ass**a**yfit pr**o**

curve fitting for
laboratory assays

About assayfit pro

Assayfit Pro from AssayCloud is an online curve fitting service and add-in for laboratory assays and other scientific data. Use fit functions like 4PL, 5PL and regression including Passing-Bablok and Deming regression.

It can be used online and in Excel for Windows and Mac Os, Libreoffice, Openoffice Python, R and others.

Assayfit pro can be used free of charge and without registration for 10-15 times each day. If you require more fits or would like to serve assayfit pro on your website for your customers to use, please visit https://www.assayfit.com/pricing.html for more information.

table of contents

using assayfit pro

use assayfit pro online

universal curve fitting online

AssayFit Pro is a service mainly focused on calculating results from laboratory test, called assays. In these assays the output signals can be compared to signals from samples of known concentration called standards or calibrators. In this way the concentration in unknown samples can be calculated.

The simplest way to use AssayFit Pro is to use it online.

You can visit the following page to start using it without registration

https://www.assayfit.com/company/assayfit/online-curve-fitting-universal.html

Enter assay data on a web page

On this page you can enter values of the known samples (calibrators or standards) with their concentration (x values) and absorbance (y value).
You can enter these manually or copy them from Excel or any other program and give them an ID.

Unknown samples with ID

The start page also contains a table for entering unknown samples. The measured value of the unknown samples can be entered or pasted. The ID of the sample can also be indicated. Samples with the same ID will be grouped and the average calculated in the results report.

You can select an empty area and then click one of the buttons above to fill the ID field with B= Blank, C= Control, S = Sample and CAL= calibrator. Replicate number can also be indicated. In this way dilutions can also be entered with the corresponding buttons.

Subtracting the blank signal or normalize

From the left menu or from the Options pull down menu you have several options under Signals to modify the signals.

Subtract blank will subtract the average of the samples marked with ID "Blank" from all the samples and calibrators.

Subtract CAL-0 will subtract the average signal of the calibrators marked with ID "CAL-0" from all the samples and calibrators.

Normalize will set the average of signals of samples marked with a specified ID to 100% and will then calculate how the signals relate to this sample.

Before blank subtraction

After blank subtraction

Selecting calibrators by their ID

The program can also identify calibrators from the sample list by their sample ID if needed.
If the ID contains "CAL-" with the concentration, the program can identify the sample as a calibrator. By selecting the cells and clicking "Toggle calibrators" the "CAL-" is placed in front of a number. This makes it mare easy to identify calibrators.

Unknown samples

	Dil	Id	Abs
1	1	CAL-500	2.295
2	1	CAL-250	1.741
3	1	CAL-62.5	0.692
4	1	CAL-31.25	0.363
5	1	CAL-7.8	0.104
		CAL-3.9	0.064
		CAL-2	0.042
			0.012
			0.382
			0.293
			0.481
		Sample 1	0.312
		Sample 2	0.191
		Sample 3	0.473
		Sample 1	0.412
		Sample 2	0.314
		Sample 3	0.471
18	1	Blank	0.000

Signals ▸
Sample ID
Dilutions ▸
Calibration curve ▸
New analysis
Clear all
Side menu on/off
Cell shading on/off

Toggle calibrator
Import from CSV
Remove sample IDs

Unknown samples

	Dil	Id	Abs
1	1	500	2.295
2	1	250	1.741
3	1	62.5	0.692
4	1	31.25	0.363
5	1	7.8	0.104
6	1	3.9	0.064
7	1	2	0.042
8	1	0	0.012
9	1	Sample 1	0.382
10	1	Sample 2	0.293
11	1	Sample 3	0.481
12	1	Sample 1	0.312
13	1	Sample 2	0.191
14	1	Sample 3	0.473
15	1	Sample 1	0.412
16	1	Sample 2	0.314
17	1	Sample 3	0.471
18	1	Blank	0.000
19			

An alternative way of generating calibrator values and IDs is by selecting empty cells and then clicking the CAL button. You will be ask to enter the highest calibrator and the dilution factor and the software will generate the dilution series for you.

Cals from sample list   Options ▾

X   B   C   S   CAL   1

1   2   Calibrator serial dilution placement in the selected cells

Unknown samples

	Dil	Id	Abs
1	1		2.295
2	1		1.741
3	1		0.692
4	1		0.363
5	1		0.104
6	1		0.064
7	1		0.042
8	1		0.012
9	1	Sample 1	0.382
10	1	Sample 2	0.293
11	1	Sample 3	0.481
12	1	Sample 1	0.312
13	1	Sample 2	0.191
14	1	Sample 3	0.473
15	1	Sample 1	0.412
16	1	Sample 2	0.314
17	1	Sample 3	0.471
18	1	Blank	0.000

When clicking the button "Cals from sample list" it will copy these values to the calibrator table.

setting dilutions

You can set the sample dilution in the column marked Dil. This can be performed manually or by a copy paste action, but you can also assing the dilutions from the menu.
There is a purple pull down menu on the left side of the page. Alternatively the top menu or the button marked options shows a similar menu.
By selecting dilutions from the list and then one of the options, the selected dilutions will be entered for all of the samples in the list.
Alternatively you can select the positions of the dilutions to be changed and then click one of the dilution buttons above the table. Then only the selected cells will be filled with the selected dilution factor.

assigning weights

Weights is a good way to set importance to the data points in the calibration curve. If you want the low dose calibrators to fit more accurately, then you can select the option from the calibration curve menu " use weights". It will then copy the $1/signal^2$ value to the weight field. This will give samples with a lower value more importance to the fit. Standard the weight is set at 1, meaning that all data points have an equal weight.
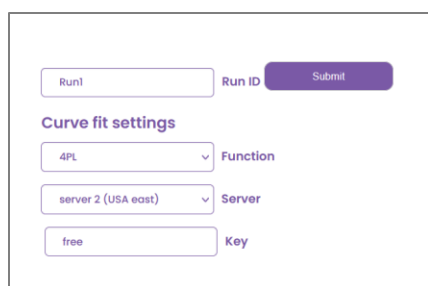
fit function and server

After entering the data you can select the fit function to be used, in this case 4PL is selected. This function will be fitted to the x and y values of concentration and signal of the calibrators.
A server can be selected. Preferably choose a server close to your location for the highest speed.
When you then click the submit button, the program will fit a curve to the data points just entered.

The unknown sample concentration will also be calculated using the fitted curve of the calibrators.

Run1	Run ID	Submit

Curve fit settings

4PL	Function
server 2 (USA east)	Server
free	Key

The fit results

After curve fitting, a graph of the data points with the fitted curve is displayed and the fit function with the calculated values of the parameters are indicated.

Fit Summary

Parameter	Value	Info	
A	3.25289506952	Function	(D + ((A - D) / (1 + ((x / C) ^ B)))) |4 parameter logistic
B	-1.07745717588	Run ID	Run1
C	215.375310183(	User	****
D	0.05742689035	Time	9/13/2023 10:00:29 AM
E			
SSq	0.00020559020	R2	0.999961902666

Calibration curve

Id	weight	Conc	Abs mean
CAL-500	1.0	500.000	2.335
CAL-250	1.0	250.000	1.781
CAL-62.5	1.0	62.500	0.732
CAL-31.25	1.0	31.250	0.403
CAL-7.8	1.0	7.800	0.145
CAL-3.9	1.0	3.900	0.104
CAL-2	1.0	2.000	0.082
CAL-0	1.0	0.000	0.053

Individual results

Pos	Id	Abs	Conc	Dil	Conc x dil
1	CAL-500	2.335	500.6	1.0	500.6
2	CAL-250	1.781	249.4	1.0	249.4
3	CAL-62.5	0.732	63.3	1.0	63.3
4	CAL-31.25	0.403	30.4	1.0	30.4
5	CAL-7.8	0.145	7.8	1.0	7.8
6	CAL-3.9	0.104	4.3	1.0	4.3
7	CAL-2	0.082	2.3	1.0	2.3
8	CAL-0	0.053	0.0	1.0	0.0
9	Sample 1	0.420	32.0	1.0	32.0
10	Sample 2	0.330	23.8	1.0	23.8
11	Sample 3	0.520	41.4	1.0	41.4
12	Sample 1	0.350	25.6	1.0	25.6
13	Sample 2	0.230	15.1	1.0	15.1
14	Sample 3	0.510	40.5	1.0	40.5
15	Sample 1	0.450	34.7	1.0	34.7
16	Sample 2	0.350	25.6	1.0	25.6
17	Sample 3	0.510	40.5	1.0	40.5

The samples will also be grouped and the mean value of the replicates will be calculated as well as the coefficient of variation (%CV).

