

# Data analysis in MyAssays with 5-Parameter Logistic Fit ( $1/y^2$ weighting)

In this Technical Note, you can find information on how to use MyAssays and learn more about how to analyze data using the 5-Parameter Logistic Fit ( $1/y^2$  weighting).

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## Introduction

A curve fitting model is needed to determine the concentrations of samples after measurement with Mercodia's immunoassays. Among the different curve fitting models available, the 5-Parameter Logistic Fit with  $1/y^2$  weighting is a curve fit suitable for calculating concentrations from sigmoidal calibrators.

In this model, data points are weighted using the expression  $1/y^2$ , meaning that points with a lower signal have a higher weight, which in turn leads to better curve fit over the entire measuring range. For this model, the inputs are the raw data signals from the reader, the already determined concentrations of calibrators and the chosen curve fitting model.

The algorithm uses the inputs to make systematic guesses and arrives at parameter values that best describe the calibrator points on the standard curve. This is done so that the distance to the points and the curve is as small as possible. The outputs are a standard curve and the calculated concentrations of samples in the assay. Figure 1 shows a schematic process of curve fitting.

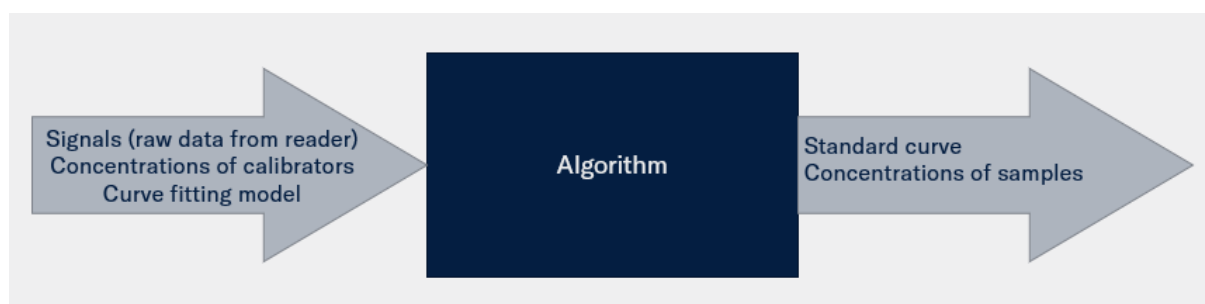


Figure 1: Schematic process of curve fitting.

## MyAssays

There are different programs where you can evaluate the results you obtained from your reader. Most of Mercodia's products are validated using either Magellan (Tecan) software or MARS (BMG Labtech). If you do not have access to these programs, you can make use of MyAssays which is a free-to-use online tool used by many organizations.

