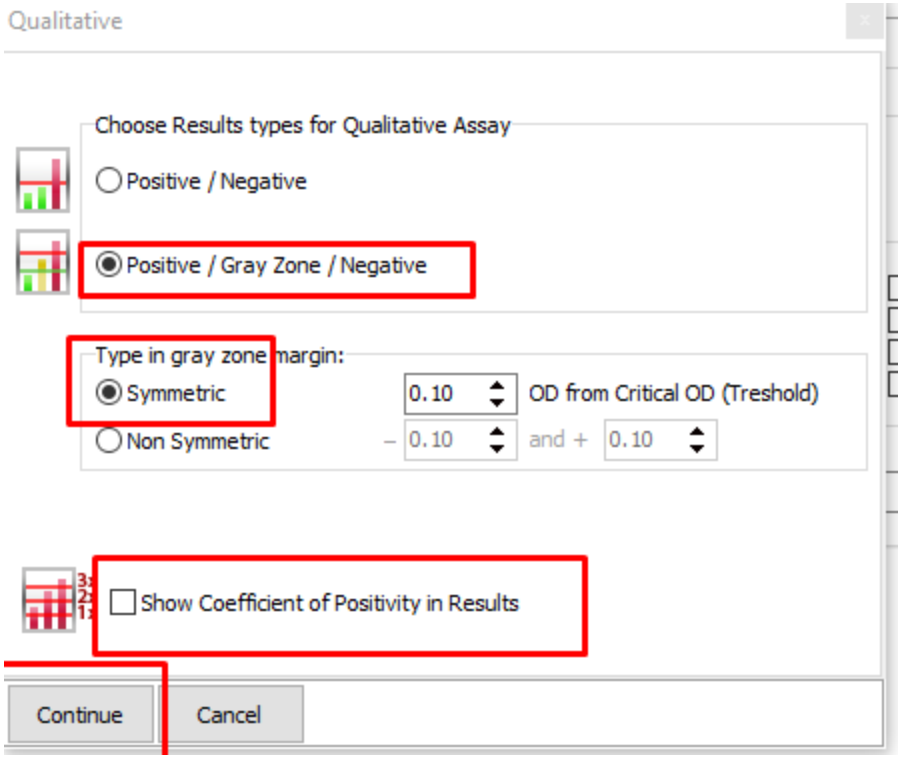
*Example shows Negative Control run in Triplicate.

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.



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



Now we need to enter the S/N ratio:

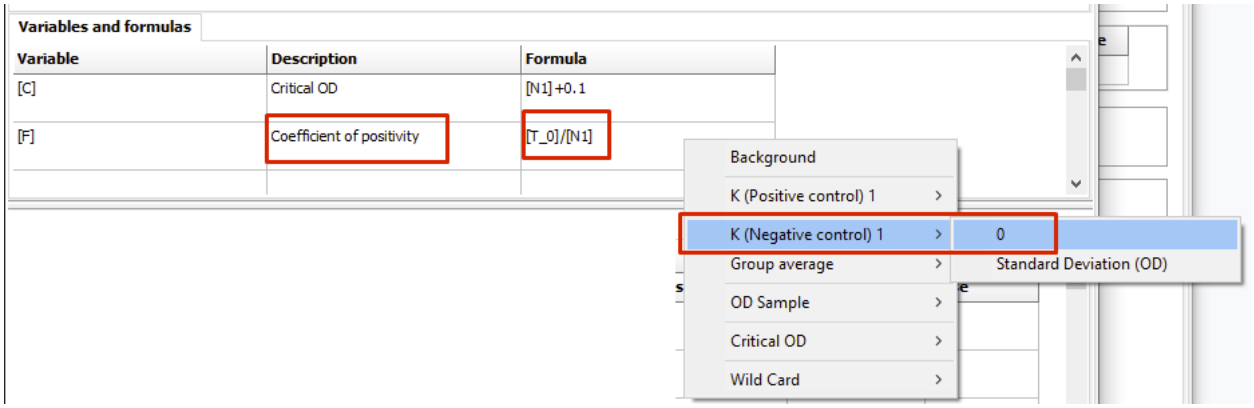
Samples

$$S/N = \frac{\text{Sample A(650)}}{NC\bar{x}}$$

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



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

$$NC\bar{x} - PC\bar{x} \geq 0.300$$

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.

Result interpretation

For variable	Conditional	Result 1	
		True	False
[P1]	[N1]-[P1]>=0.3	OK	Error
[N1]	[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 ≤ 0.70	S/N ≤ 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 interpretation

For variable	Conditional	Result 1		Res
		True	False	Tru
[T]	[F]>0.7	Negative		
[T]	([F]>0.6) && ([F]<=0.7)	Suspect		
[T]	[F]<=0.6	Positive		
[P1]	[N1]-[P1]>=0.3	OK	Error	
[N1]	[N1]-[P1]>=0.3	OK	Error	

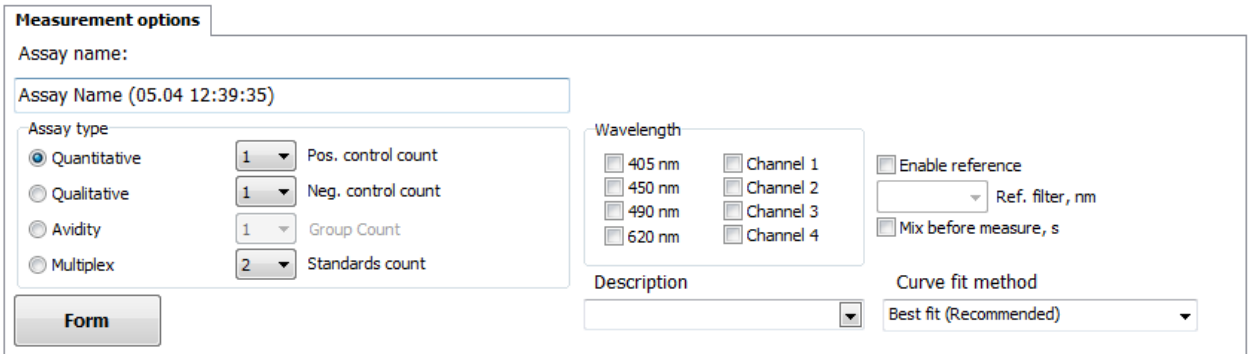
Your assay is good to go!

Create a Quantitative Assay

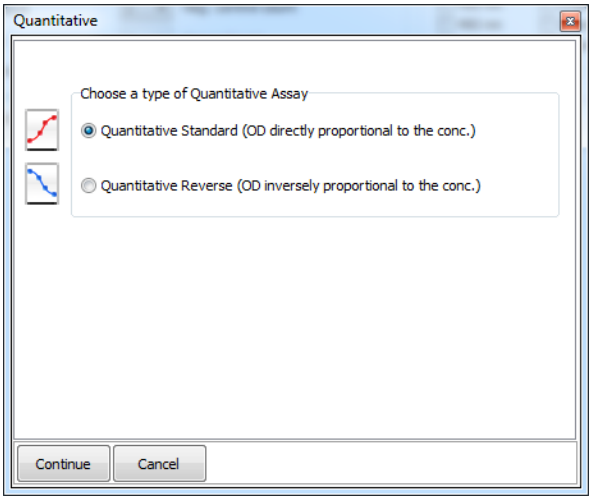
We want to create a quantitative assay with following criteria:
Measurement channel is 450 nm., with reference channel at 620 nm and mix before measuring.
6 standards with concentrations of: 0, 5, 10, 25, 100, 500 International Units (IU)are being used.
Calibration curve should be fitted automatically by choosing the best fitting curve (based on R^2 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

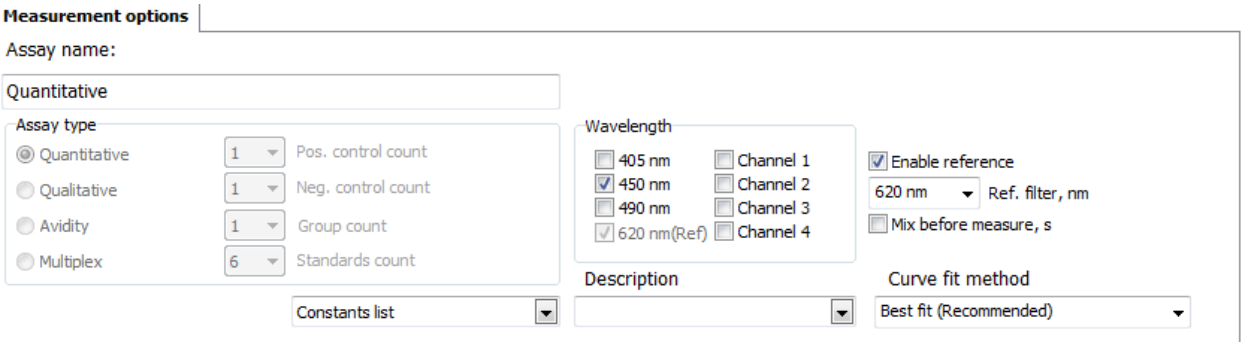
The following steps show procedure of creation of this assay

1. Click on “Create” button. Following window will appear:



2. 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**”

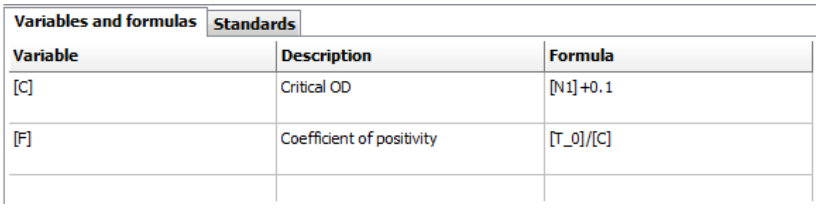




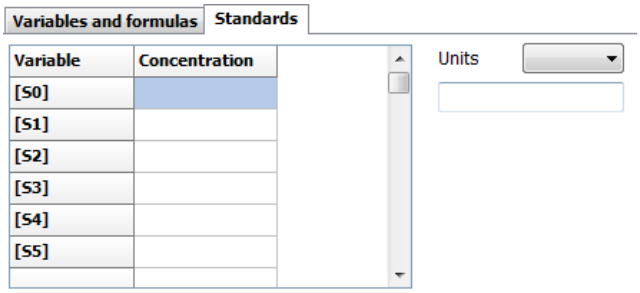
3. In the next window you can select the type of the quantitative assay: “Quantitative Standard” or “Quantitative Reverse” (“Reverse” means that with the increase of concentration the OD is decreasing, “Standard” means that with the increase of concentration the OD is also increasing)

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:

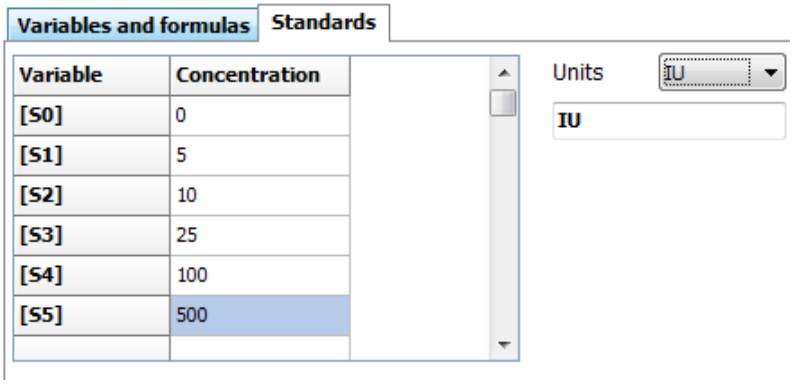
5. Tab “Variables and formulas”



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



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).