Results

ID	Abs 1	Abs 2	Abs 3	Abs mean	Abs CV	N	ID	Conc 1	Conc 2	Conc 3	Conc mean	Dilution	Conc x dilu	Conc CV	Position
CAL-0	0.053			0.053		1	CAL-0	0.0			0.0	1.0	0.0		8
CAL-2	0.082			0.082		1	CAL-2	2.3			2.3	1.0	2.3		7
CAL-3.9	0.104			0.104		1	CAL-3.9	4.3			4.3	1.0	4.3		6
CAL-7.8	0.145			0.145		1	CAL-7.8	7.8			7.8	1.0	7.8		5
CAL-31.25	0.403			0.403		1	CAL-31.25	30.4			30.4	1.0	30.4		4
CAL-62.5	0.732			0.732		1	CAL-62.5	63.3			63.3	1.0	63.3		3
CAL-250	1.781			1.781		1	CAL-250	249.4			249.4	1.0	249.4		2
CAL-500	2.335			2.335		1	CAL-500	500.6			500.6	1.0	500.6		1
Sample 1	0.420	0.350	0.450	0.407	12.6	3	Sample 1	32.0	25.6	34.7	30.8	1.0	30.8	15.2	9,12,15
Sample 2	0.330	0.230	0.350	0.303	21.2	3	Sample 2	23.8	15.1	25.6	21.5	1.0	21.5	26.1	10,13,16
Sample 3	0.520	0.510	0.510	0.513	1.1	3	Sample 3	41.4	40.5	40.5	40.8	1.0	40.8	1.4	11,14,17

By clicking the buttons below the graph the axis can be displayed as linear or logarithmic
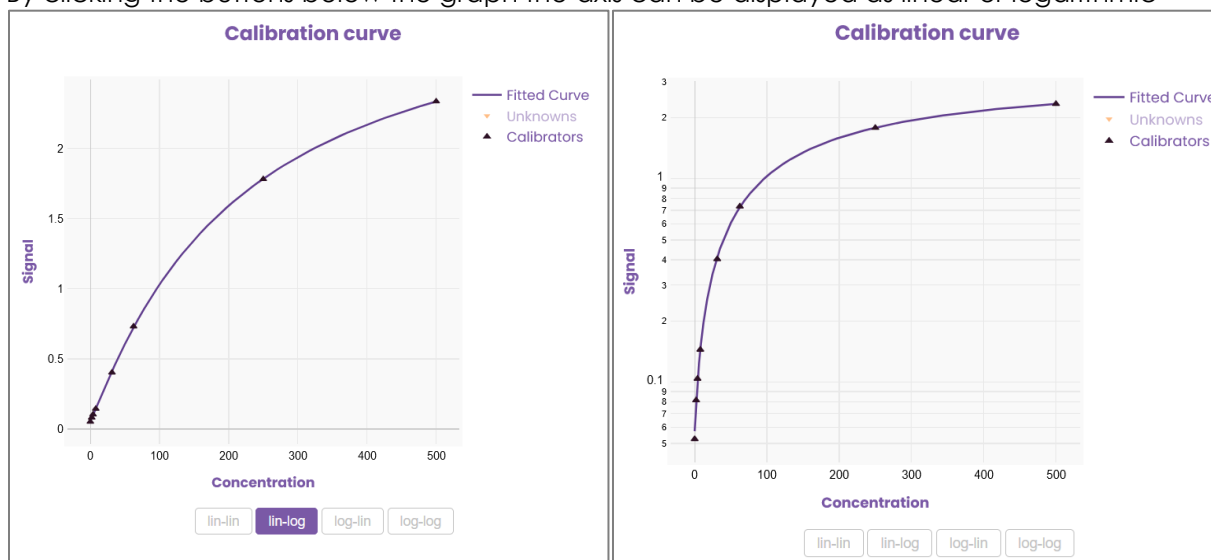
By right clicking in the graph and selecting a region, this region can be enlarged for better viewing of the calibrators. Double clicking in the graph resets the view again.
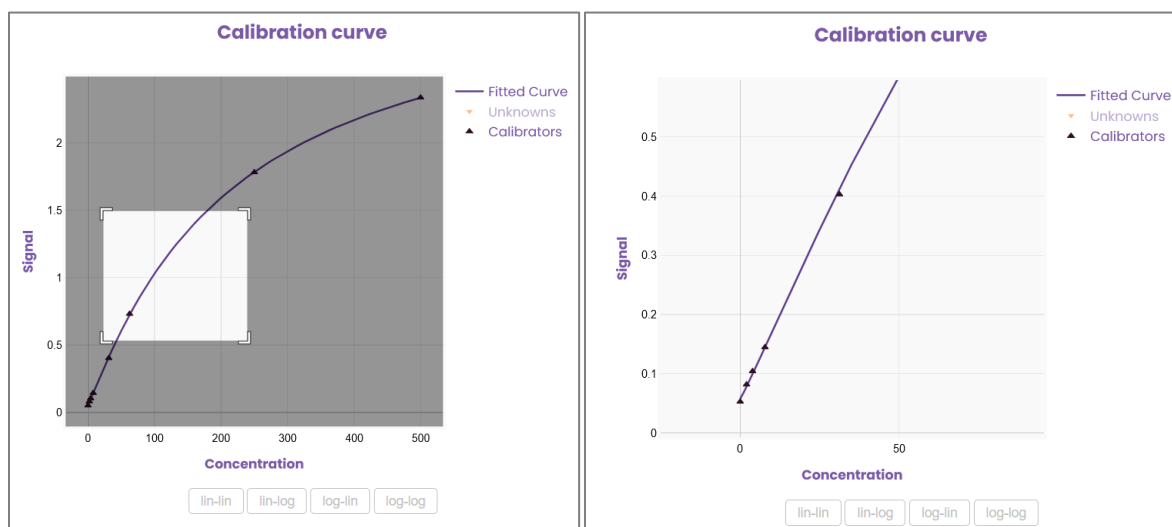
Saving or printing data

Next to the calibration curve table the button export shows a list with options to save the data. You can select "save" to save the data in html format. You can also choose, for CSV or PDF output or choose to print.

Calibration curve

Id	weight	Conc	Abs mean
CAL-500	0.2	500.000	2.335
CAL-250	0.3	250.000	1.781
CAL-62.5	1.9	62.500	0.732
CAL-31.25	6.2	31.250	0.403
CAL-7.8	47.9	7.800	0.145
CAL-3.9	92.5	3.900	0.104
CAL-2	150.6	2.000	0.082
CAL-0	362.8	0.000	0.053

Export ▾

Save

Download CSV

Print

PDF

Expand all hidden data

Online curve fitting for ELISA

Similar to the curve fitting online page above you can use curve fitting for your results in 96 well (ELISA) format.

This page Is located at:
https://www.assayfit.com/company/assayfit/online-curve-fitting-elisa.html

The page shows a 96 well table and results can be copied in this table.
The cell color changes based on its value. This can be switched off by clicking cells shading off from the menu.

assayfit pro

Options▾ home download fit online ▾ info ▾ pricing

online ELISA assay results calculator

Perform online curve fitting and calculation of your ELISA assay data using this page using AssayFit Pro version 5.3. You can use this service for free 15 times each day. read the terms for use and distribution.
Alternatively use the Microsoft Excel add-in to perform the calculation. Previous version 5.2 Check the menu for other online options and previous versions.
For Research Use Only. Not for use in diagnostic procedures.

Paste ELISA results here

Enter your ELISA data in the spreadsheet like table below, you can copy and paste from Excel. Optional: you can subtract blank values or normalize, select this from the options menu.

	1	2	3	4	5	6	7	8	9	10	11	12
A	2.318	2.356	0.063	0.053	0.059	0.053						
B	1.767	1.781	0.068	0.057	0.055	0.055						
C	0.722	0.741	0.093	0.069	0.059	0.071						
D	0.402	0.404	0.086	0.07	0.058	0.059						
E	0.142	0.147	0.173	0.116	0.06	0.077						
F	0.098	0.11	0.382	0.347	0.059	0.088						
G	0.084	0.079	0.067	0.061	0.058	0.056						
H	0.052	0.053	0.188	0.199	0.076	0.134						

Clear signal input   Options ▾

Sample IDs in ELISA format

The position of calibrators, samples and blanks can be indicated as described in the sections above. X deletes the entries, B=blank, C=control, S=sample and CAL= calibrator. The cells can be selected and subsequently one of these buttons.
The S> and Rv will indicate the sample fill direction (in this case to the right) and the replicate fill direction(in this case top to bottom). The number of replicates can be indicated by clicking the button next to the Rv button.

ELISA layout and sample IDs

Select a region and click the B, C, S, CAL buttons to fill the region with blank, control, sample or calibrator values. You can use the S>, Rv buttons to specify the direction of the filling process and the number of replicates. Sample IDs are kept on your local computer and are not sent over the internet.

	1	2	3	4	5	6	7	8	9	10
A	CAL-500	CAL-500	Blank	Control	Sample 8	Sample 8				
B	CAL-250	CAL-250	Sample 1	Sample 1	Sample 9	Sample 9				
C	CAL-62.5	CAL-62.5	Sample 2	Sample 2	Sample 10	Sample 10				
D	CAL-31.3	CAL-31.3	Sample 3	Sample 3	Sample 11	Sample 11				
E	CAL-7.8	CAL-7.8	Sample 4	Sample 4	Sample 12	Sample 12				
F	CAL-3.9	CAL-3.9	Sample 5	Sample 5	Sample 13	Sample 13				
G	CAL-2.0	CAL-2.0	Sample 6	Sample 6	Sample 14	Sample 14				
H	CAL-0	CAL-0	Sample 7	Sample 7	Sample 15	Sample 15				

| Toggle calibrators | Import IDs from CSV | X | B | C | S | CAL | S> | Rv | 1 |

setting dilutions in ELISA format

The dilutions are in a separate table also in 96 well format. Data can be pasted in this table or entered manually. Alternatively one of the buttons can be used which will enter the dilution in the selected cells after clicking.

Sample dilution

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	1	1	1	1	1	1	1	1	1	1	1
B	1	1	1	1	1	1	1	1	1	1	1	1
C	1	1	1	1	1	1	1	1	1	1	1	1
D	1	1	1	1	1	1	1	1	1	1	1	1
E	1	1	1	1	1	1	1	1	1	1	1	1
F	1	1	1	1	1	1	1	1	1	1	1	1
G	1	1	1	1	1	1	1	1	1	1	1	1
H	1	1	1	1	1	1	1	1	1	1	1	1

| Cals from layout | Options ▾ | 1 | 2 | 5 | 10 | 100 |
| | | 1000 | | | | |

Entering calibrator values

The values for the calibrators can be easily obtained from the above tables if the position of the calibrators is indicated with the use of CAL- before their concentration in the sample ID table. Then clicking the "Cals from layout" button will copy all the values from the calibrators into the curve fit input table.