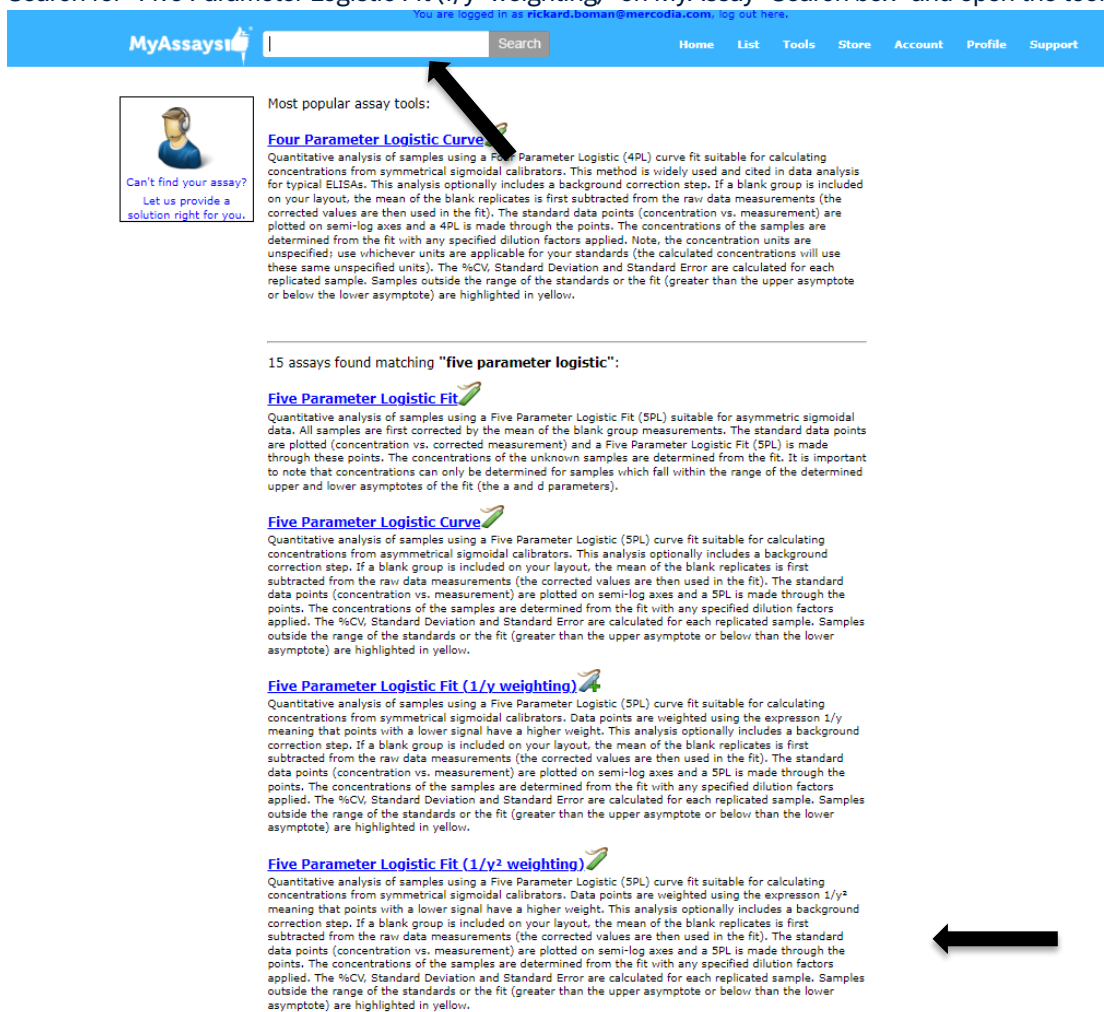
There are however a few things you should keep in mind when using MyAssays:

- Mercodia's products are not validated using MyAssays.
- Mercodia's products are not validated with blank reduction. Therefore, please do not include Calibrator 0 in the standard curve when using MyAssays. Specify that the negative control (calibrator 0) equals to zero for the blank in the plate layout. The reason is that blank is obligatory to add in the plate layout in MyAssays.

Different programs have different algorithms, which means that even if you use the same curve fitting model with the same data input, the output parameter values may differ from program to program.

**Step-by-step guide on how to use MyAssays**

- 1) Open an account at MyAssays.com
- 2) Search for "Five Parameter Logistic Fit (1/y<sup>2</sup> weighting)" on MyAssay "Search box" and open the tool.



The screenshot shows the MyAssays website interface. At the top, there is a navigation bar with the MyAssays logo, a search bar, and links for Home, List, Tools, Store, Account, Profile, and Support. Below the navigation bar, there is a section titled "Most popular assay tools:" with a list of tools. The first tool is "Four Parameter Logistic Curve". Below this, there is a section titled "15 assays found matching 'five parameter logistic':" with a list of tools. The first tool is "Five Parameter Logistic Fit", the second is "Five Parameter Logistic Curve", the third is "Five Parameter Logistic Fit (1/y weighting)", and the fourth is "Five Parameter Logistic Fit (1/y<sup>2</sup> weighting)". A black arrow points to the search bar, and another black arrow points to the "Five Parameter Logistic Fit (1/y<sup>2</sup> weighting)" tool.

