Mercodia

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Data analysis in MyAssays with 5-Parameter Logistic Fit (1/y² 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/y² 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:

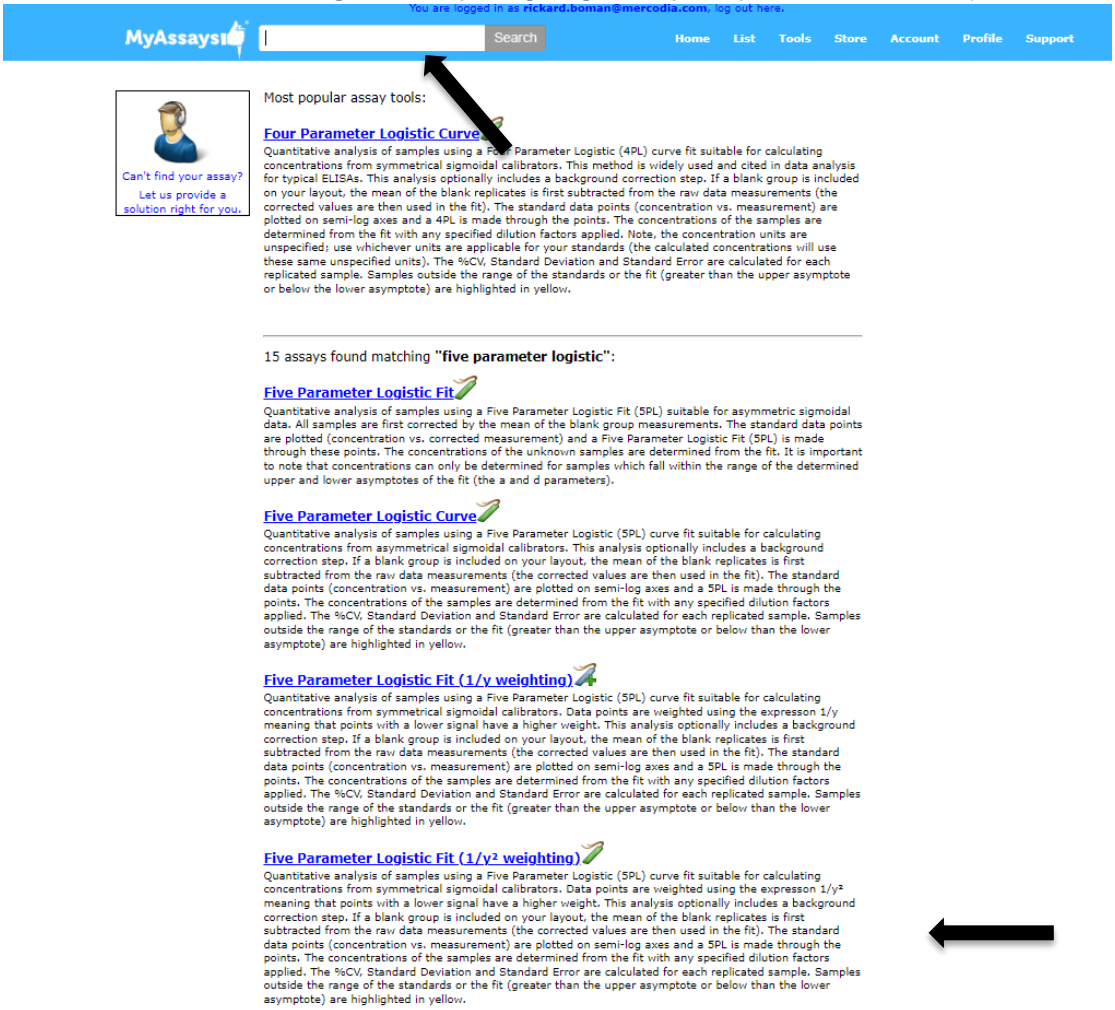
- Mercodia's products are not validated using MyAssays.
- Mercodia's products are not validated with blank reduction. Therefore, please do not include Calibrator 0 in the standard curve when using MyAssays. Specify that the negative control (calibrator 0) equals to zero for the blank in the plate layout.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