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	
		True	False
[S0]	[S0]<[S1]	OK	Error
[S1]	[S1]<[S2]	OK	Error
[S2]	[S2]<[S3]	OK	Error
[S3]	[S3]<[S4]	OK	Error
[S4]	[S4]<[S5]	OK	Error
[S5]	[S4]<[S5]	OK	Error
[T]	([SMin]<[T])&&([T]<[SMax])	In Range	Out of Range
[P1]	[P1]>1	OK	Error
[N1]	[N1]<0.2	OK	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”.

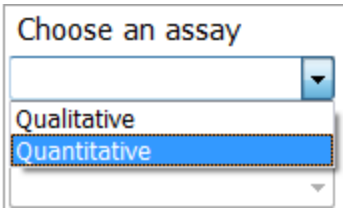
Further:
Analysis of test samples:

[T]	([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**.

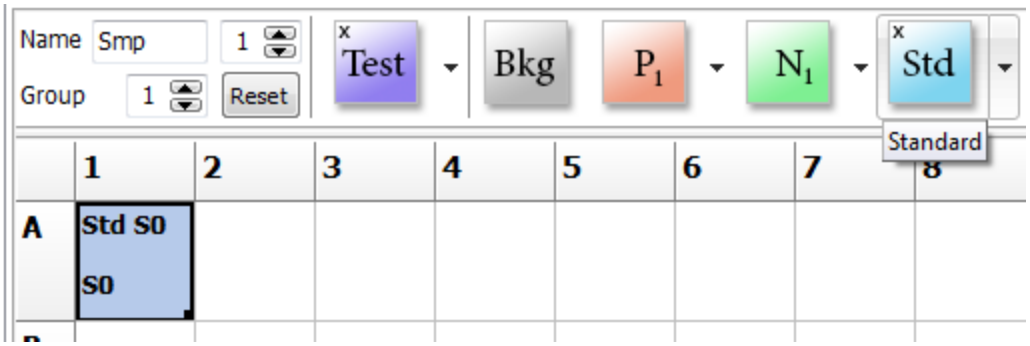
For the negative control ([N1_0] is the same as [N1]) is written a condition [N1] <0.2, which means that if the OD of neg. control is less than 0.2 OD, then “Result 1” outputs "Ok", else outputs "Error".



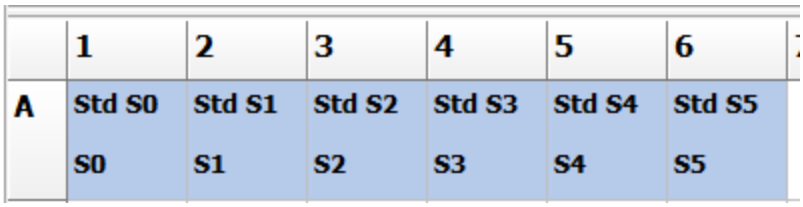
For the positive control ([P1_0] is the same as [P1]) the condition is [P1]>1.0 that is, if the OD of Positive control is more than 1.0, then “Result 1” outputs "Ok", else "Error".

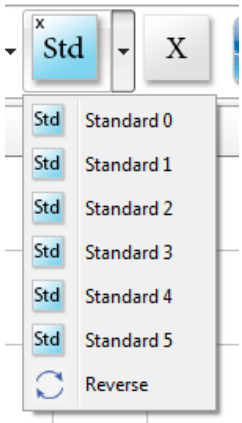
7. Save the assay and close the window of “Assay Editor”"
8. Choose your newly created assay and run it:

9. In order to add Standards do the following:
   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

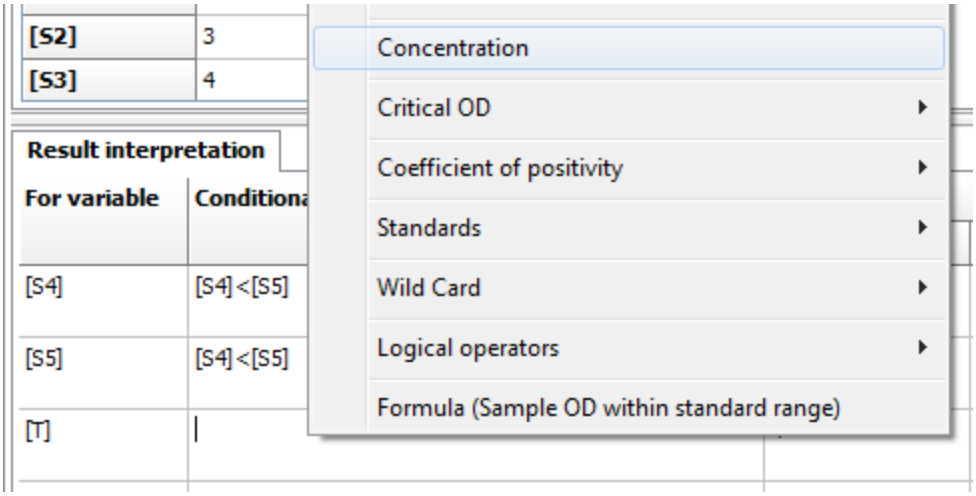




   Or manually chose a well and add a needed standard by choosing from drop box
   c. Setting samples and controls, and obtaining results is the same as in Qualitative assay

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])



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.

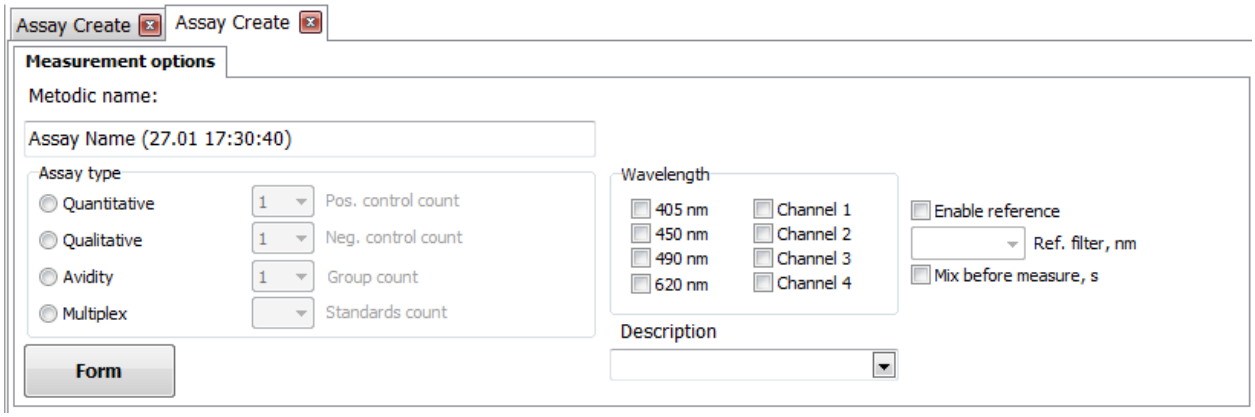
[T]	[O]>3	+	-

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.

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

Create an avidity assay



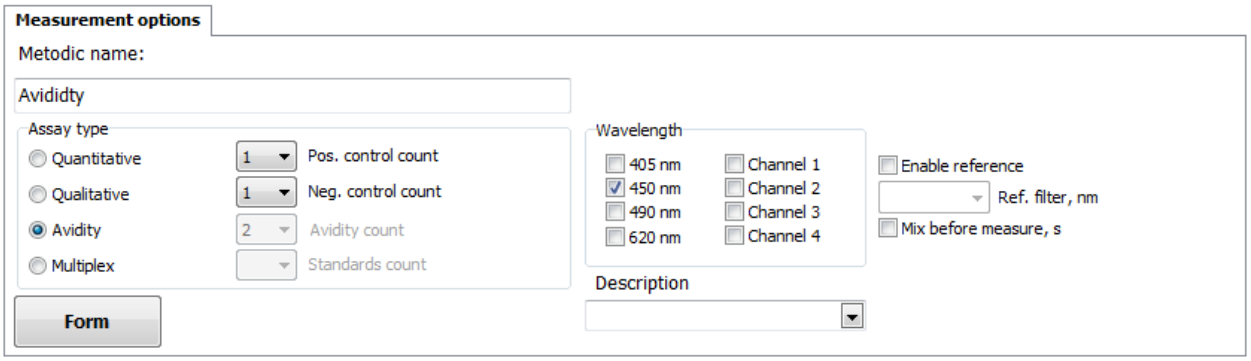
1. Avidity assays analyses the samples by calculating the Index of Avidity (IA) of positive test samples. Index of Avidity is the ratio of optical density of a sample in the presence of a dissociating agent (dissociation ELISA) to an optical density of the same sample without dissociating agent (direct 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 $OD_{\text{of direct ELISA}}$ 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:
   = $OD_{\text{dissociation ELISA}}$/ $OD_{\text{direct ELISA}}$
   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)

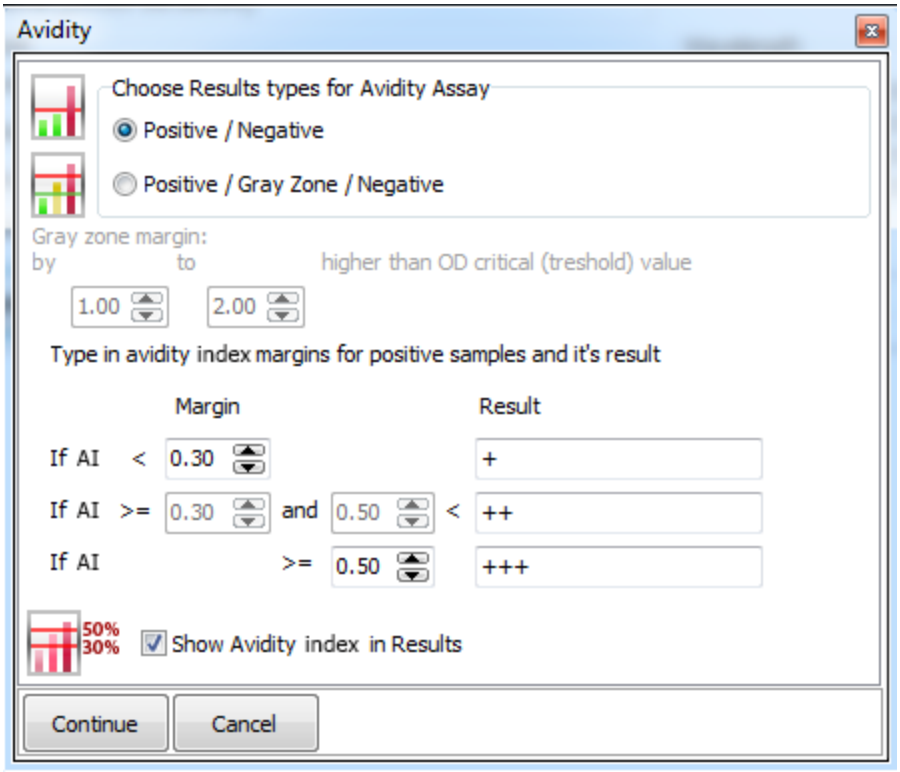
   Quality control of negative and positive controls should meet following criteria:
   - $OD_{\text{direct ELISA}}$ of the positive control must be greater than 1 OD and the index of avidity greater than 0.30 (30%);
   - OD value of the negative control must be less than 0.1 OD

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”**



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

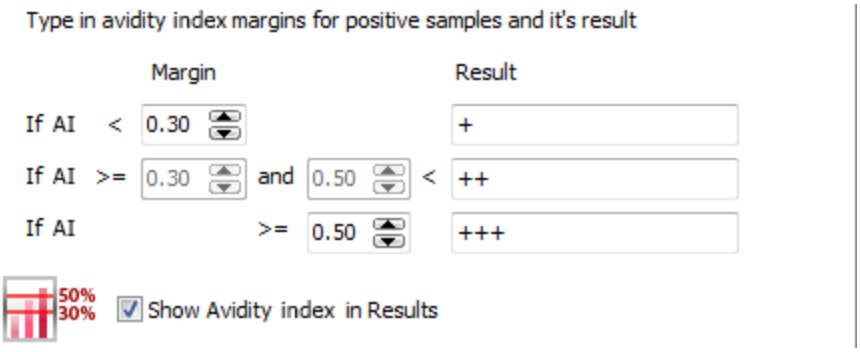


1) Positive / Negative, e.g. according if the OD of the sample is greater than or less than the threshold OD, program will output results "Positive" or "Negative", Avidity index will be calculated only for positive test samples