In this table also the weight can be indicated. The weight can be changed from the menu as described above. Also alternative values for the weight can be typed into the table.

It is not required to obtain the values from the above tables. In practice any calibrator can be entered in this table.

| Cals from layout | | Options ▾ | | 1 | 2 | 5 | 10 | 100 |
| 1000 |

Curve fit input values

You can copy the values from Excel, enter them manually or use the 'Cals from layout' button to copy all calibrator values marked with CAL- from the layout.

	Id	weight	Conc	Abs 1	Abs 2	Abs 3	Abs 4	Abs 5	Abs 6
1	CAL-500	1.0	500.000	2.337					
2	CAL-250	1.0	250.000	1.781					
3	CAL-62.5	1.0	62.500	0.732					
4	CAL-31.25	1.0	31.250	0.403					
5	CAL-7.8	1.0	7.800	0.145					
6	CAL-3.9	1.0	3.900	0.104					
7	CAL-2	1.0	2.000	0.082					
8	CAL-0	1.0	0.000	0.053					
9									

After the calibrators have been entered select a fit function from the Curve fit settings section. In these boxes also a server and a key (you can obtain one from AssayCloud) can be entered. Hitting the submit button will perform the actual curve fitting and the results will be presented.

Curve fit settings

4PL ⌄		1	**Run ID**
Function		**Submit**	
server 2 (USA east) ⌄	**Server**		
free	**Key**		

Fit results in ELISA format

The fit results are in ELISA format too with a summary table.

Dilution

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	1	1	1	1	1	1	1	1	1	1	1
B	1	1	1	1	1	1	1	1	1	1	1	1
C	1	1	1	1	1	1	1	1	1	1	1	1
D	1	1	1	1	1	1	1	1	1	1	1	1
E	1	1	1	1	1	1	1	1	1	1	1	1
F	1	1	1	1	1	1	1	1	1	1	1	1
G	1	1	1	1	1	1	1	1	1	1	1	1
H	1	1	1	1	1	1	1	1	1	1	1	1

Conc x Dilution

	1	2	3	4	5	6	7	8	9	10	11	12
A	487.5	514.3	0.6	0.0	0.2	0.0						
B	245.3	249.3	1.1	0.0	0.0	0.0						
C	62.3	64.4	3.3	1.2	0.2	1.4						
D	30.3	30.5	2.7	1.3	0.1	0.2						
E	7.6	8.0	10.2	5.3	0.3	1.9						
F	3.8	4.8	28.5	25.3	0.2	2.9						
G	2.5	2.1	1.0	0.4	0.1	0.0						
H	0.0	0.0	11.5	12.4	1.8	6.9						

Also a fit overview is created with the samples, the replicate results and their calculated concentrations with average and CV%. The position of the replicates in the plate is also indicated.

Results

ID	Abs 1	Abs 2	Abs 3	Abs mean	Abs CV	N	ID	Conc 1	Conc 2	Conc 3	Conc mean	Dilution	Conc x dilu	Conc CV	Position
CAL-0	0.052	0.053		0.053	1.3	2	CAL-0	0.0	0.0		0.0	1.0	0.0		H1,H2
CAL-2.0	0.084	0.079		0.082	4.3	2	CAL-2.0	2.5	2.1		2.3	1.0	2.3	13.7	G1,G2
CAL-3.9	0.098	0.110		0.104	8.2	2	CAL-3.9	3.8	4.8		4.3	1.0	4.3	17.1	F1,F2
CAL-7.8	0.142	0.147		0.145	2.4	2	CAL-7.8	7.6	8.0		7.8	1.0	7.8	3.9	E1,E2
CAL-31.3	0.402	0.404		0.403	0.4	2	CAL-31.3	30.3	30.5		30.4	1.0	30.4	0.4	D1,D2
CAL-62.5	0.722	0.741		0.732	1.8	2	CAL-62.5	62.3	64.4		63.3	1.0	63.3	2.3	C1,C2
CAL-250	1.767	1.781		1.774	0.6	2	CAL-250	245.3	249.3		247.3	1.0	247.3	1.2	B1,B2
CAL-500	2.318	2.356		2.337	1.1	2	CAL-500	487.5	514.3		500.9	1.0	500.9	3.8	A1,A2
Sample 1	0.068	0.057		0.063	12.4	2	Sample 1	1.1	0.0		0.5	1.0	0.5	141.4	B3,B4
Sample 2	0.093	0.069		0.081	21.0	2	Sample 2	3.3	1.2		2.3	1.0	2.3	67.8	C3,C4
Sample 3	0.086	0.070		0.078	14.5	2	Sample 3	2.7	1.3		2.0	1.0	2.0	51.4	D3,D4

Saving and printing results

Results can be printed or exported in various ways by clicking the export button.

assayfit pro use in Microsoft excel

index

AssayFit pro curve fitting can also be used in Microsoft Excel on Mac Os and Windows.

In this section we will explain

1. Installing the Excel Add-in
2. How to obtain a free key
3. How to enter data in the Excel or Libreoffice sheet for Assayfit Pro
4. How to perform a curve fit and view the results
5. How to calculate an unknown value from the curve fit
6. How to view the results in excel

the microsoft excel add-in

The use of Assayfit Pro in Microsoft Excel for Mac OS and Windows is easiest using the Add-in. For Libreoffice and openoffice calc templates are available.

The Excel add-in can be downloaded from:

https://www.assayfit.com/content/examples/assayfitproaddin.zip

To install the add-in follow the following procedure:

After downloading you can copy the add-in somewhere on your hard drive or network. Use a location which is writeable. As an example we made a folder C:\add-in and copied the AssayFitProAddin.xlam to this location.
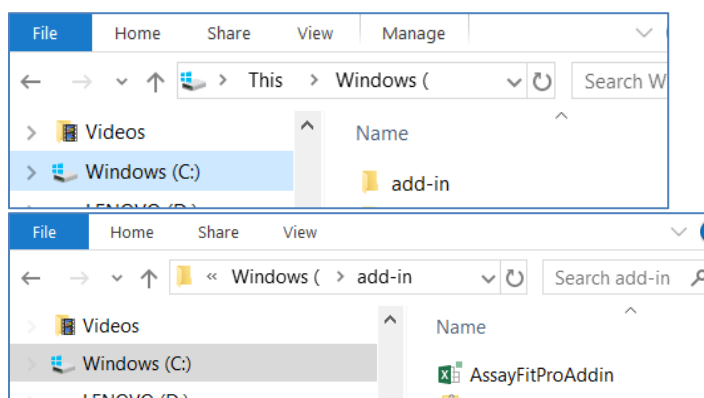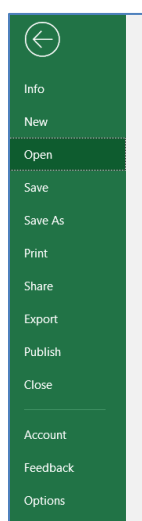

You may need to set the security settings for Excel for this folder to trust the folder.
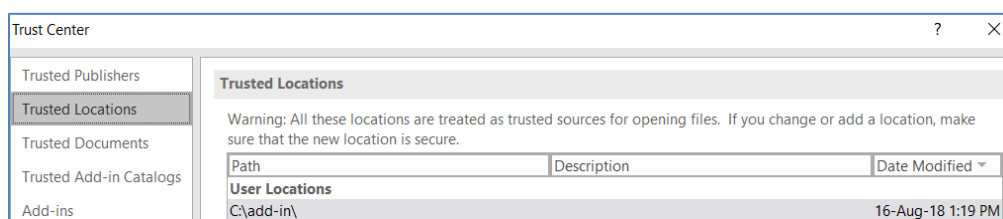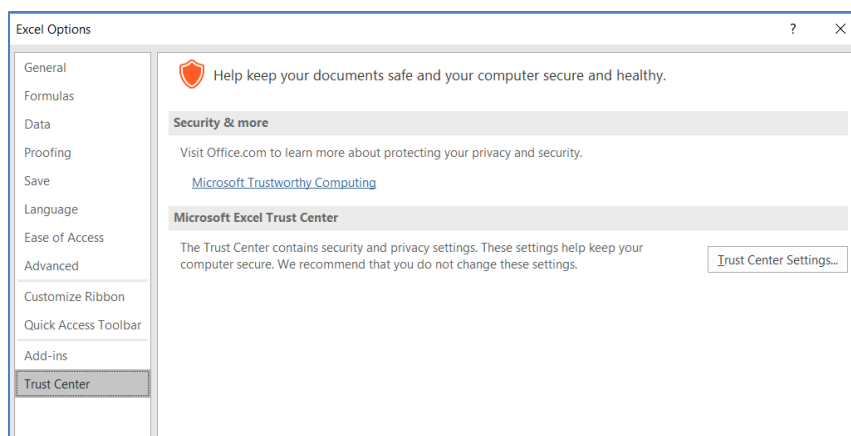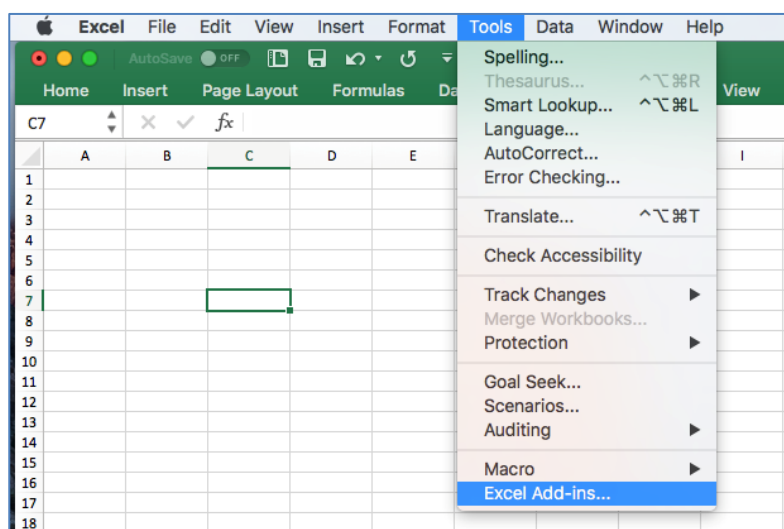
To install, open excel and select options from the menu on Windows. On Mac OS select Excel add-ins from the Tools menu.