You are logged in as [rickard.boman@mercodia.com](#), [log out here](#).

**MyAssays** Search Home List Tools Store Account Profile Support

Most popular assay tools:

**Four Parameter Logistic Curve**  
Quantitative analysis of samples using a Four Parameter Logistic (4PL) curve fit suitable for calculating concentrations from symmetrical sigmoidal calibrators. This method is widely used and cited in data analysis for typical ELISAs. This analysis optionally includes a background correction step. If a blank group is included on your layout, the mean of the blank replicates is first subtracted from the raw data measurements (the corrected values are then used in the fit). The standard data points (concentration vs. measurement) are plotted on semi-log axes and a 4PL is made through the points. The concentrations of the samples are determined from the fit with any specified dilution factors applied. Note, the concentration units are unspecified; use whichever units are applicable for your standards (the calculated concentrations will use these same unspecified units). The %CV, Standard Deviation and Standard Error are calculated for each replicated sample. Samples outside the range of the standards or the fit (greater than the upper asymptote or below the lower asymptote) are highlighted in yellow.

15 assays found matching "five parameter logistic":

**Five Parameter Logistic Fit**  
Quantitative analysis of samples using a Five Parameter Logistic Fit (5PL) suitable for asymmetric sigmoidal data. All samples are first corrected by the mean of the blank group measurements. The standard data points are plotted (concentration vs. corrected measurement) and a Five Parameter Logistic Fit (5PL) is made through these points. The concentrations of the unknown samples are determined from the fit. It is important to note that concentrations can only be determined for samples which fall within the range of the determined upper and lower asymptotes of the fit (the a and d parameters).

**Five Parameter Logistic Curve**  
Quantitative analysis of samples using a Five Parameter Logistic (5PL) curve fit suitable for calculating concentrations from asymmetrical sigmoidal calibrators. This analysis optionally includes a background correction step. If a blank group is included on your layout, the mean of the blank replicates is first subtracted from the raw data measurements (the corrected values are then used in the fit). The standard data points (concentration vs. measurement) are plotted on semi-log axes and a 5PL is made through the points. The concentrations of the samples are determined from the fit with any specified dilution factors applied. The %CV, Standard Deviation and Standard Error are calculated for each replicated sample. Samples outside the range of the standards or the fit (greater than the upper asymptote or below than the lower asymptote) are highlighted in yellow.

**Five Parameter Logistic Fit (1/y weighting)**  
Quantitative analysis of samples using a Five Parameter Logistic (5PL) curve fit suitable for calculating concentrations from symmetrical sigmoidal calibrators. Data points are weighted using the expression 1/y meaning that points with a lower signal have a higher weight. This analysis optionally includes a background correction step. If a blank group is included on your layout, the mean of the blank replicates is first subtracted from the raw data measurements (the corrected values are then used in the fit). The standard data points (concentration vs. measurement) are plotted on semi-log axes and a 5PL is made through the points. The concentrations of the samples are determined from the fit with any specified dilution factors applied. The %CV, Standard Deviation and Standard Error are calculated for each replicated sample. Samples outside the range of the standards or the fit (greater than the upper asymptote or below than the lower asymptote) are highlighted in yellow.

**Five Parameter Logistic Fit (1/y<sup>2</sup> weighting)**  
Quantitative analysis of samples using a Five Parameter Logistic (5PL) curve fit suitable for calculating concentrations from symmetrical sigmoidal calibrators. Data points are weighted using the expression 1/y<sup>2</sup> meaning that points with a lower signal have a higher weight. This analysis optionally includes a background correction step. If a blank group is included on your layout, the mean of the blank replicates is first subtracted from the raw data measurements (the corrected values are then used in the fit). The standard data points (concentration vs. measurement) are plotted on semi-log axes and a 5PL is made through the points. The concentrations of the samples are determined from the fit with any specified dilution factors applied. The %CV, Standard Deviation and Standard Error are calculated for each replicated sample. Samples outside the range of the standards or the fit (greater than the upper asymptote or below than the lower asymptote) are highlighted in yellow.