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:



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:

   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
[T]	[T_0]<[C]	-			
[T]	[R]<0.3 && [T_0]>=[C]	+		[R]	
[T]	[R]>=0.3 && [R]<0.5 && [T_0]>=[C]	++		[R]	
[T]	[R]>=0.5 && [T_0]>=[C]	+++		[R]	

   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 $_{\text{of direct ELISA}}$ ([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 $_{\text{of direct ELISA}}$ 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 $_{\text{of direct ELISA}}$ 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 $_{\text{of direct ELISA}}$ 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

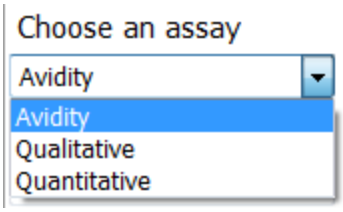
Result interpretation

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

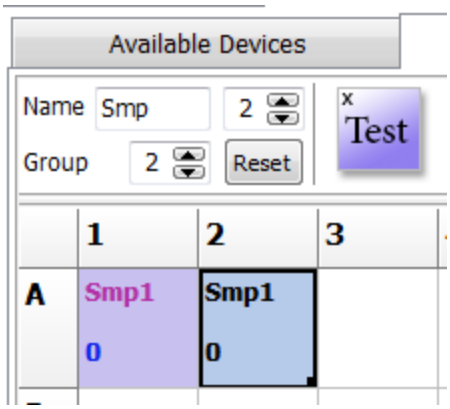
For the positive controls ([P1_0] and [P1_1]) we need to check if $OD_{\text{of direct ELISA}}$ is greater than the threshold OD ([P1_0]>=[C]) **AND** the avidity index should be greater or equal to 0.3, since we do not have a variable for positive controls' avidity indexes, we need to specify it separately 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 $OD_{\text{of direct ELISA}}$ 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:

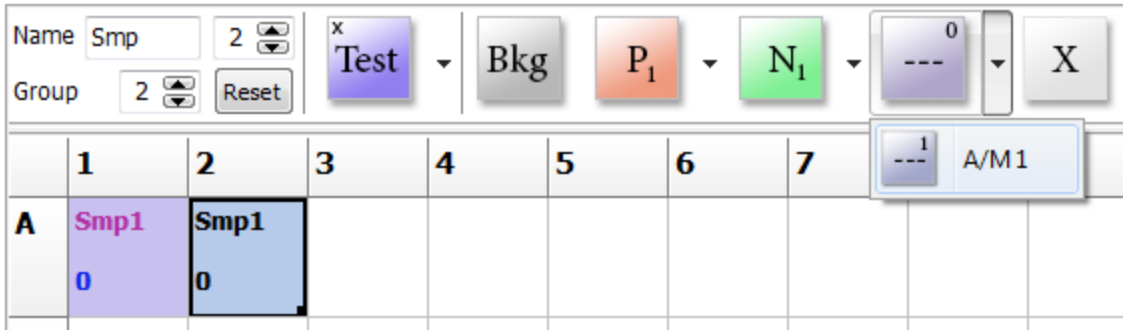


12. In order to fill the samples do the following:
    Set 2 samples in two adjacent wells (or controls) and select the sample with protein dissociating agent (e.g. urea).

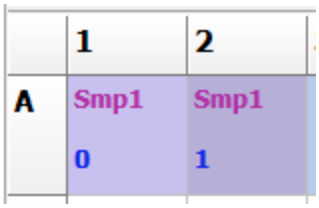


Click on the droplist near the "---^{0}" button, A/M 1 button

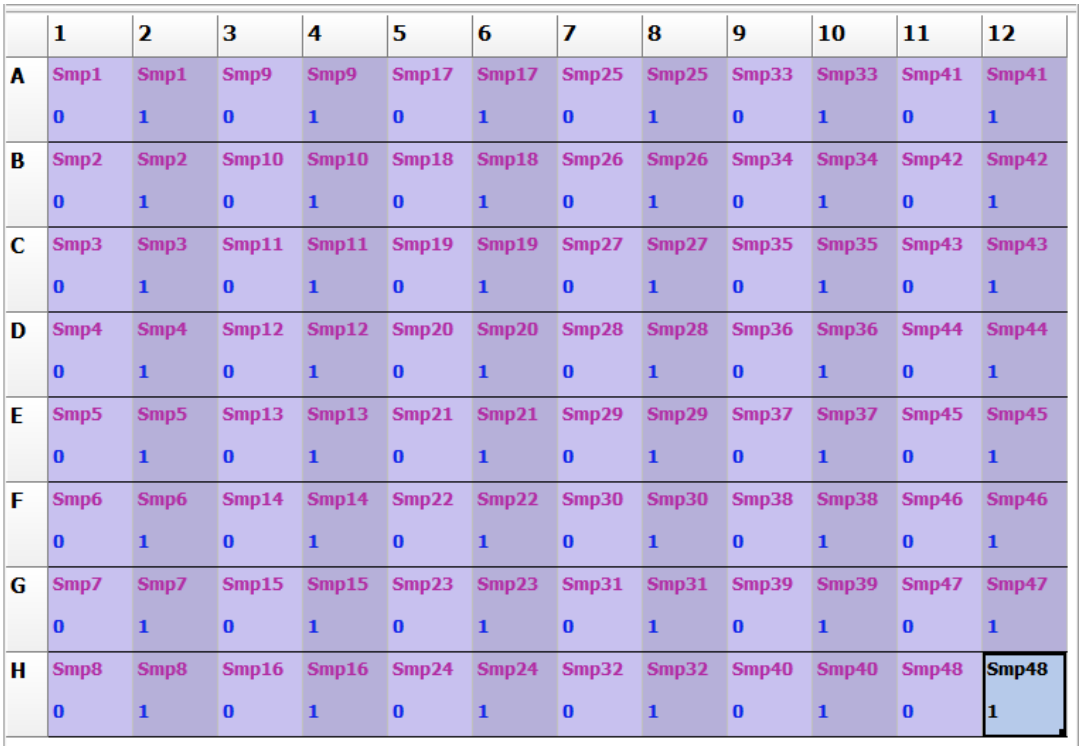
will appear, click on it.



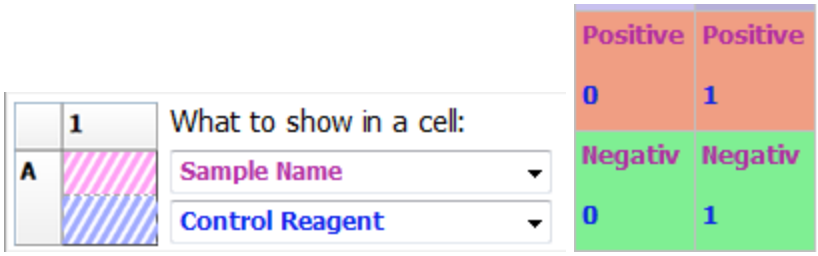
13. Now the sample in A2 is with urea, the bottom part of the well will now show “Control Reagent” type. **Note:** The well with urea will now appear with a slightly different color. Select any other well in order to see it. Controls will not appear with different color.



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



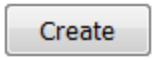
15. When setting controls, be sure to put “A/M 1” again. **Note** Controls with urea will not appear with different color, to check if you have entered control with urea, choose “Control Reagent” in “What to show in a cell”, controls with urea will have “1” in the bottom part of the cell



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.

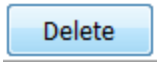


- Creates a new assay

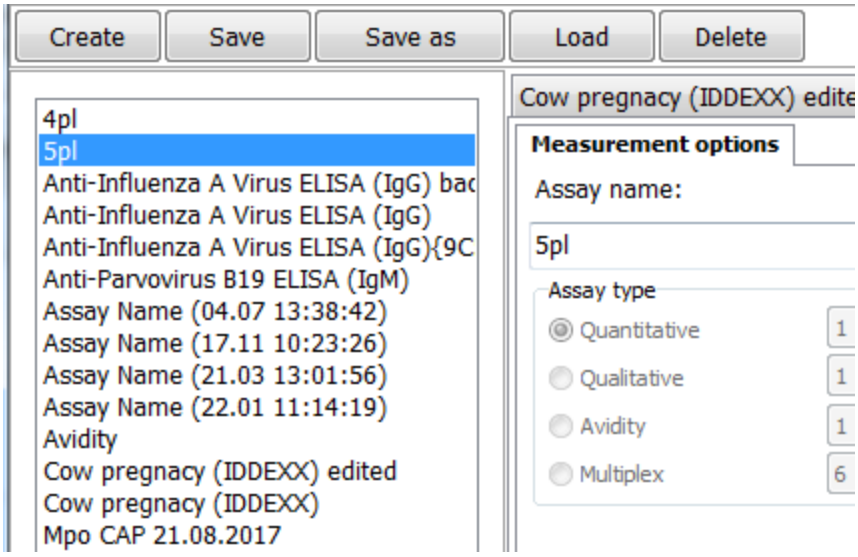
- Saves the assay

- Saves a copy of an assay (including all referenced templates)

- Loads an assay



- Deletes a selected assay from the list of assays.

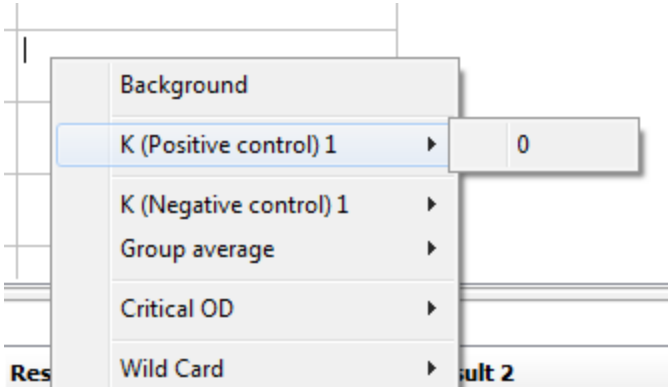


Using new variables: Wildcards

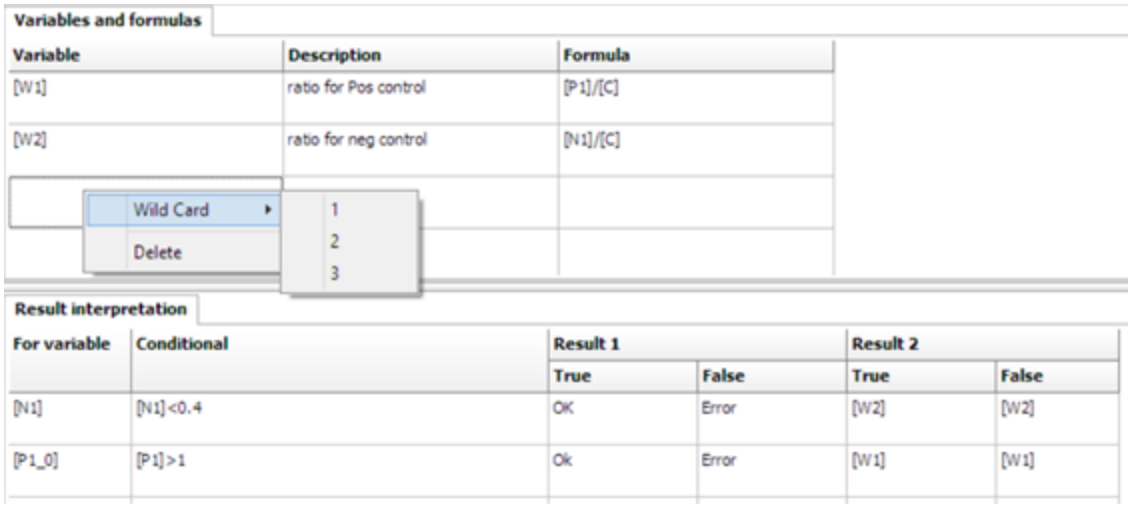
While creating new assays, users can use other than preset variables like Critical OD [C] or Coefficient of positivity [F]. Those variables are called Wildcards [W], and user can use 7 new variables per assay.