You can also check this link https://support.office.com/en-us/article/Add-or-remove-add-ins-in-Excel-0af570c4-5cf3-4fa9-9b88-403625a0b460#OfficeVersion=Windows

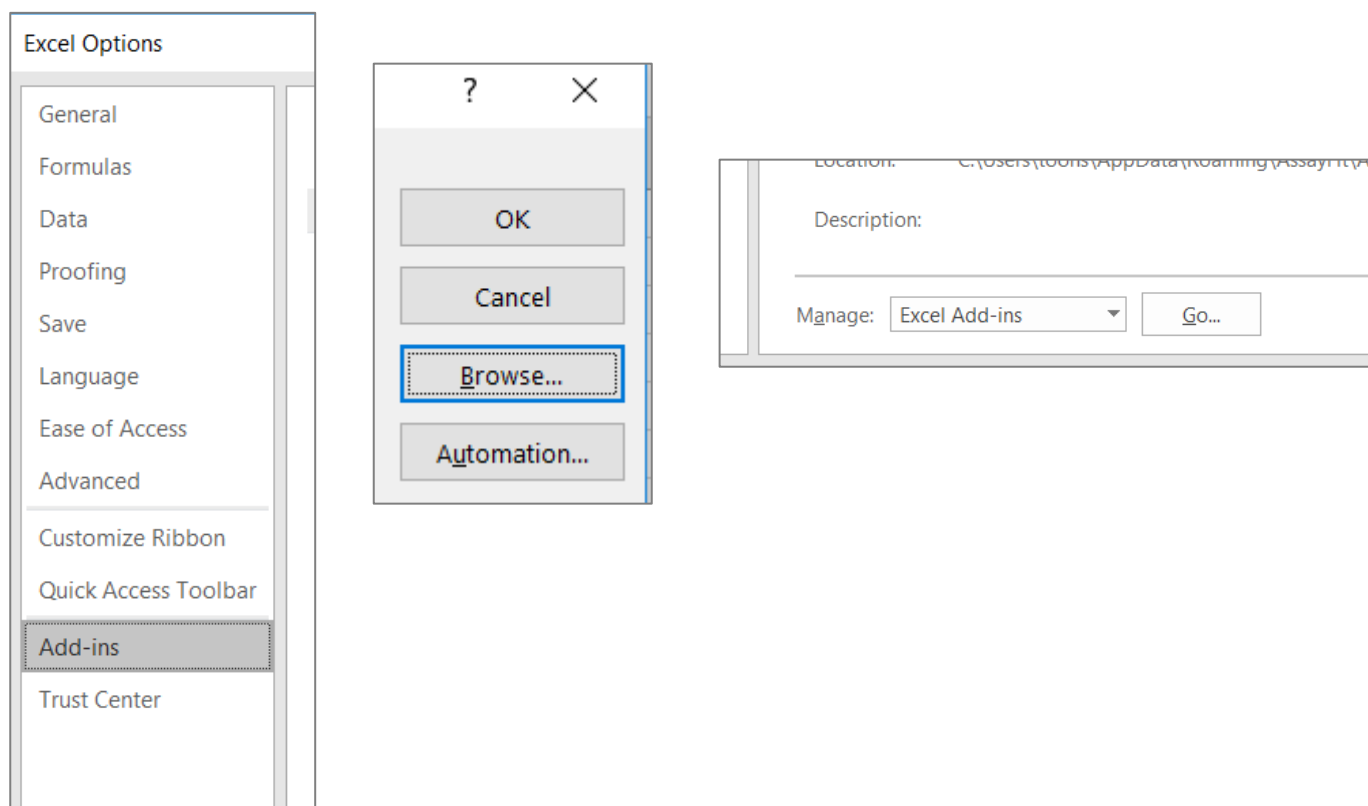Windows   Mac Os
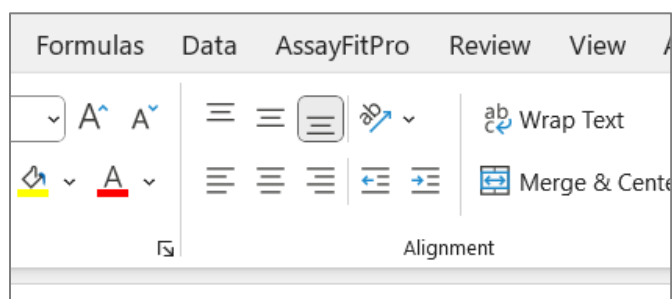
In Windows follow the following menus

Click Add-ins

Press the go button in the bottom of the windows

Then click browse and select the add-in.

Once it is installed a Menu named AssayFitPro appears.

If you click the menu all Assayfit Pro buttons are visible.
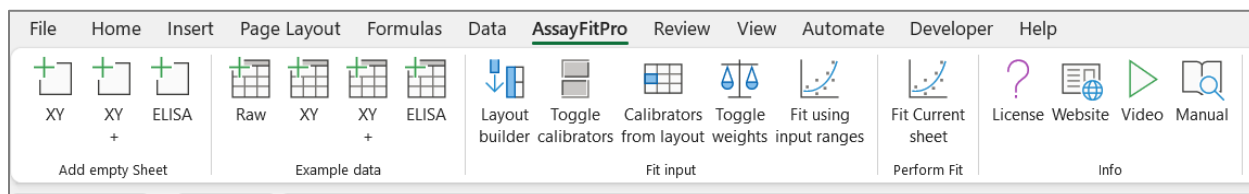
By clicking the right ELISA button a curve fitting sheet will appear with experimental data.
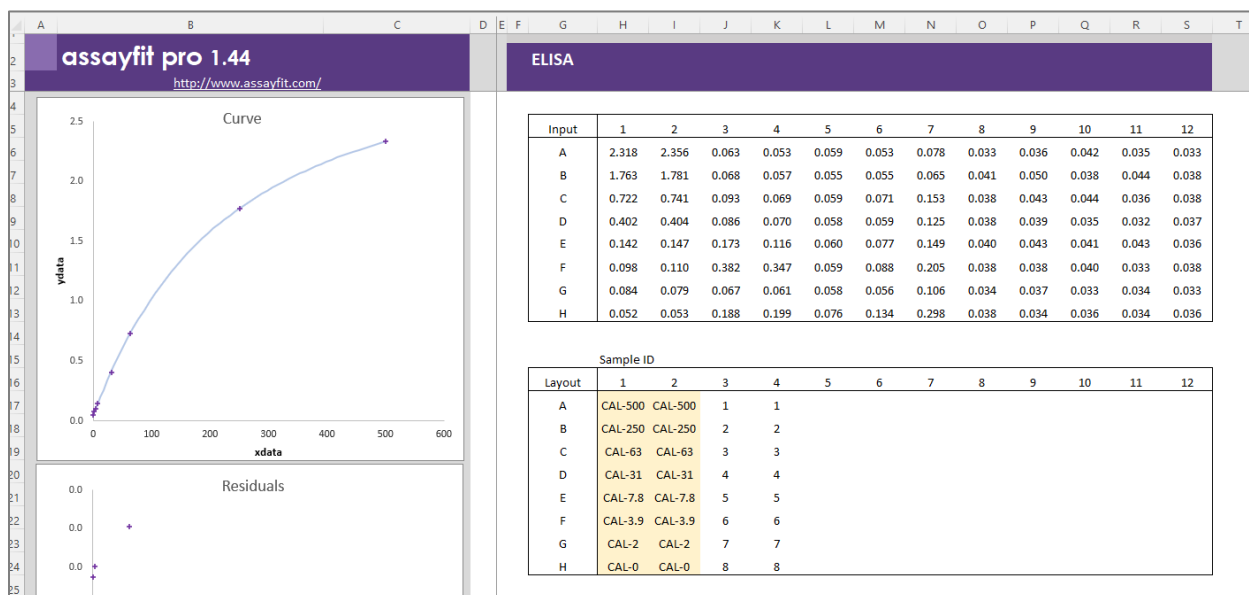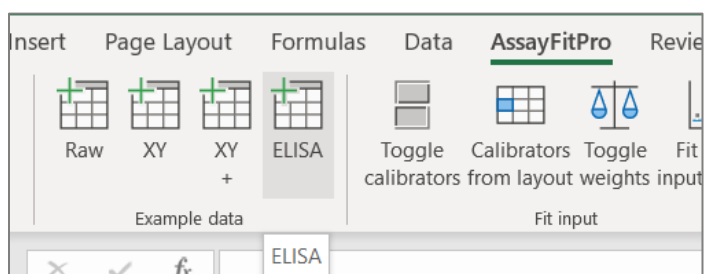
In the top table called input you can paste your data in 96 well format.

In the second table you can identify where in the 96 well plate calibrators, controls, blanks and samples are located.

This can be performed by typing or copying the identifies in the table or by using the plate layout builder

Using the plate layout builder to assign sample IDs

Assigning calibrator, sample control and blank positions

Assayfit pro version 1.3 and higher has an option to create a plate layout to identify sample positions.

In the paragraph above, an ELISA sample curve fitting sheet is shown. Some identifiers contain the suffix CAL-. This indicates the position in the plate is a calibrator. The concentration of the calibrator is indicated after CAL-. CAL-500 means a calibrator with concentration 500. The layout may contain more than one CAL-500 and the program will calculate the average of the corresponding input values and copy this average to the input section.