- 3) Add your measurement data, which is the raw data from your reader output. Remember to specify that the negative control (Calibrator 0) equals to zero. Use the dot "." as the decimal separator.

**Five Parameter Logistic Fit (1/y<sup>2</sup> weighting)**
Play Video Guide  
(3 min 24 sec)

SPL weighting

Quantitative analysis of samples using a Five Parameter Logistic (5PL) curve fit suitable for calculating concentrations from symmetrical sigmoidal calibrators. Data points are weighted using the expression 1/y<sup>2</sup> meaning that points with a lower signal have a higher weight.

This analysis optionally includes a background correction step. If a blank group is included on your layout, the mean of the blank replicates is first subtracted from the raw data measurements (the corrected values are then used in the fit).

The standard data points (concentration vs. measurement) are plotted on semi-log axes and a 5PL is made through the points. The concentrations of the samples are determined from the fit with any specified dilution factors applied.

The %CV, Standard Deviation and Standard Error are calculated for each replicated sample.

Samples outside the range of the standards or the fit (greater than the upper asymptote or below than the lower asymptote) are highlighted in yellow.

[See also](#)

$$y = d + \frac{a - d}{\left[1 + \left(\frac{x}{c}\right)^b\right]^m}$$

**1**

**Measurements**

Supply your measurement data: 2

Raw  File

0	4474	122748	608617	9329	420601
0	4580	137083	645867	8336	435166
0	4044	134996	711112	15759	523783
0	3884	127567	616732	9637	483659
2521	37046	358891	896797	114064	111081
2280	36425	435629	801949	100551	107749
3032	41730	372954	808182	128250	107574
3084	35762	430180	757922	109878	97310

Paste Flag Positions

**2** Microplate

**3** Standard Concentrations

**4** Dilution Factors

**5** Sample IDs

**6** Run Notes

↑↓

↑↓


↑↓

↑↓

↑↓

Calculate...

4) Adjust the Microplate so that it corresponds to the microplate set up out of your experiment.

See also 


1

**Measurements**

↑

2

**Microplate**

Select how your samples are arranged on the microplate: 

6 Standards (with Background Correction)

11 Standards

Custom Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	1	1	1	9	9	17	17	25	25	33	33
B	2	2	2	2	10	10	18	18	26	26	34	34
C	3	3	3	3	11	11	19	19	27	27	35	35
D	4	4	4	4	12	12	20	20	28	28	36	36
E	5	5	5	5	13	13	21	21	29	29	37	37
F	6	6	6	6	14	14	22	22	30	30	38	38
G	1	1	7	7	15	15	23	23	31	31	39	39
H	1	1	8	8	16	16	24	24	32	32	40	40

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	2	3	4	5	6	7	8	9	10	11	1
B	1	2	3	4	5	6	7	8	9	10	11	1
C	1	2	3	4	5	6	7	8	9	10	11	12
D	1	2	3	4	5	6	7	8	9	10	11	12
E	13	14	15	16	17	18	19	20	21	22	23	24
F	13	14	15	16	17	18	19	20	21	22	23	24
G	25	26	27	28	29	30	31	32	33	34	35	36
H	25	26	27	28	29	30	31	32	33	34	35	36