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.



Logical operations in the interpretation of results

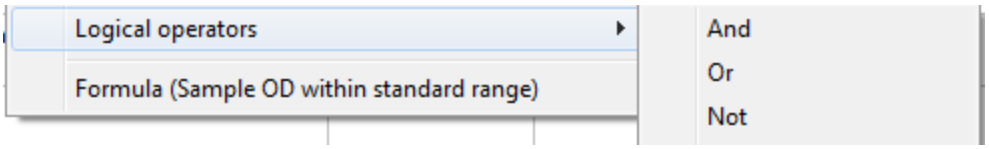
Logical expressions can take one of two values - "true" or "false". Logic operations are used for complex logical expressions. We use logical operations as conditions for determining the results of the program.

For example, Condition:

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

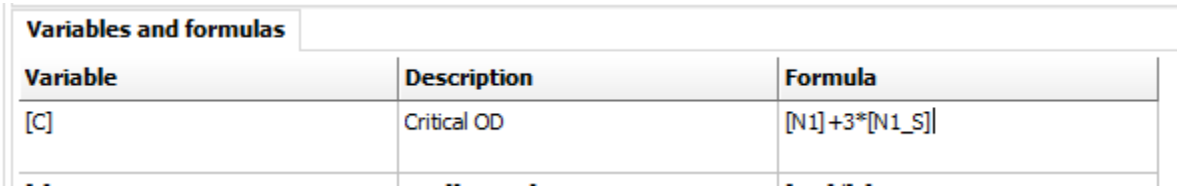
Here we have two conditions: OD sample type T_0 and OD sample type T_1, if the OD of both samples is greater than or equal to 1, then the conditions of "Yes" and as a result in front of the sample It will be written the result of "laid." If not, the result will be written "Neg."

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



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.



Models for quantitative analysis

For building calibration curves we

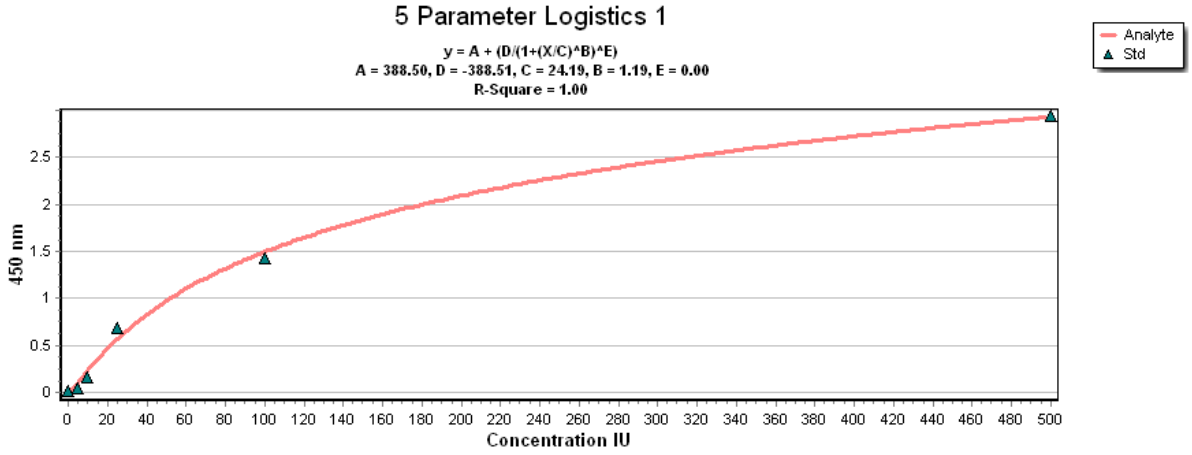
1. 5-parameter logistic model
2. 4-parameter logistic model
3. linear model
4. Piecewise linear model

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:

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

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

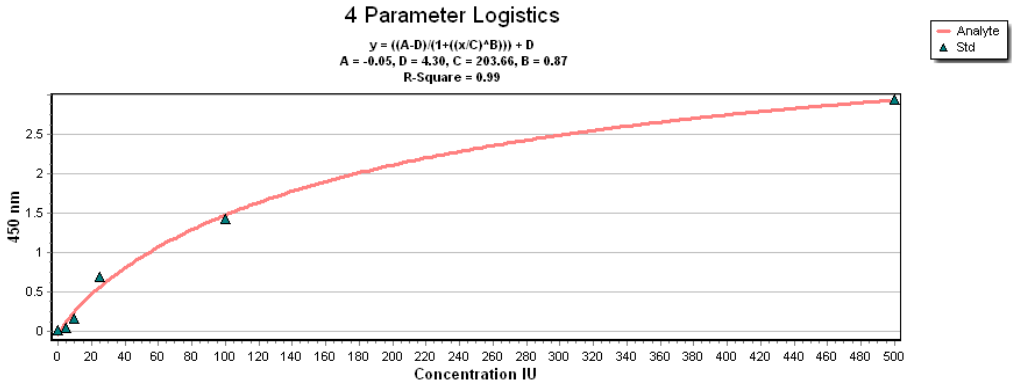
where:

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

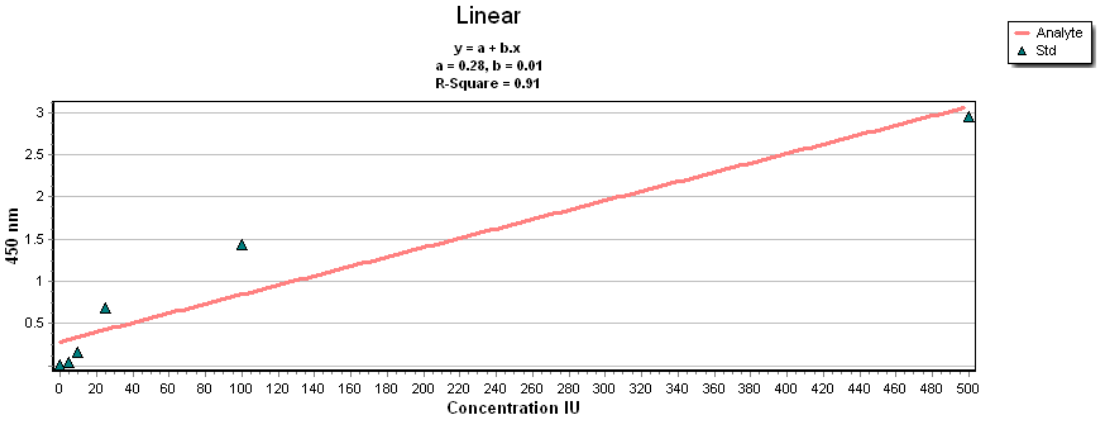


Linear model

linear function - the function of the form

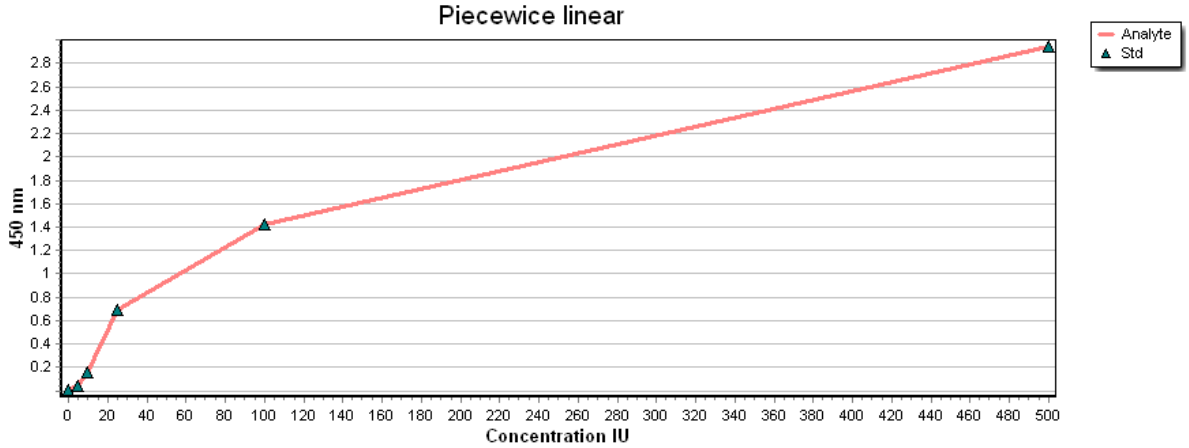
$$y = kx + b$$

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

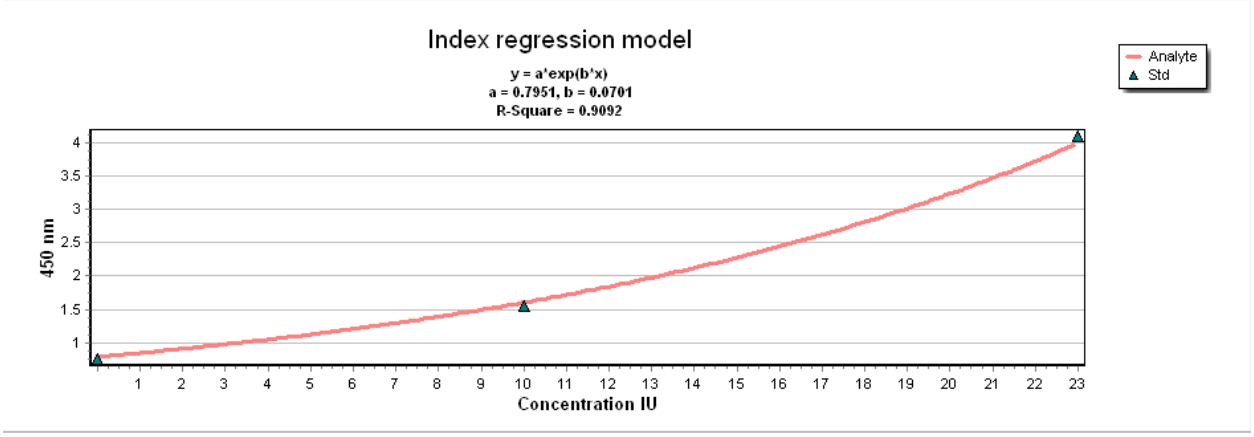


The piecewise linear model

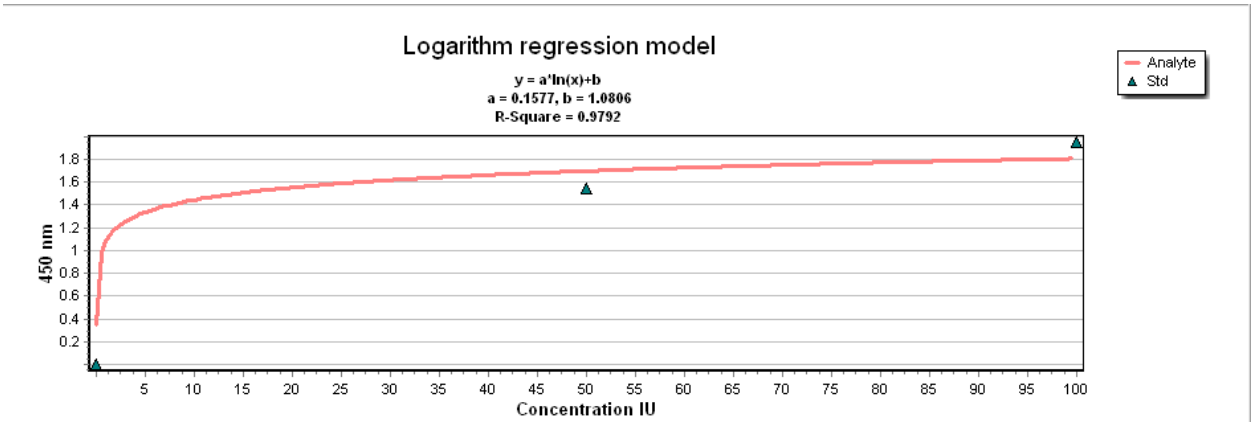
A piecewise linear function is a function defined on the set of points and is linear between each interval.