The CAL-500 replicates may be anywhere on the plate not necessarily next to each other. You can also add other sample ID information in the layout field for your information. The sample ID information will not be sent over the internet.

Calibrator values can be entered manually or using copy-paste, or you can use the layout builder to enter these. By clicking the layout builder from the assayfit pro menu a form will appear with buttons.

By clicking the Sample -> and Replicate -> the fill direction in the layout of the sample numbers and replicates can be assigned. Also the number of replicates can be defined.

Then select the region in the sample ID table you wish to assign as a calibrator.
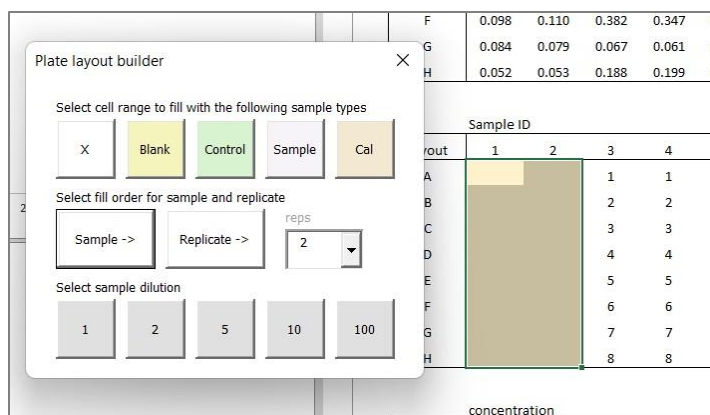
If you then click on the Cal button of the Layout builder. The software will ask you for the highest concentration of the calibrator and the dilution factor. Suppose we would type in 500 and 2 respectively then the software will fill the region starting with replicates of the highest calibrator (500) and then replicates of a 2x diluted calibrator = CAL-250 and continue for each dilution until the selected region is filled.
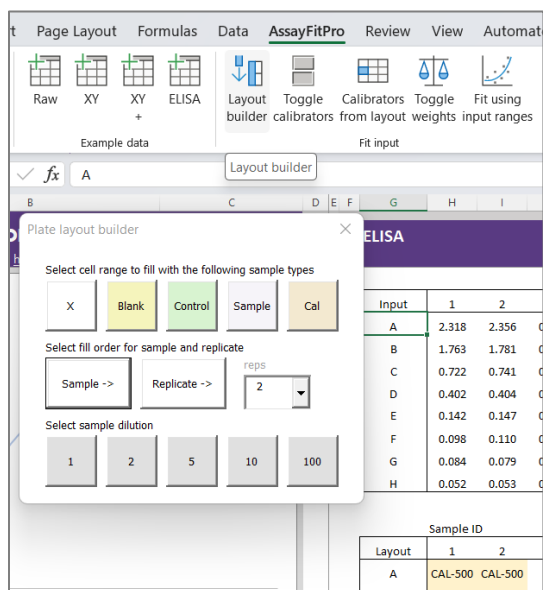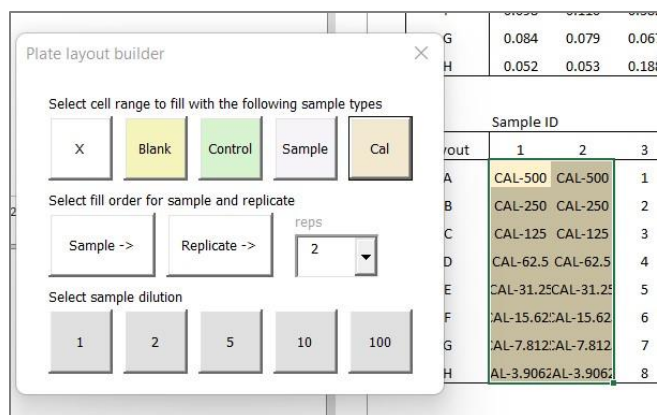
You can use the layout builder also to add Blank, control and sample IDs into the table to identify their location.

The "CAL-" identifier before the value can be removed by clicking the toggle calibrators button. When a cell contains a number representing the calibrator concentration, a calibrator can be assigned by selecting this cell or cells and clicking the toggle calibrators button. It will then place "CAL-" before the value.

Dilution factors

Usually samples are diluted when they are measured. The dilution can be indicated in the dilution table.

Dilutions can be copied or typed into this table or alternatively the layout builder can be used to assign a dilution factor to certain cells.

Copy the values of the standards to the calibration input table

By clicking on the Calibrators from layout button the concentration of the calibrators and the average calibrator values from the corresponding input field will be copied to the input field. The input field contains values needed to perform curve fitting and calculate unknown values.

The input columns can be found from column "AL" in the worksheet.

Assigning weights to the calibrators

If you like to use weights in the curve fitting, you can apply weights by hitting the weights button. This button will copy weight formulas to the weight column. In this case it will use $1/(signal)^2$ By clicking this button again, the weight formulas will disappear again.
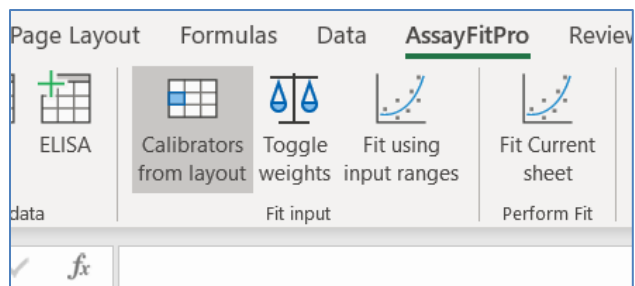
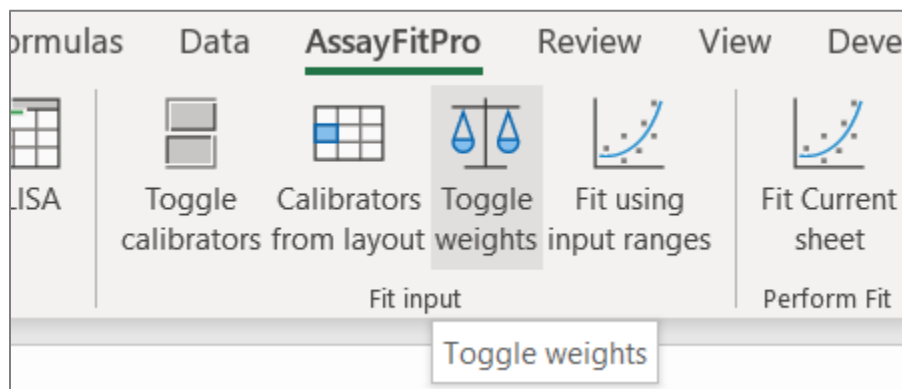| Input							Output	
xdata	ydata	weights	xlimits	start	xknown	yknown	xdata	
500	2.337	0.183				2.3	500	
250	1.772	0.318				1.8	250	
63	0.7315	1.869				0.7	63	
31	0.403	6.157				0.4	31	
7.8	0.1445	47.892				0.1	7.8	
3.9	0.104	92.456				0.1	3.9	
2	0.0815	150.551				0.1	2	
0	0.0525	362.812				0.1	1.00E-06	
						2.4		
						1.8		

Alternative weight calculation can be applied by entering any formula or number in the weights column before curve fitting.

You can now perform curve fitting in the template or online by hitting the Fit Current Sheet button.
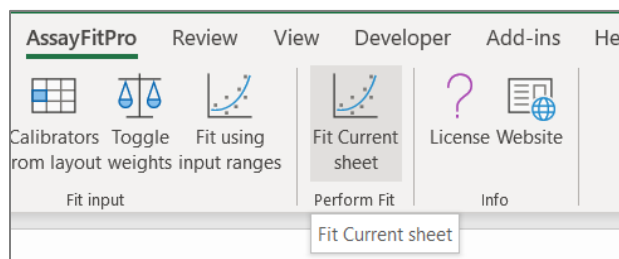
A form will appear in which you can enter several fit settings.

The fit function is the function to which the calibrator data points will be fitted. A good start is a 4PL fit.

The email field is only for information, but you can leave this blank or type in any name you like. The key in this example is entered as "free" and this this will enable you to perform 10-15 free curve fits per day. If you would like to use this service more frequently then you can register for a free or paid key at www.assaycloud.com.

In the "Fit current sheet" form, can also select a server close to you for improved speed. There are also offline options. You can get more information about offline fitting at www.assayfit.com or contact info@assaycloud.com.

When hitting the perform fit button the values in the input field will be used to fit a calibration curve. This calibration curve will be used to backfit all the values in the 96 well plate. The calculated concentration will be shown in a table. Also a concentration x dilution table will be shown.