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

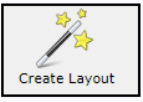
Custom Layout

Custom Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	1	9	17	25	33	41	49	57	65	73	81
B	1	2	10	18	26	34	42	50	58	66	74	82
C	2	3	11	19	27	35	43	51	59	67	75	83
D	3	4	12	20	28	36	44	52	60	68	76	84
E	4	5	13	21	29	37	45	53	61	69	77	85
F	5	6	14	22	30	38	46	54	62	70	78	86
G	6	7	15	23	31	39	47	55	63	71	79	87
H	7	8	16	24	32	40	48	56	64	72	80	88

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



■ Standard    ■ Control    ■ Unknown    ■ Blank

3


**Standard Concentrations**

↑

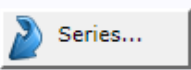

5) Fill in the standard concentrations (Calibrators). The concentrations and units should be stated on the standard vials

3

**Standard Concentrations**

Review or edit the concentration values here: 

Calibrator	Conc.
Standard1	24.5
Standard2	36.3
Standard3	98.4
Standard4	226
Standard5	525
Standard6	1226
Standard7	2208

- 6) Add dilution factors if you have any.

**4 Dilution Factors**

Review or edit the dilution factors here: ?

Sample	Factor
Unknown1	1.000000
Unknown2	1.000000
Unknown3	1.000000
Unknown4	1.000000
Unknown5	1.000000
Unknown6	1.000000
Unknown7	1.000000
Unknown8	1.000000
Unknown9	1.000000
Unknown10	1.000000

Series...

Paste

- 7) Add Sample IDs if you have any.

**5 Sample IDs**

Optionally provide an ID for each of your Unknown samples. ?

Sample	ID
Unknown1	Unknown1
Unknown2	Unknown2
Unknown3	Unknown3
Unknown4	Unknown4
Unknown5	Unknown5
Unknown6	Unknown6
Unknown7	Unknown7
Unknown8	Unknown8
Unknown9	Unknown9
Unknown10	Unknown10

Series...

Paste

8) Add run notes if needed and press “Calculate”.

**6**

**Run Notes**

Optionally provide additional data to store alongside the results for this assay:

Run Name:

Notes:

Calculate...

9) Click on the results file.

See also

- 1

**Measurements**
- 2

**Microplate**
- 3

**Standard Concentrations**
- 4

**Dilution Factors**
- 5

**Sample IDs**
- 6

**Run Notes**

Optionally provide additional data to store alongside the results for this assay:

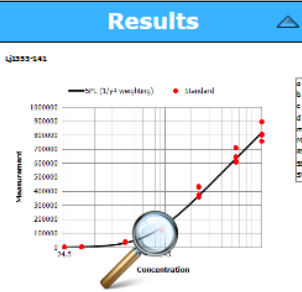
Run Name:

Notes:

Calculate...

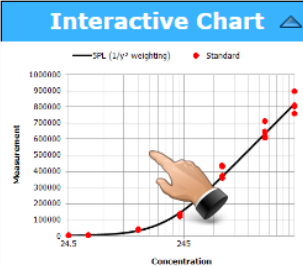
Results

Lj1353-141



Calibrator	Well	Conc.	Raw (Corrected)	SD	Recovery	Recovery %
Standard 1	F1	24.5	2212	106	24.41	99.23
	F1	24.5	2280	123	23.39	95.4
	F1	24.5	2320	128	23.29	95.48
Standard 2	F1	24.5	2380	143	24.43	100
	F1	24.5	2410	147	24.24	99.23
	F1	24.5	2480	158	23.34	100.7
Standard 3	F1	24.5	2490	162	23.32	92.49
	F1	24.5	2590	175	23.29	91.18
	F1	24.5	2620	179	23.19	89.8
Standard 4	F1	24.5	26100	1300	24.5	100.8
	F2	24.400	2539	113.9	23.9	100.8
	F2	24.200	2420	111.2	23.4	100

Interactive Chart



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- 10) Here you can evaluate your data. You can find the measurements of the standard curve and the concentrations of calibrators. The concentrations of controls and samples will be expressed in the same units as the calibrators (units used in point 5). You can also export the data to Excel.