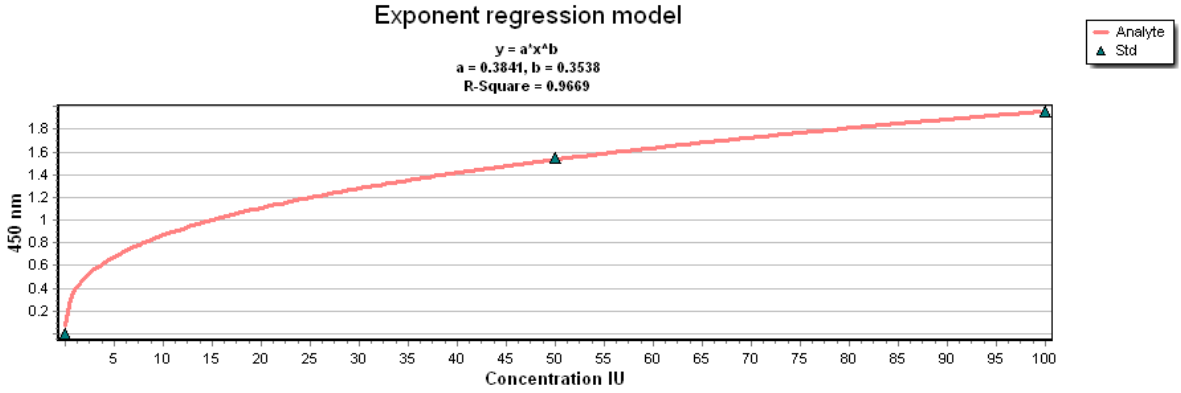
The index regression model



The logarith regression model



The exponent regression model



The cubic spline model

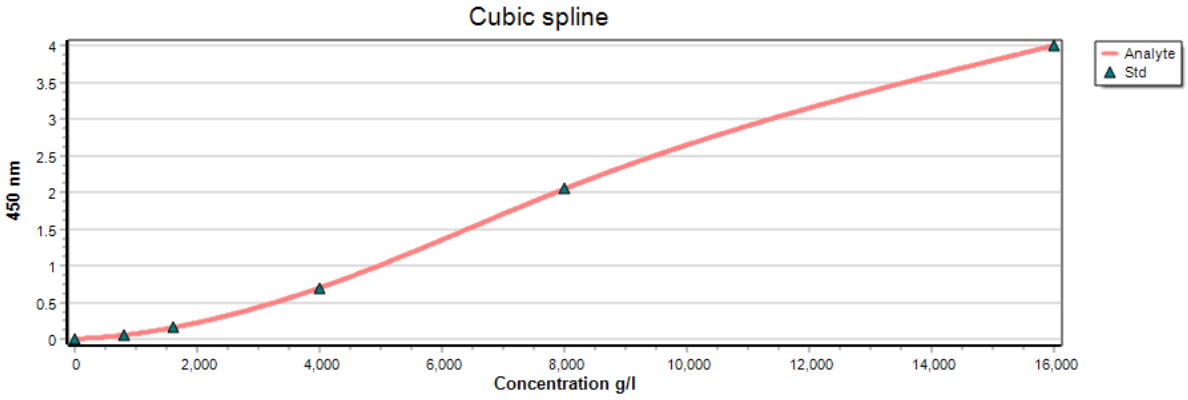
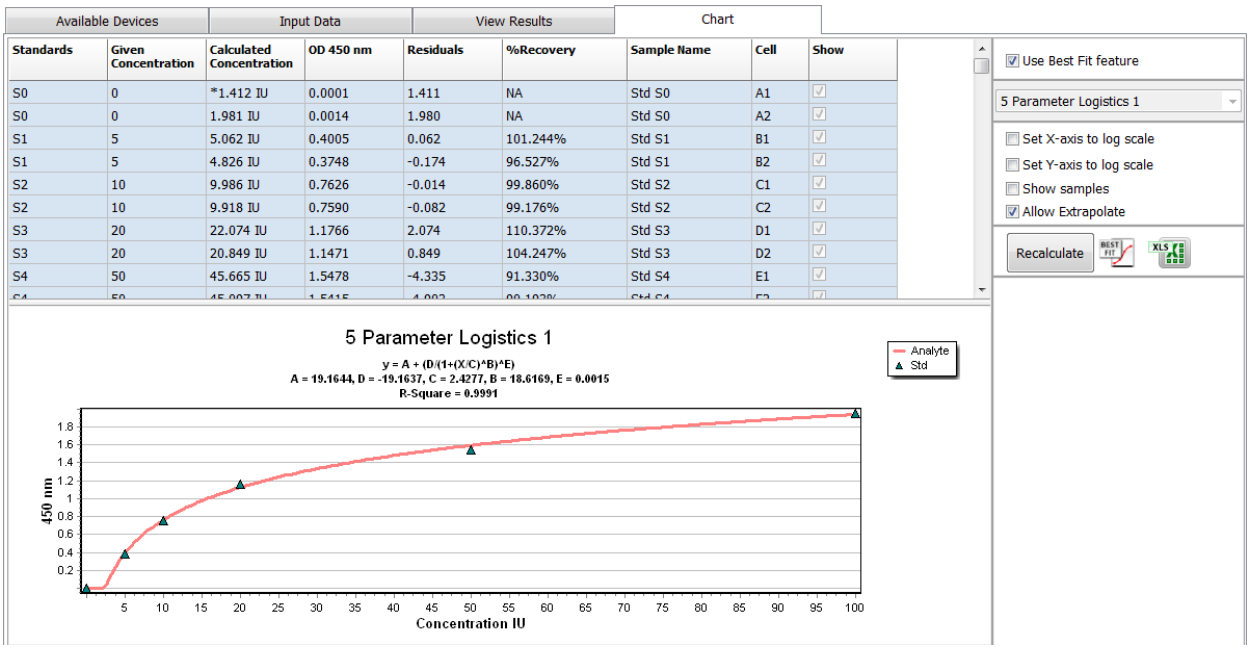
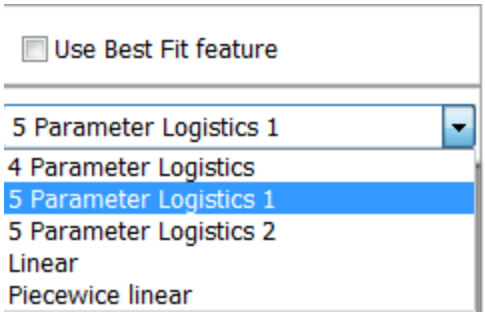
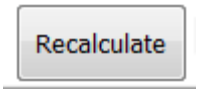


Chart [calibration] tab



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.



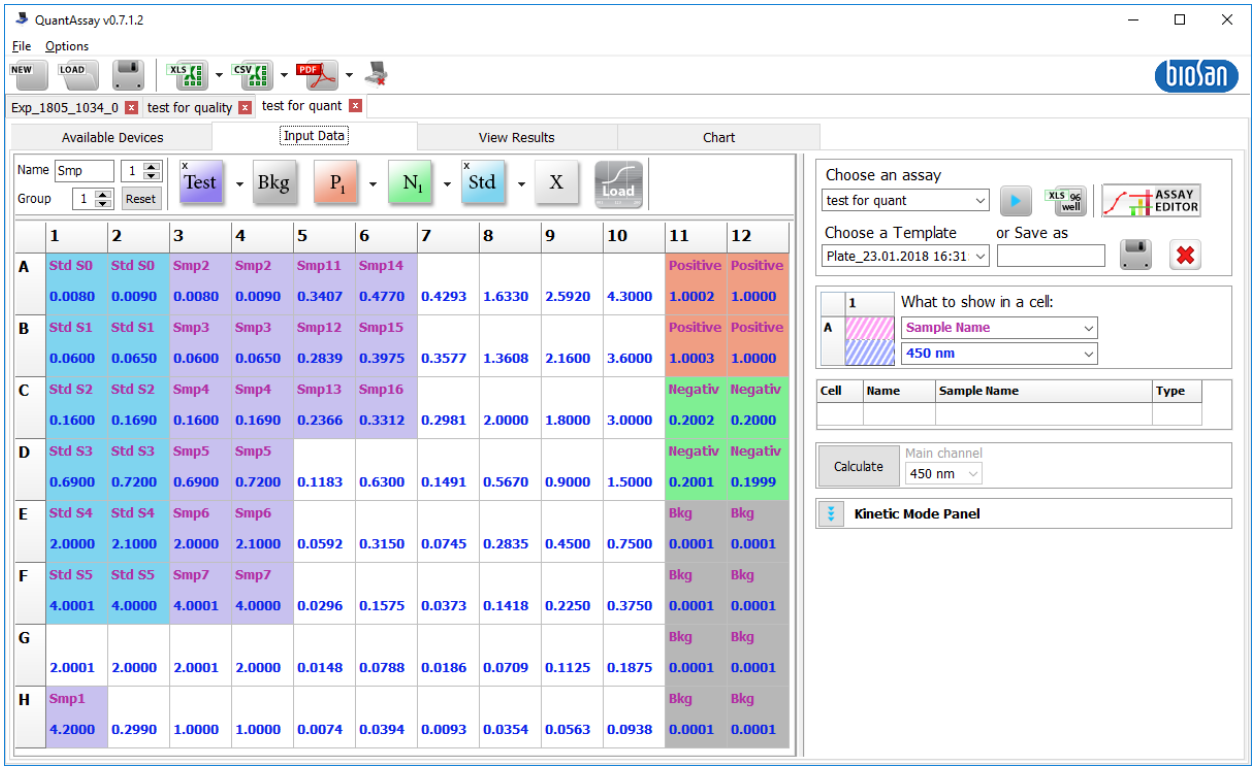
Then click on the “Recalculate” button. 