Output	concentration 1	2	3	4	5	6	7	8	9	10	11	12
A	474.0	496.2	0.8	0.0	0.4	0.0	2.0	0.0	0.0	0.0	0.0	0.0
B	252.8	257.9	1.2	0.3	0.1	0.1	0.9	0.0	0.0	0.0	0.0	0.0
C	64.0	66.3	3.2	1.2	0.4	1.4	8.1	0.0	0.0	0.0	0.0	0.0
D	30.2	30.4	2.6	1.3	0.4	0.4	5.8	0.0	0.0	0.0	0.0	0.0
E	7.1	7.6	9.7	5.0	0.5	1.9	7.7	0.0	0.0	0.0	0.0	0.0
F	3.6	4.5	28.3	25.0	0.4	2.8	12.4	0.0	0.0	0.0	0.0	0.0
G	2.4	2.0	1.1	0.6	0.4	0.2	4.2	0.0	0.0	0.0	0.0	0.0
H	0.0	0.0	11.0	11.9	1.8	6.5	20.6	0.0	0.0	0.0	0.0	0.0

Dilution	dilution factor 1	2	3	4	5	6	7	8	9	10	11	12
A	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
B	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
C	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
D	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
E	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
F	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
G	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
H	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

Conc x dil	concentration x dilution factor 1	2	3	4	5	6	7	8	9	10	11	12
A	474.0	496.2	0.8	0.0	0.4	0.0	2.0	0.0	0.0	0.0	0.0	0.0
B	252.8	257.9	1.2	0.3	0.1	0.1	0.9	0.0	0.0	0.0	0.0	0.0
C	64.0	66.3	3.2	1.2	0.4	1.4	8.1	0.0	0.0	0.0	0.0	0.0
D	30.2	30.4	2.6	1.3	0.4	0.4	5.8	0.0	0.0	0.0	0.0	0.0
E	7.1	7.6	9.7	5.0	0.5	1.9	7.7	0.0	0.0	0.0	0.0	0.0
F	3.6	4.5	28.3	25.0	0.4	2.8	12.4	0.0	0.0	0.0	0.0	0.0
G	2.4	2.0	1.1	0.6	0.4	0.2	4.2	0.0	0.0	0.0	0.0	0.0
H	0.0	0.0	11.0	11.9	1.8	6.5	20.6	0.0	0.0	0.0	0.0	0.0

A report will also be generated on the right of these tables. The report contains the calibrator values with the fitted curve. The fit function and the values of the fit parameters (A, B, C, D). It will also show goodness of fit parameters R2 and sum of squares (SSQ).

The replicate signal values of the samples will be shown as well as the calculated concentration values with average and CV%.

Report

assayfit pro

ELISA result report

| Operator | Kit | Units |
| | | pg/mL |

| Date | Lot | LOQ |
| ####### | | |

Fit function **Fit parameters**

4 parameter logistic

$(D + ((A - D) / (1 + ((x / C)^B))))$ 4 para   A 3.640

 B -1.005

 C 277.534

| R2 | SSQ | D | 0.053 |
| 0.9988 | 4.57E-03 | | |

Calibration Curve

	Signal						Concentration						
ID	Rep1	Rep2	Rep3	Rep av	Rep CV%	ID	Conc1	Conc2	Conc3	Conc Av	Dilution	Conc x Di	Conc CV%
CAL-0	0.052	0.053		0.053	1.347	CAL-0	0.0	0.0		0.0	1	0.0	
CAL-2	0.084	0.079		0.082	4.338	CAL-2	2.4	2.0		2.2	1	2.2	12.6
CAL-3.9	0.098	0.110		0.104	8.159	CAL-3.9	3.6	4.5		4.0	1	4.0	16.9
CAL-7.8	0.142	0.147		0.145	2.447	CAL-7.8	7.1	7.6		7.4	1	7.4	4.0
CAL-31	0.402	0.404		0.403	0.351	CAL-31	30.2	30.4		30.3	1	30.3	0.4
CAL-63	0.722	0.741		0.732	1.837	CAL-63	64.0	66.3		65.1	1	65.1	2.4
CAL-250	1.763	1.781		1.772	0.718	CAL-250	252.8	257.9		255.4	1	255.4	1.4
CAL-500	2.318	2.356		2.337	1.150	CAL-500	474.0	496.2		485.1	1	485.1	3.2
1	0.063	0.053		0.058	12.191	1	0.8	0.0		0.4	1	0.4	141.4

Data in XY format

Assayfit pro also has other options for curve fitting than the frequently used 96 well format.

Sometimes the data is presented as x and y and the values are not in 96 well format, but just in one column.
For this you can use XY or XY+ button in the assayfit pro menu.

Using these options you can directly enter the standard curve data points in the xdata and ydata columns. The unknown samples can be entered into the yknown column.

Cal	xdata	ydata	weights	xlimits	start	xknown	yknown
Input							
Cal-1	200.0	116.1					29.0
Cal-2	150.0	147.0					38.0
Cal-3	100.0	192.0					100.0
Cal-4	75.0	231.0					
Cal-5	50.0	298.0					
Cal-6	37.0	347.0					
Cal-7	25.0	417.0					
Cal-8	20.0	446.0					
Cal-9	15.0	495.0					
Cal-10	10.0	536.0					

Data in any other format

By clicking the Raw button, a raw example appears as an example of an output file from an assay reader. The data can be anywhere and can have any format, not just 96 wells.

The top example has a top to bottom calibration, the bottom a left to right calibration. The add-in can work both with left to right and top to bottom layouts.

With the Fit input ranges button you can select regions which will be used for the curve-fitting
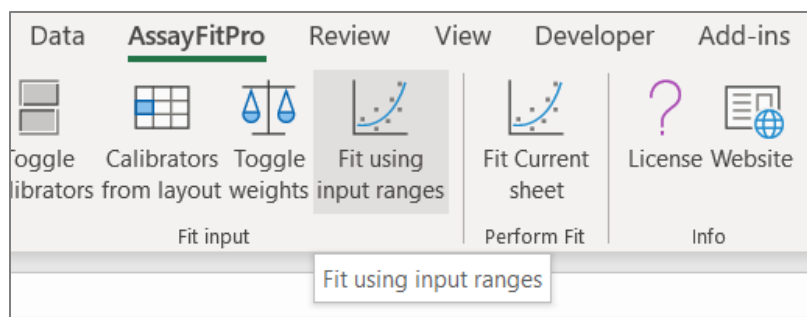
A window will appear. Selecting the icon on the right of the input boxes will display a select box. Selecting a region will then copy the location of this region into the field.

You can also select the regions from left to right as is indicated in the following example.

B	C	D	E	F	G	H	I	J	K	L	M	N
		standard curve from left to right									Inputbox	
	x	500	250	62.5	31.25	7.8125	3.90625	1.953125	0		Select input range	
	Input	1	2	3	4	5	6	7	8	9	X values	
	A	2.318	1.763	0.722	0.402	0.142	0.098	0.084	0.052	0.0	$D$17:$K$17	
	B	2.356	1.781	0.741	0.404	0.147	0.11	0.079	0.053	0.0		
	C	0.063	0.068	0.093	0.069	0.059	0.071	0.153	0.038	0.0	Y values	
	D	0.053	0.057	0.086	0.07	0.058	0.059	0.125	0.038	0.0	$D$19:$K$20	
	E	0.059	0.055	0.173	0.116	0.06	0.077	0.149	0.04	0.0		
	F	0.053	0.055	0.382	0.347	0.059	0.088	0.205	0.038	0.0	Weights	
	G	0.078	0.065	0.067	0.061	0.058	0.056	0.106	0.034	0.0		
	H	0.033	0.041	0.188	0.199	0.076	0.134	0.298	0.038	0.0	Known y (calculate x)	
											$D$19:$O$26	
											Known x (calculate y)	
											Start values	
											Limits	

By clicking on the Absolute click box the selection will make the reference absolute. This will allow to enter values from another sheet than the active sheet.

Inputbox

Select input ranges for curve fitting

X values   Absolute

RAW!$B$5:$B$12   ☑

Y values

With the buttons Clear input, you can clear the input ranges.

With the save range button you can save the regions on your computer. This is however usually done automatically. The settings will be loaded every time you open the window.

With save settings you can save your username, key, server and fit function on your computer.

Clear input   Save range   Save settings

After fitting the results are shown with graphs and data

More advanced fit options in Excel or LIBREOFFICE

There are several more advanced features that assayfit pro can use. The columns marked weights, xlimits, start

	Input						
Cal	xdata	ydata	weights	xlimits	start	xknown	yknown
Cal-1	200.0	116.1					29.0
Cal-2	150.0	147.0					38.0
Cal-3	100.0	192.0					100.0
Cal-4	75.0	231.0					
Cal-5	50.0	298.0					
Cal-6	37.0	347.0					
Cal-7	25.0	417.0					
Cal-8	20.0	446.0					
Cal-9	15.0	495.0					
Cal-10	10.0	536.0					

1. Weights: already explained before, this column can assign weights to the individual calibrators.
2. Xlimits: you can set limits what concentration can be calculated. You can use this to calculate values exactly between the LOQ and the top of range and not beyond these. Alternatively if these are set extra wide extrapolation can be performed with most functions, but not all functions.
3. Start. You can supply your own start values for curve fitting of the parameters A to E. These will then be used to start optimization of the curve fitting. These fields can also contain formulas.
4. Xknown: by entering a value in the xknown field the corresponding y value will be calculated after curve fitting
5. Yknown: this is used to calculate concentration from signal.