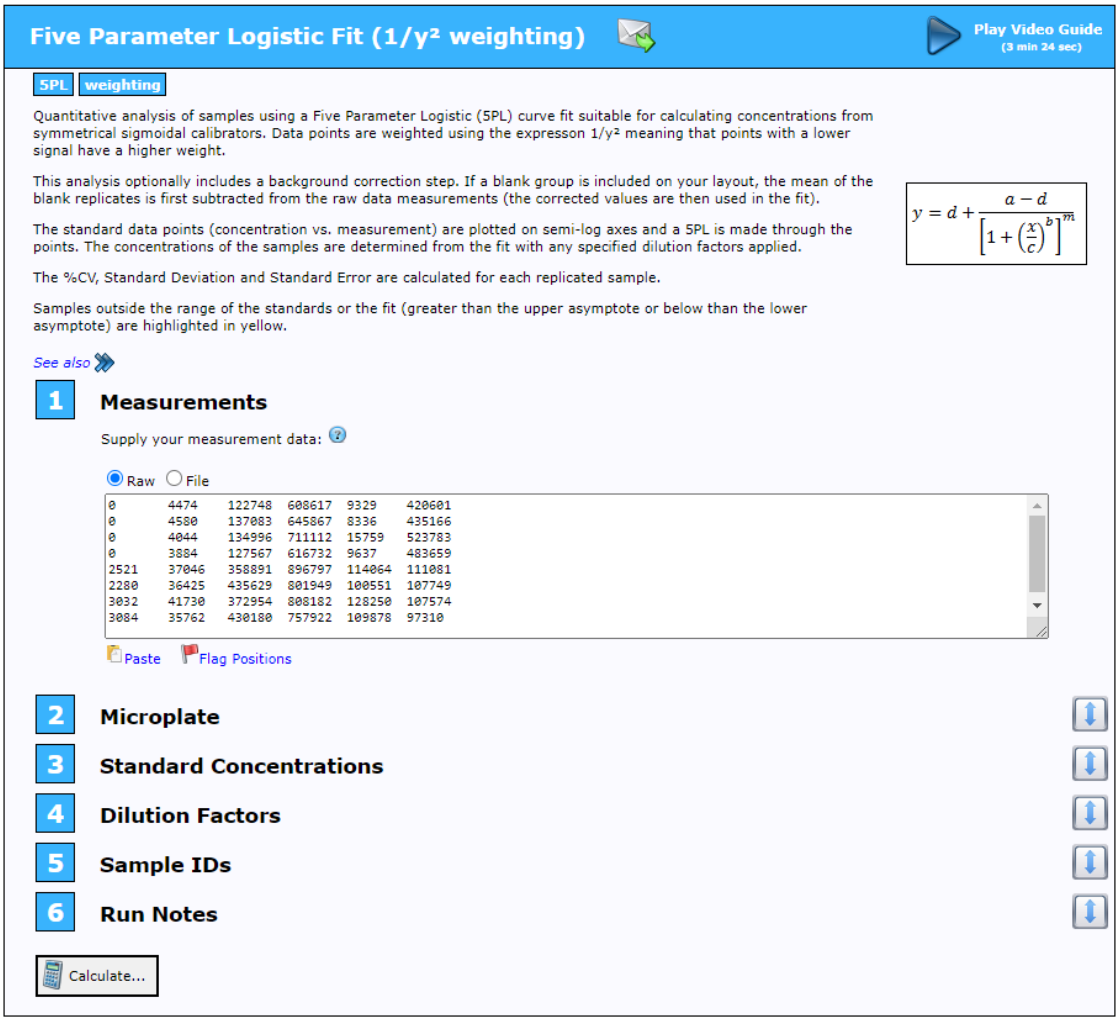
Step-by-step guide on how to use MyAssays