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

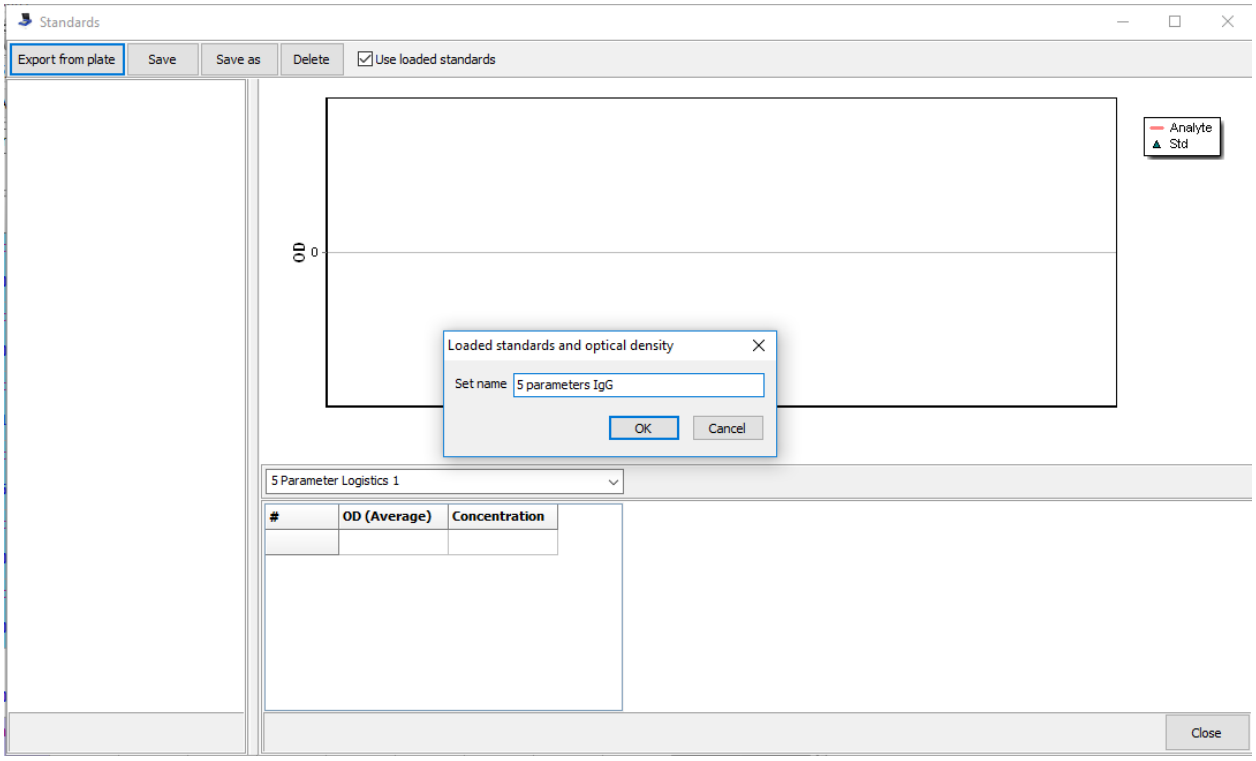
First you need to create your curve.
Open the program and load a quantitative experiment, like below:

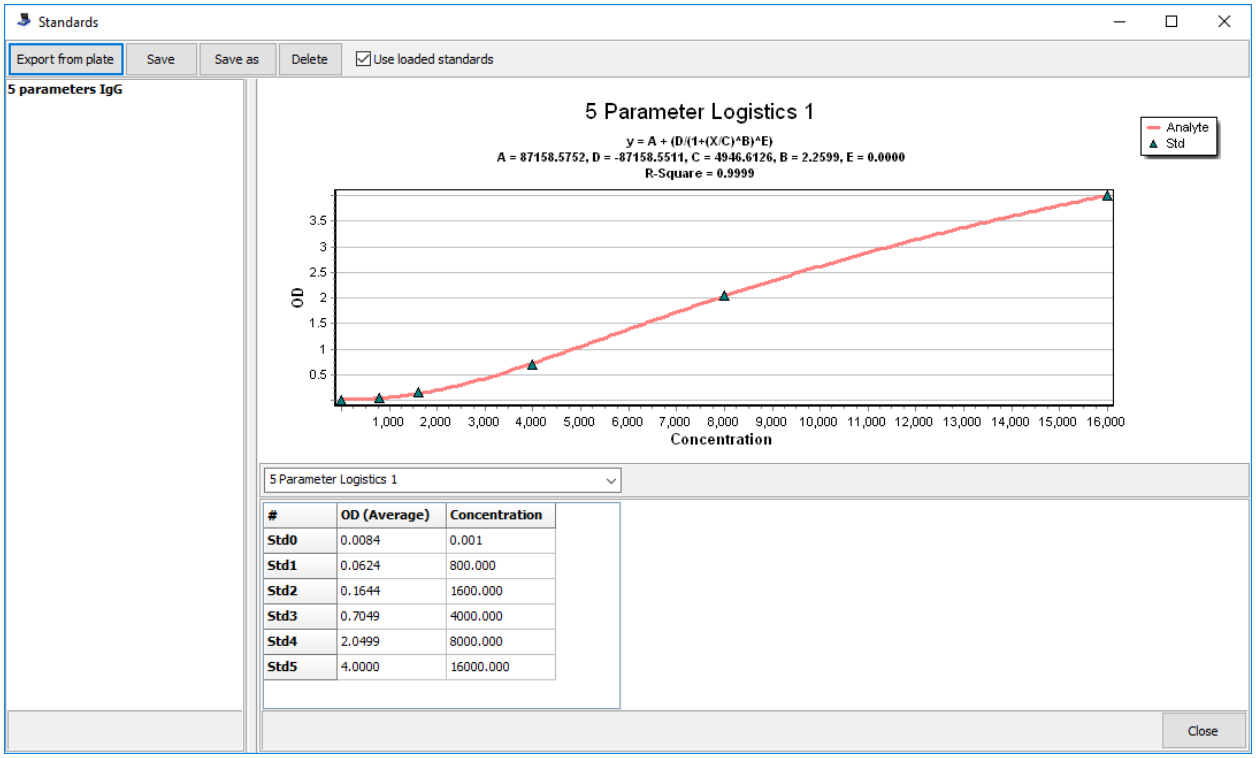


	1	2	3	4	5	6	7	8	9	10	11	12
A	Std S0	Std S0	Smp2	Smp2	Smp11	Smp14					Positive	Positive
	0.0080	0.0090	0.0080	0.0090	0.3407	0.4770	0.4293	1.6330	2.5920	4.3000	1.0002	1.0000
B	Std S1	Std S1	Smp3	Smp3	Smp12	Smp15					Positive	Positive
	0.0600	0.0650	0.0600	0.0650	0.2839	0.3975	0.3577	1.3608	2.1600	3.6000	1.0003	1.0000
C	Std S2	Std S2	Smp4	Smp4	Smp13	Smp16					Negativ	Negativ
	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
	0.6900	0.7200	0.6900	0.7200	0.1183	0.6300	0.1491	0.5670	0.9000	1.5000	0.2001	0.1999
E	Std S4	Std S4	Smp6	Smp6							Bkg	Bkg
	2.0000	2.1000	2.0000	2.1000	0.0592	0.3150	0.0745	0.2835	0.4500	0.7500	0.0001	0.0001
F	Std S5	Std S5	Smp7	Smp7							Bkg	Bkg
	4.0001	4.0000	4.0001	4.0000	0.0296	0.1575	0.0373	0.1418	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
H	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

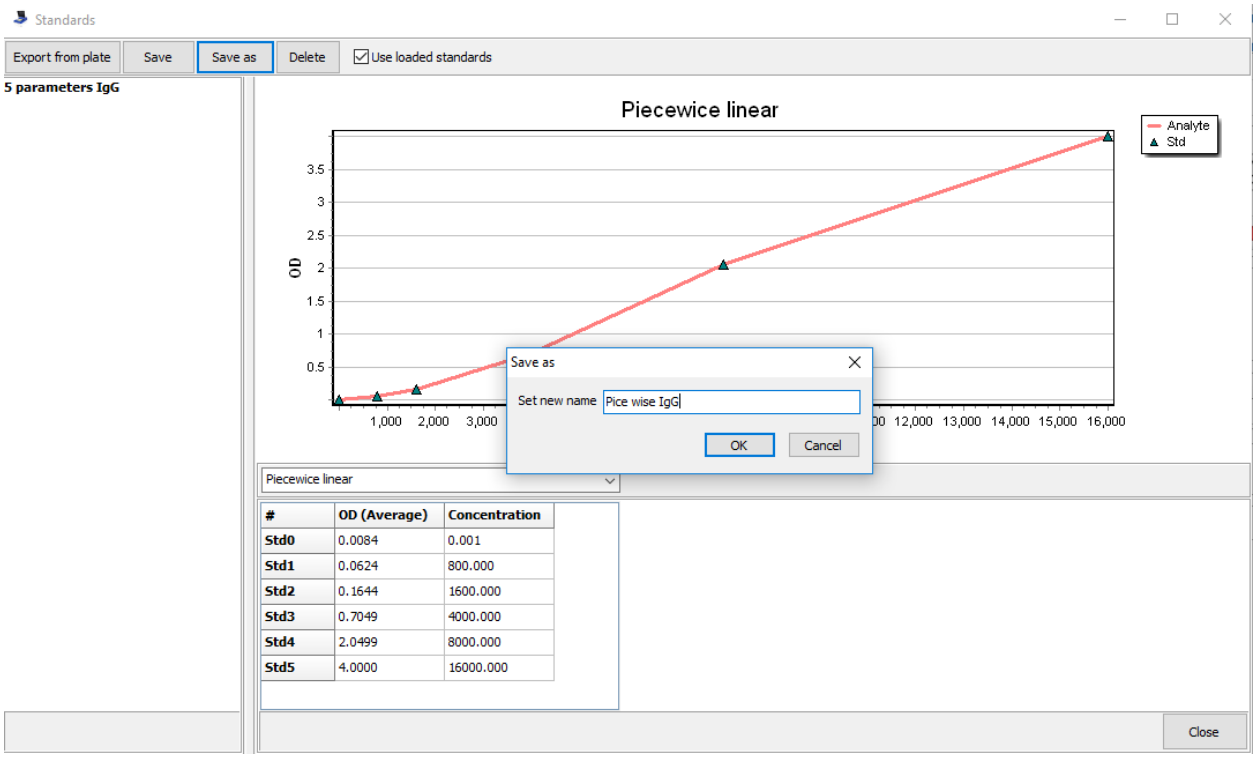
Press on load curve icon:

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
Export from plate
Save
Save as
Delete
Use loaded standards
Analyte
Std
OD
0
Loaded standards and optical density
Set name
5 parameters IgG
OK
Cancel
5 Parameter Logistics 1
OD (Average) Concentration
Close


Standards
Export from plate
Save
Save as
Delete
Use loaded standards
5 parameters IgG
5 Parameter Logistics 1
y = A + (D/(1+(X/C)^B))^E)
A = 87158.5752, D = -87158.5511, C = 4946.6126, B = 2.2599, E = 0.0000
R-Square = 0.9999
Analyte
Std
OD
Concentration
5 Parameter Logistics 1
OD (Average) Concentration
Std0 0.0084 0.001
Std1 0.0624 800.000
Std2 0.1644 1600.000
Std3 0.7049 4000.000
Std4 2.0499 8000.000
Std5 4.0000 16000.000
Close

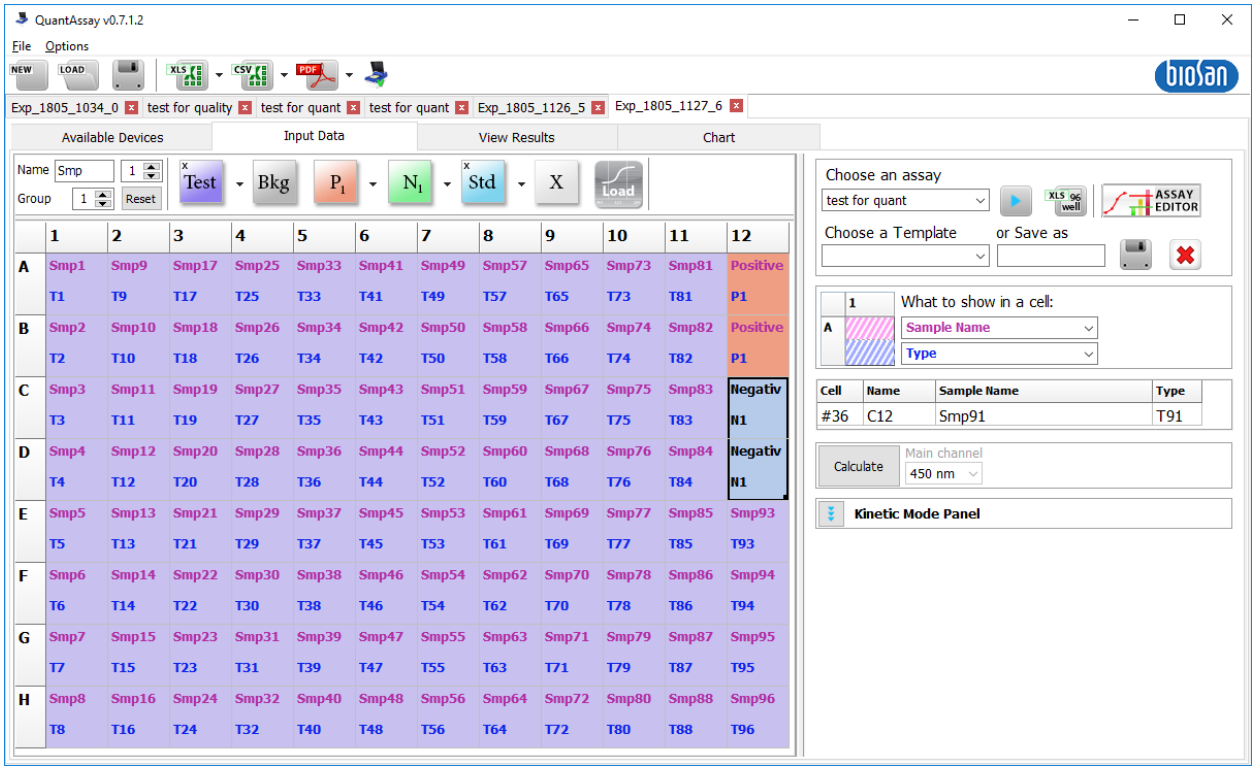
Here you can now set other type of curve and Save it as new curve.