Writing your own software to use Assayfit Pro

Assayfit Pro can work with any software that can send Http GET or POST commands. To understand how to interact with the service we will first explain how the commands are sent to the server.
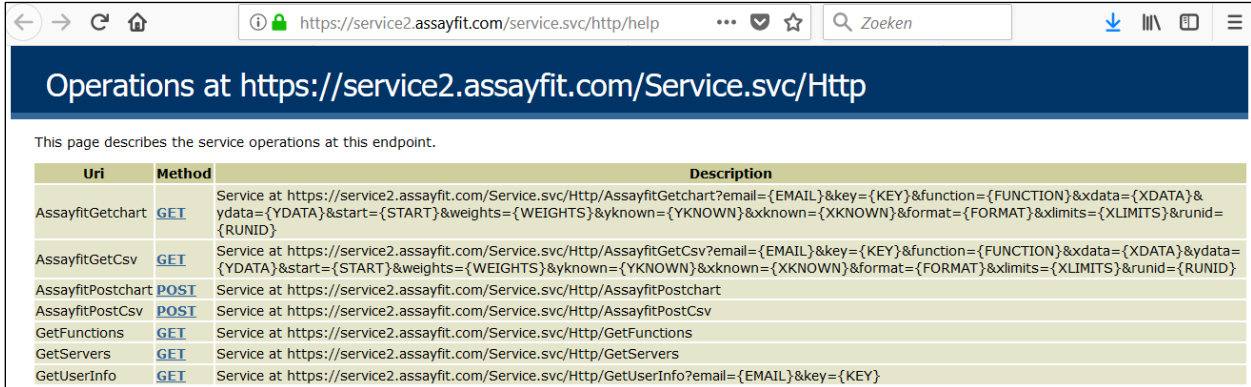
Server help files and commands

The server endpoint commands are shown if you type in the following url in a browser. An endpoint is actually a program running on the server which you can call.

https://service2.assayfit.com/service.svc/http/help

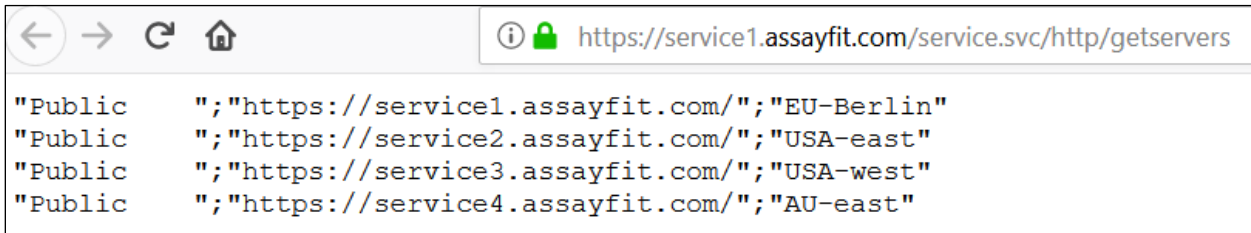This will return all the methods running on the server and if they use a GET or POST command to activate them.

The GetServers endpoint uses a GET method to send data to the server and the location is

https://service1.assayfit.com/service.svc/http/getservers

If we type this in a browser we get the following data returned. It is a list of servers.

This was an easy example as the service endpoint did not need any additional data to work.

Another command is GetUserInfo. This requires the inputs email and key and also uses a GET method. It does not matter in which order you supply the input parameters

We will try it using the following command

https://service2.assayfit.com/service.svc/http/getuserinfo?email=testuser@assayfit.com&key=free

or

https://service2.assayfit.com/service.svc/http/getuserinfo?key=free&email=testuser@assayfit.com

We will get this reply:

```
←  →  C  ⌂              ⓘ 🔒  https://service2.assayfit.com/service.svc/http/getuserinfo?email=testuser@assayfit.com&key=free        ···  ▽

Status; Your are using a free key which has some limitations. Get more options and credits with a payed key on www.assayfit.com
Email;testuser@assayfit.com
Key;free
Is your key used today;True
Your remaining credits for today;23
Current IP;85.148.227.82
UTC time;1/22/2018 7:51:47 PM
```

HTTP GET and POST

In the previous example we supplied two parameters after the question mark. This is the way the GET method works. Data is sent in the URL of the browser or other software. There is however a 2000 character restriction to the URL, so if you are sending a large dataset to the server this option does not work. The GET method however is very easy to troubleshoot your program as the command to send data to the server and receiving back the reply are so easy to view.

With the POST command it is different. Data is sent in the body of the message to the server and it is not so easy to view. It is therefore recommended if you write your software to first try it with the AssayfitGetCSV command and then transform it into a POST command using the AssayfitPostCSV command.

Your first Assayfit Pro script

To explain you how the software performs curve fitting we will use a very simple data set:

xdata	ydata
1	6
2	7
3	8
4	9
5	10

If you would plot it in excel it looks like this:
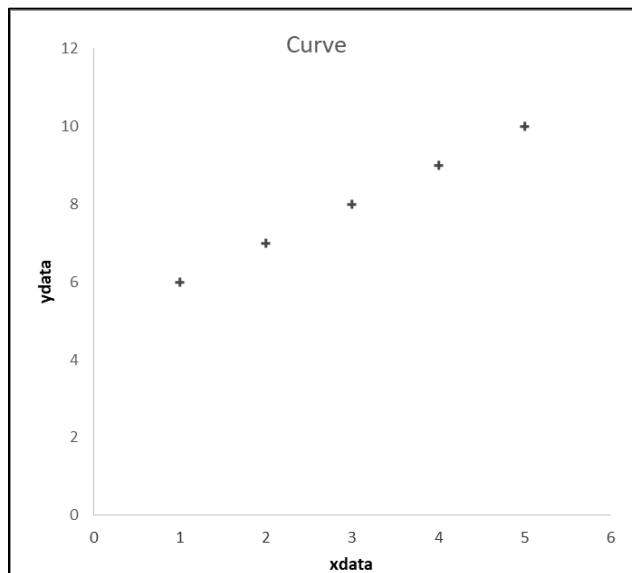
If you want to fit these data points to a curve you send the xdata points and the ydata points to the Assayfit Pro server with this command:

https://service1.assayfit.com/service.svc/http/assayfitgetcsv?email=testuser@assayfit.com&key=free&xdata=1;2;3;4;5&ydata=6;7;8;9;10

https://service1.assayfit.com/service.svc/http/assayfitgetchart?email=testuser@assayfit.com&key=free&xdata=1;2;3;4;5&ydata=6;7;8;9;10

This may look complicated but if you know what the elements mean it is actually quite simple. This example shows the minimal data you need to provide to perform a curve fitting.

The data you are providing is the following:

server location	https://service1.assayfit.com/
service and script	service.svc/http/assayfitgetcsv?
Email (not required)	email=testuser@assayfit.com
key	&key=free
xdata	&xdata=1;2;3;4;5
ydata	&ydata=6;7;8;9;10;11

The server location and service and scripts identify where the service is located.

The email and key identify you as a user. You can use the free service, but in case of a paid service the server can identify if you have access to the paid services.

The xdata and ydata are the actual data sent to the service.

If you are using the get scripts, the string above can be entered in a browser and you can view the response. It will send an http GET command to the server and return text with the calculated data formatted as a comma separated file (CSV) with a semicolon as the separator.

If you require headers add the &format=h to the request string.

This file does not make that much sense in the browser, but if the data is in a spreadsheet it is much easier to read. The headers and column numbers are not returned, but these are indicated in the image below for explanation.

Output

xdata	ydata	weights	percent	yfitted	resid	paraminfo	param	knowι	xfromyknown	knowιηxkr	xcurve	xcurve	infoname	info
1.00E-06	1	1	16.1	1.00208657	-0.00208657	A	7.2305747	2	0.443642941		1.00E-06	1.00209	Run ID:	Assayfit
	3.5	1	56.5	3.479976979	0.020023021	B	-1.528299	3	0.802780813		1.29E-06	1.00209		
	5	1	80.6	5.087003709	-0.087003709	C	1.3115657	4	1.248965066		1.68E-06	1.00209	Function:	401
	6	1	96.8	5.85906043	0.14093957	D	1.0020866	6	3.281432321		2.17E-06	1.00209		
	6.2	1	100	6.271872323	-0.071872323	E					2.81E-06	1.00209	UTC DateTime:	2/21/2018 19:37
											3.63E-06	1.00209		
											4.70E-06	1.00209	User:	excel10@assaycloud.com
					Fit function:						6.09E-06	1.00209		
					(D + ((A - D) / (1 + ((x / C) ^ B)))) |4 parameter logistic						7.88E-06	1.00209	IP address:	
											1.02E-05	1.00209		
					Inv function:						1.32E-05	1.00209	Kernel time:	0.015
					(((((A - D) / (y - D)) - 1) ^ (1 / B)) * C)						1.71E-05	1.00209	Service time:	0.263
											2.21E-05	1.00209	Application time:	0.796875
											2.86E-05	1.00209		
											3.70E-05	1.00209	Status code:	0
											4.79E-05	1.00209		
											6.20E-05	1.00209	Info:	http://www.assaycloud.com/
											8.03E-05	1.00209		
											0.0001039	1.00209	Intended use	For research use only
											0.0001345	1.00209		
											0.0001741	1.00209	License:	the software license on https://w
											0.0002254	1.0021		
											0.0002917	1.0021	Sum of Squares:	0.033004514

In our example the service returns not only the original input data (xdata and ydata), but also the xcurve and ycurve data. These columns contain 100 points of the fitted curve. We can also plot these in excel. It will look like this:
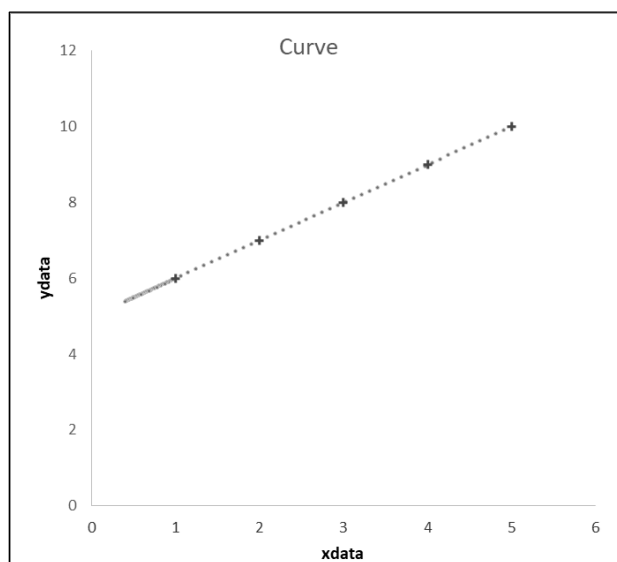
You can see there are more data points in the low end of the curve. This is useful when plotting the data on logarithmic axis.

Usually you do not plot the fitted curve as data points but as a line.
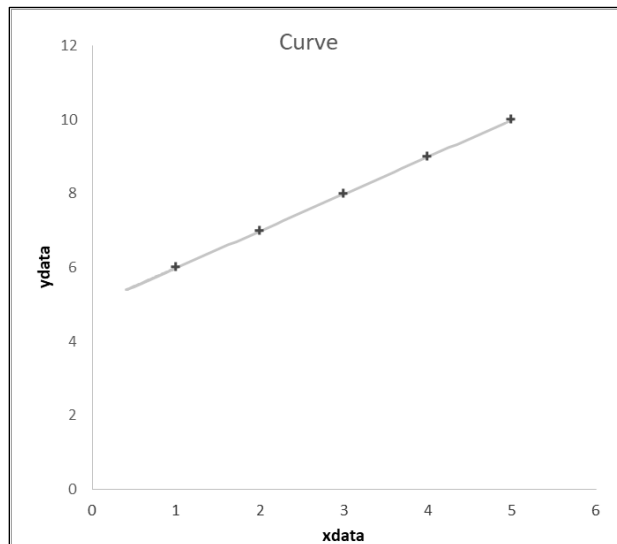
You can use the plot from the fitted curve to see how well the curve fits to the data.

Now suppose you have a measurement of 7.5 and you want to know what the fitted x value of 7 is. You know in this case what the y value is, but you do not know what the x value is.

You can then enter an extra value to the string

&yknown=7.5

https://service3.assayfit.com/service.svc/http/assayfitgetcsv?email=testuser@AssayCloud.com&key=free&xdata=1;2;3;4;5&ydata=6;7;8;9;10;11&yknown=7.5

https://service3.assayfit.com/service.svc/http/assayfitgetchart?email=testuser@AssayCloud.com&key=free&xdata=1;2;3;4;5&ydata=6;7;8;9;10;11&yknown=7.5

Part of the returned object will look like this

xdata	ydata	weights	resid	params start	params	ss params	ss - statuscode	yknown	xfromyknown
1	6	1	-0.000126379	10	993.6327961		2.25E-07	7.5	2.500127943
2	7	1	0.000300639	0	-1.005425197				
3	8	1	-8.36E-05	3	957.1763479		0		
4	9	1	-0.000281454	6	5.006024213				
5	10	1	0.000180819						

You can see 7.5 indicated in the yknown column and the corresponding xfromyknown value, which is the x value calculated from the curve is 2.500127943.
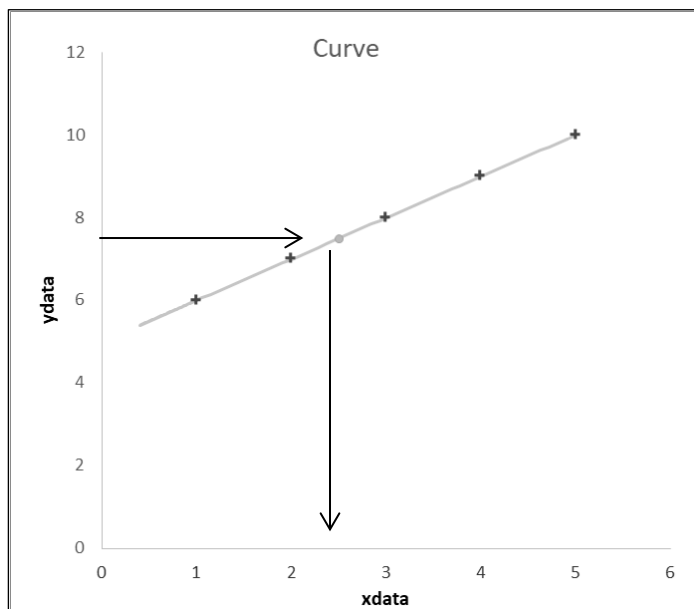
This example explained the minimal commands to return the fitted curve, however there is much more Assayfit Pro can do. You can also enter the following parameters:

functio n	&function=401	The function used for the fit. A large number of fits is available. Use the getfunctions command to send to the server to see what these are
start	&start=10;0;3;6	The start values of the function. If a function is for example y = b + a*x there are two parameters a and b. The software optimises these to get the best possible fit by modifying a and b, however the function works best if it starts with parameter a and b values which are close the the final values. You can provide your own values, if not provided Assayfit will calculate the start values.
weights	&weights=1;1;1;1; 1	The weight value indicates the relative importance of a data point in the fit. You can specify these. If you enter 100, the value will be considered 100x more important than the other values.
yknown	&yknown=7.5;8.5	yknown are values of which you know the y value and want to calculate

|		the x value read from the fitted curve
xknown	&yknown=2.5;3.5	xknown are the values of which you know the x value and want to calculate the y values read from the fitted curve
format	&format=h	If you require headers add the &format=h to the request string.
xlimits	&xlimits=2;5	Limits when provided restrict the calculation of unknown values to a certain interval of the curve. In this case if you provide 2 and 5 only values within this range will be calculated. Values below will return 0, values above will return 110% of the maximum limit. If not provided Assayfit Pro will use the maximum range possible.
runid	&runid=test_run	This option can be used to identify your run. In excel the tab name is used as runid. Do not use spaces on Mac as it will prevent running of the fit.

Below is a list of what the columns mean. For every fit easy or complicated the return object is the same.

1	xdata	The xdata sent and processed by the server. In the case of a free account only 5 data points are used.
2	ydata	The ydata sent and processed by the server. In the case of a free account only 5 data points are used.
3	weights	The weights per data point. If not supplied the program will enter 1 for each data point, meaning no weights are used.
4	percent	The percent of each data point of the maximum of y
5	yfitted	The y value calculated from the x value fitted on the curve.
6	resid	The residuals per data point, this is the distance of the point to the fitted curve.
7	paramsinfo	The name of the parameters and the fit function
8	params	The optimized parameter values
9	yknown	The values of y which need to be fitted back to an x value
10	xfromyknown	The values of x calculated from the yknown by backfitting
11	xknown	the values of x which need to be fitted to a y value
12	xfromyknown	the values of y calculated from the xknown values
13	xcurve	100 x value points, the x values of the fitted curve
14	ycurve	100 y value points , the y values of the fitted curve
15	infovalue	The names of the different items in the info column
16	info	The values of the information. Run, user, speed limit and fit statistics and quality of fit information is indicated.

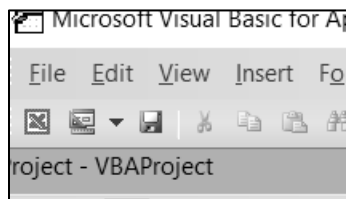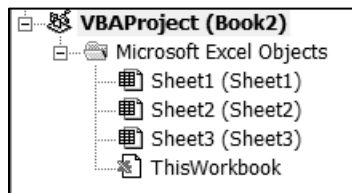## Writing a simple Assayfit Pro VBA script in Excel VBA

Assayfit Pro provides templates to create your own application in Excel, R, Python, C# and others.

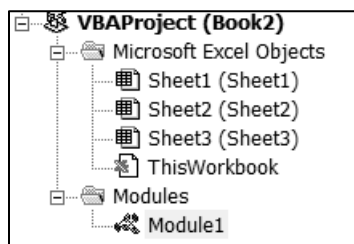To explain how to create a script in Excel this example will show how this is done.
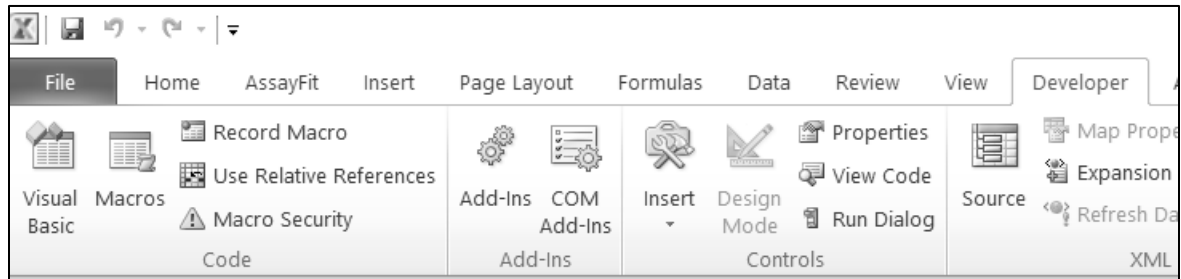
Click on Visual basic

You will see the object explorer. In this case of spreadsheet Book2.

Click the insert button and choose module

It will create a module in the file

VBA script in Excel and call the assayfit pro add-in functions

You can now write any script in this module that gets data from the spreadsheet. It is easiest to write the script to copy your data into an existing assayfit pro sheet.

If you do this, you only need to add the following code to your VBA script and have assayfit pro handle the fitting and reporting.

```
' part of a VBA script to call the assayfit pro curve fitter

''set the fit settings in the activesheet

        Range("B5").Value = "anyname"
        Range("B7").Value = "free"
        Range("B9").Value = "101"
        Range("B13").Value = https://service1.assayfit.com

 ''Call the addin scripts
        Application.Run "AssayfitpostcsvELISA"
        Application.Run "createreportelisa3"
```

You can also write scripts from scratch, however this is more difficult.

Python, Java, R, C# or any other programming language

Assayfit pro can be used from essentially any program that can make an API call. You can download example files how to use assayfit pro from these languages from https://www.assayfit.com/examples.html

Purchasing Assayfit Pro

Assayfit pro is free to use for 10-15 times each day, however If you require more data points or more fits per day you can go to our website on https://www.assayfit.com/pricing.html to view other options.

With a yearly license of the group key you can also serve the online version of assayfit pro on your own website for your customers to use. Please go to the assayfit pro website for more details.

licence agreement

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