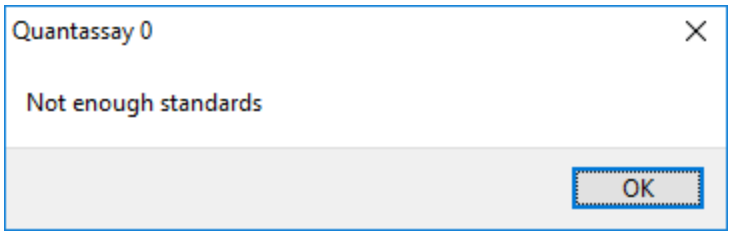
#	OD (Average)	Concentration
Std0	0.0084	0.001
Std1	0.0624	800.000
Std2	0.1644	1600.000
Std3	0.7049	4000.000
Std4	2.0499	8000.000
Std5	4.0000	16000.000

Now you need to load it in the new experiment.

Set you samples and measure the plate.



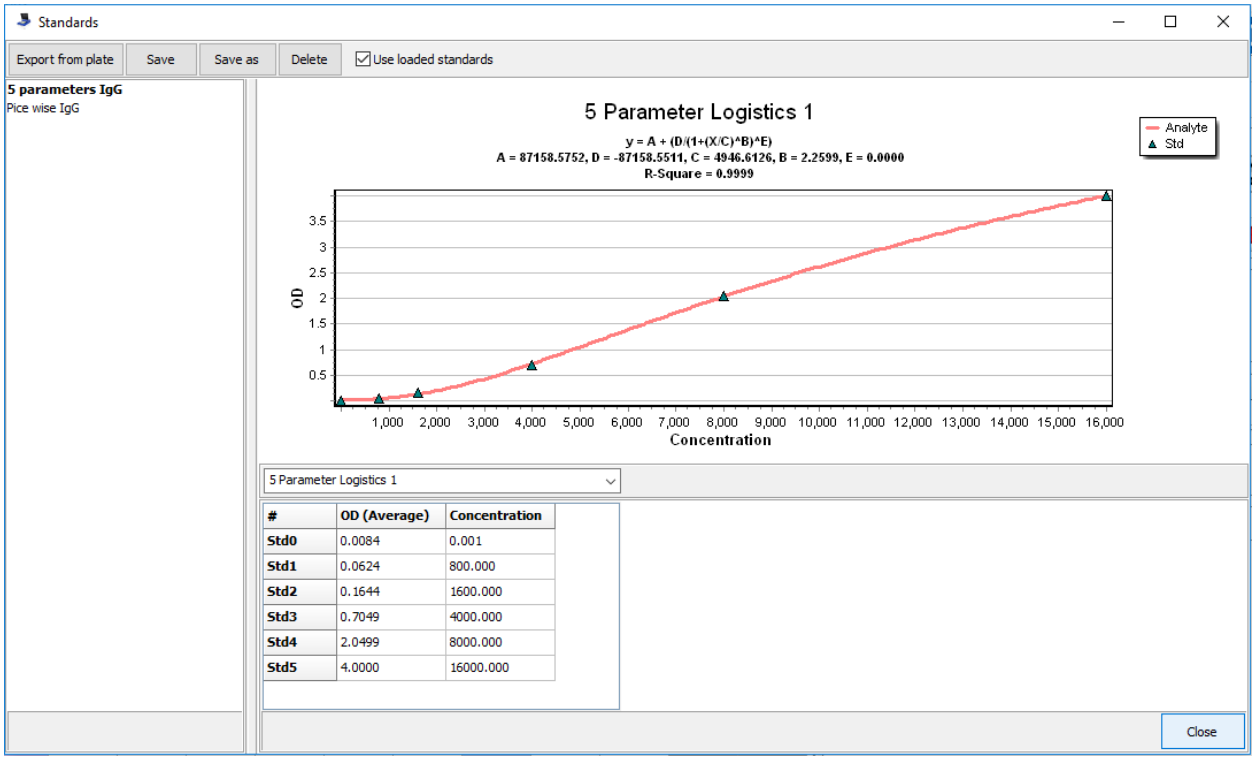
You will get following message:

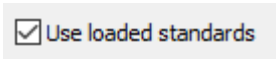


Press ok, go to Input Data tab.

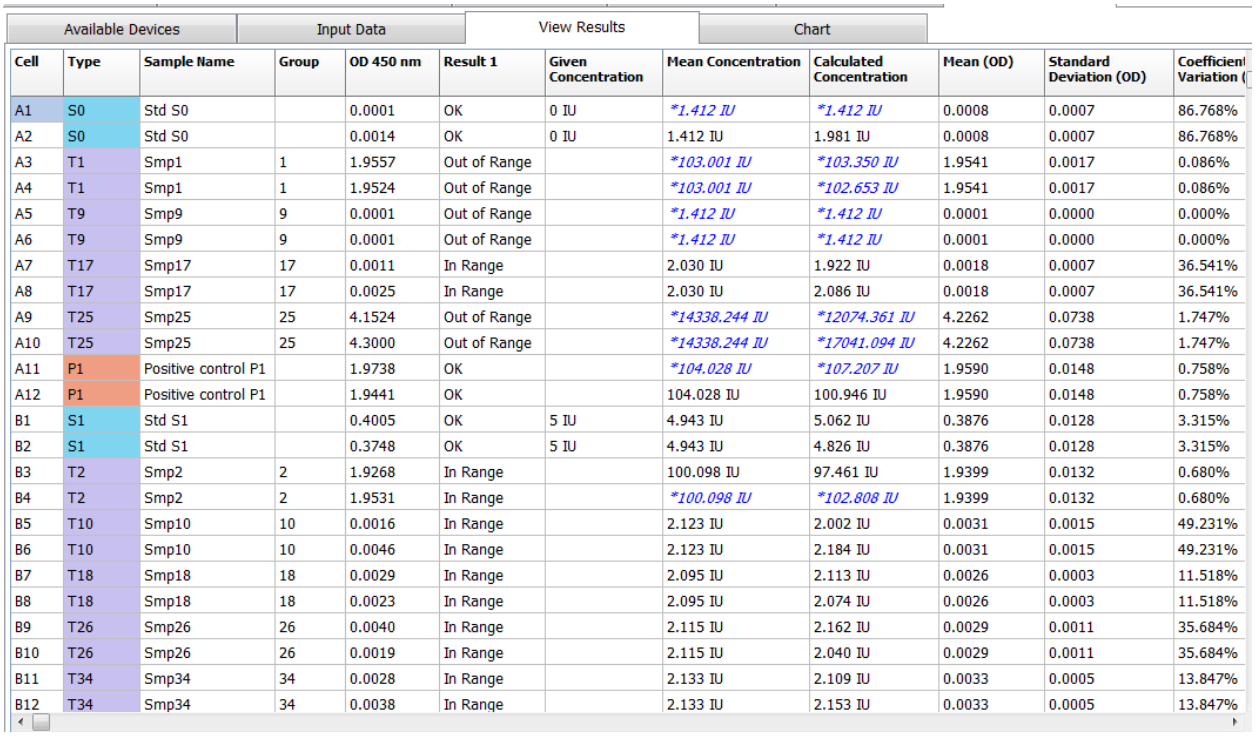
Press on load curve icon:

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 | Input Data | View Results | Chart

Cell	Type	Sample Name	Group	OD 450 nm	Result 1	Given Concentration	Mean Concentration	Calculated Concentration	Mean (OD)	Standard Deviation (OD)	Coefficient Variation
A1	S0	Std S0		0.0001	OK	0 IU	*1.412 IU	*1.412 IU	0.0008	0.0007	86.768%
A2	S0	Std S0		0.0014	OK	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	T9	Smp9	9	0.0001	Out of Range		*1.412 IU	*1.412 IU	0.0001	0.0000	0.000%
A6	T9	Smp9	9	0.0001	Out of Range		*1.412 IU	*1.412 IU	0.0001	0.0000	0.000%
A7	T17	Smp17	17	0.0011	In Range		2.030 IU	1.922 IU	0.0018	0.0007	36.541%
A8	T17	Smp17	17	0.0025	In Range		2.030 IU	2.086 IU	0.0018	0.0007	36.541%
A9	T25	Smp25	25	4.1524	Out of Range		*14338.244 IU	*12074.361 IU	4.2262	0.0738	1.747%
A10	T25	Smp25	25	4.3000	Out of Range		*14338.244 IU	*17041.094 IU	4.2262	0.0738	1.747%
A11	P1	Positive control P1		1.9738	OK		*104.028 IU	*107.207 IU	1.9590	0.0148	0.758%
A12	P1	Positive control P1		1.9441	OK		104.028 IU	100.946 IU	1.9590	0.0148	0.758%
B1	S1	Std S1		0.4005	OK	5 IU	4.943 IU	5.062 IU	0.3876	0.0128	3.315%
B2	S1	Std S1		0.3748	OK	5 IU	4.943 IU	4.826 IU	0.3876	0.0128	3.315%
B3	T2	Smp2	2	1.9268	In Range		100.098 IU	97.461 IU	1.9399	0.0132	0.680%
B4	T2	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 IU	2.113 IU	0.0026	0.0003	11.518%
B8	T18	Smp18	18	0.0023	In Range		2.095 IU	2.074 IU	0.0026	0.0003	11.518%
B9	T26	Smp26	26	0.0040	In Range		2.115 IU	2.162 IU	0.0029	0.0011	35.684%
B10	T26	Smp26	26	0.0019	In Range		2.115 IU	2.040 IU	0.0029	0.0011	35.684%
B11	T34	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 #
Type
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)

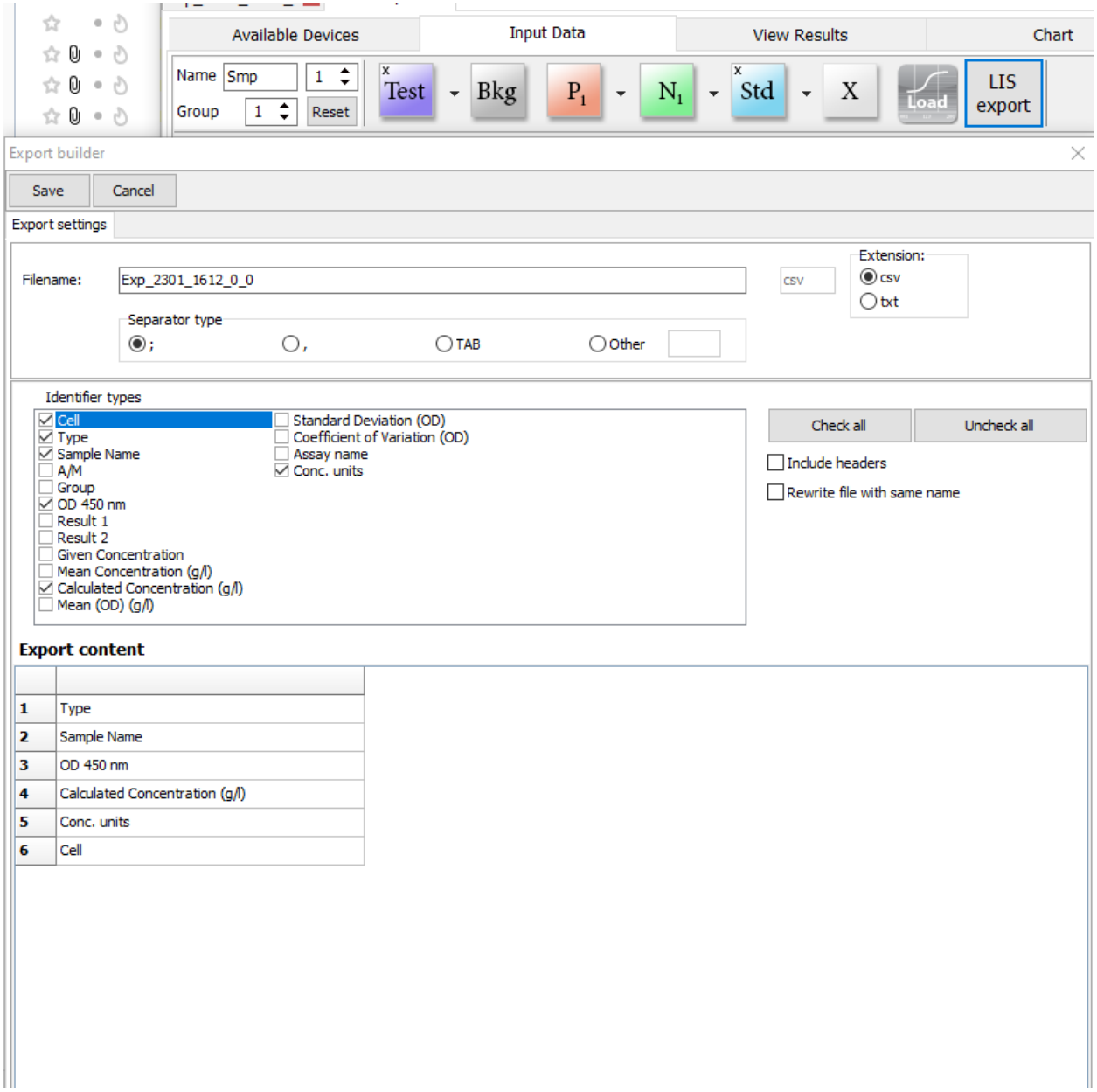
For multiplex, avidity and qualitative assays columns relating to the concentration are not displayed.
For avidity methods column A/M indicates what sample was diluted with a dissociating agent (0 -- not diluted, 1 -- diluted)

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

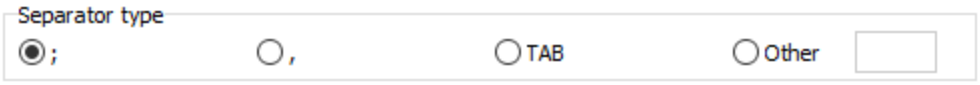
LIS export

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

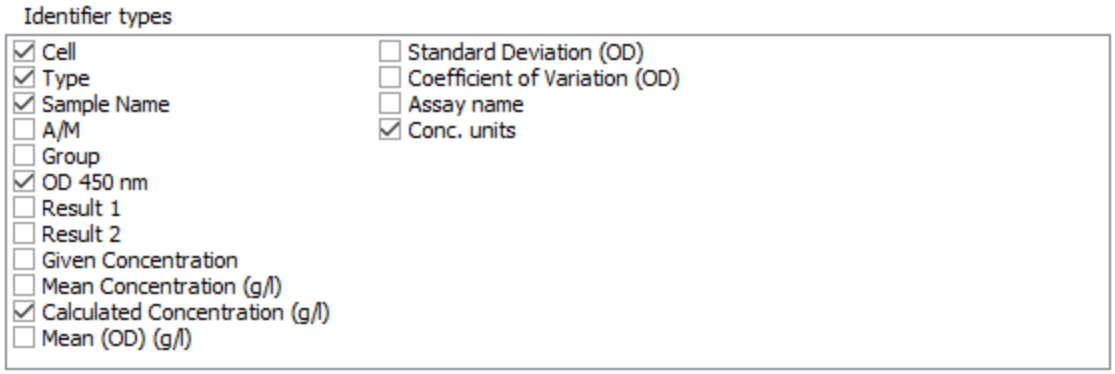


Select the file name extension for your data. You can choose either .csv or .txt format.

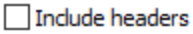
Choose the needed separator type:



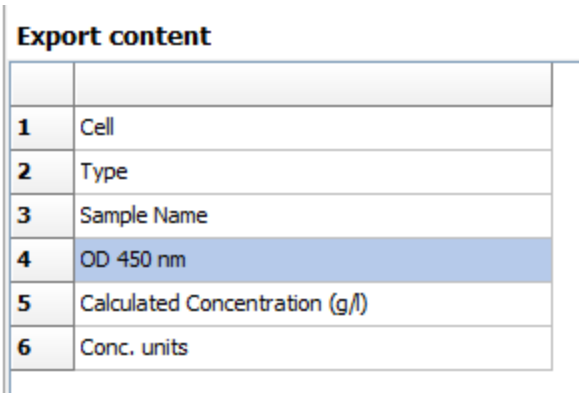
Select the identifiers (headers) you want to export.



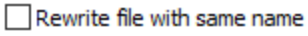
Select if you want to export the header names



Export content panel is visualizing the the exportable headers.



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



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

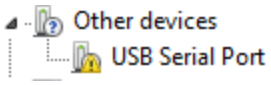
World practice shows that software vendors in the case of software malfunctioning indicate that user have accepted of the license agreement by which the software was provided as is or/and the shortcomings of operating system compatibility with the PC hardware, which leads to errors of or reduced productivity 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 software@biosan.lv,so that we can identify the cause and, possibly, make the program better.

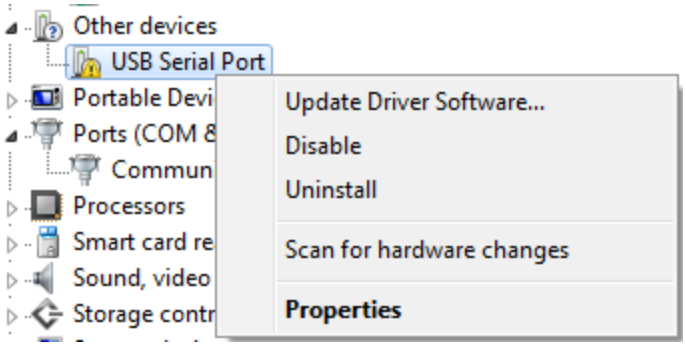
1. - The device can not connect to the computer.
   1.1 Check that the USB cable is firmly connected to the PC and to the instrument, try to eject and inster both ends.
   1.2 Try restarting your devices/software/computer, if it does not help, then reinstall the software.
   1.3 If the problem persists, 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”

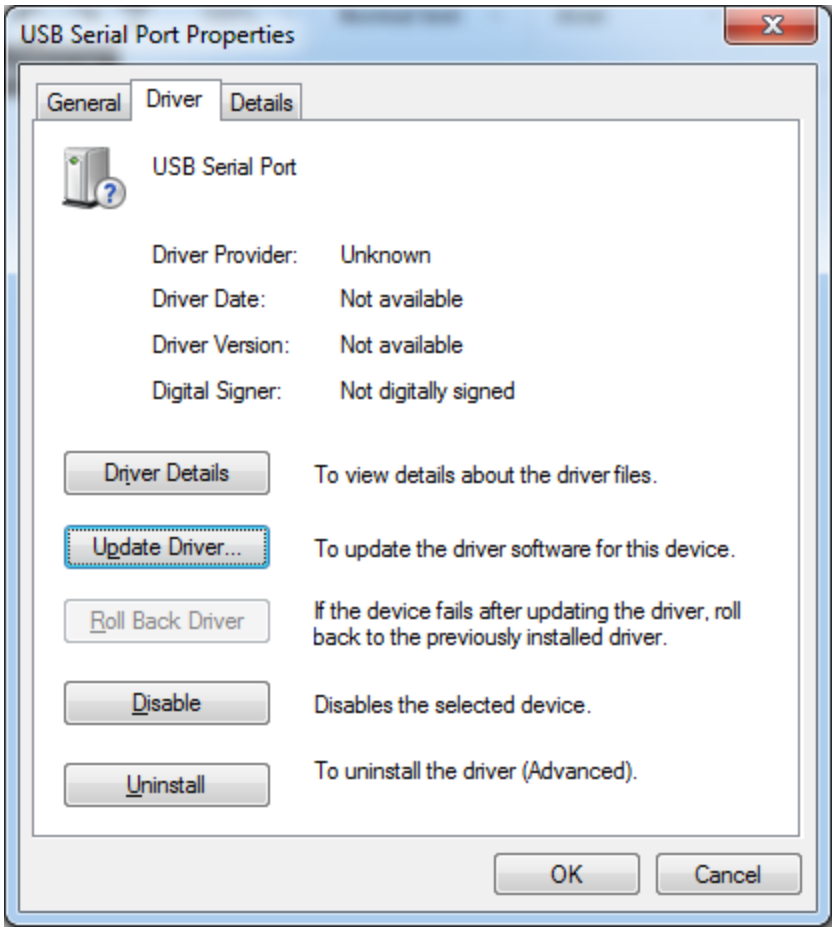
3. - Device does not respond to the program
   - Try to turn on or off the device, if necessary, try to plug it off and then on.

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:

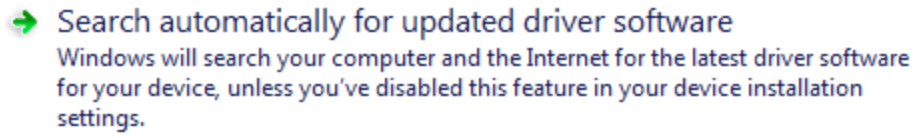




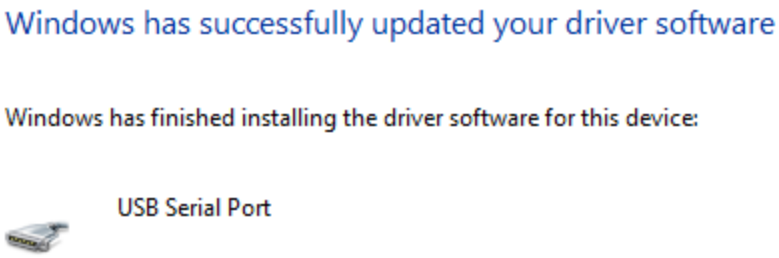
Click on Properties, Select Driver tab, click on the Update Driver:



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



After installing the driver, following message should appear:



If you do not have internet connection, please use computer with internet to download latest drivers from: http://www.ftdichip.com/Drivers/VCP.htm 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: https://support.microsoft.com/en-us/help/2654149/error-usb-device-not-recognized-when-you-try-to-access-a-usb-external-hard-drive.

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.

5. - 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 software@biosan.lv , we will be happy to guide you!