1) Open an account at MyAssays.com

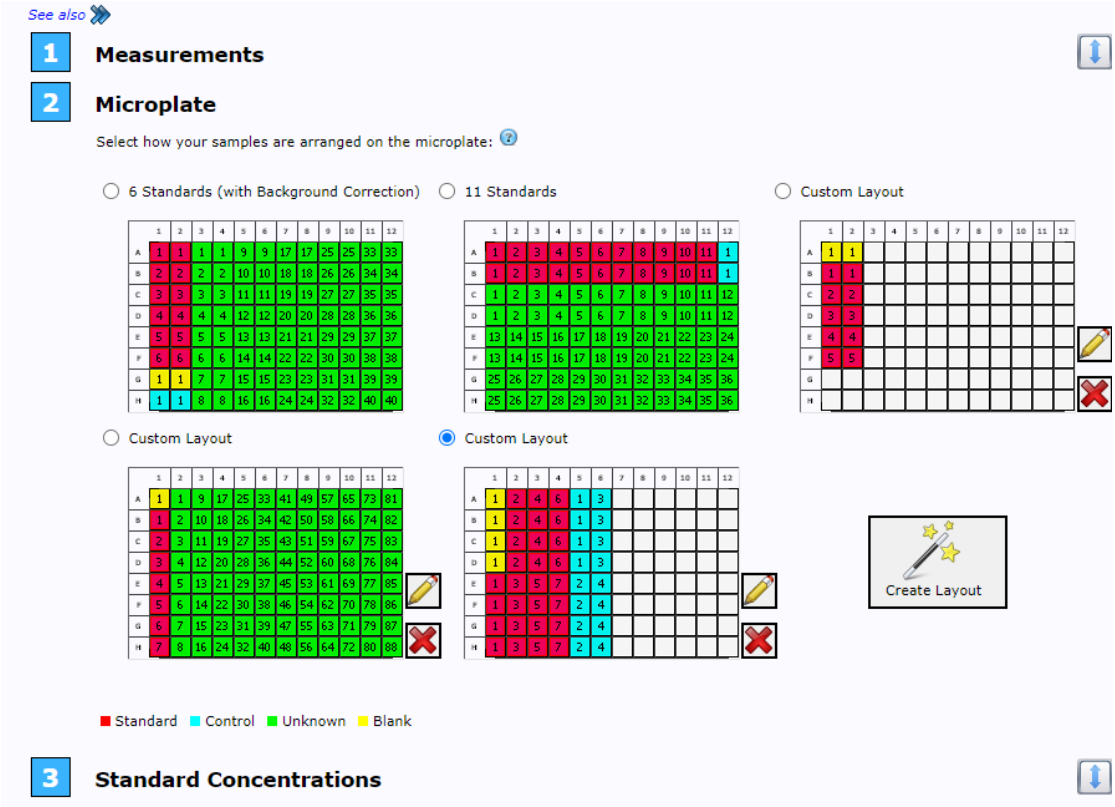
2) Search for ”Five Parameter Logistic Fit (1/y² weighting)” on MyAssay “Search box” and open the tool.



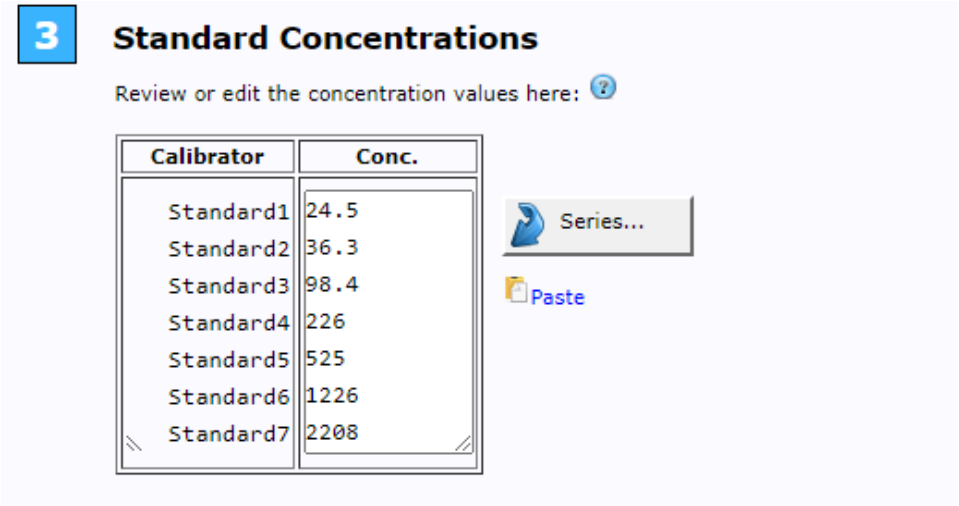
3) Add your measurement data, which is the raw data from your reader output. Remember to specify that the negative control (Calibrator 0) equals to zero. Use the dot "." as the decimal separator.



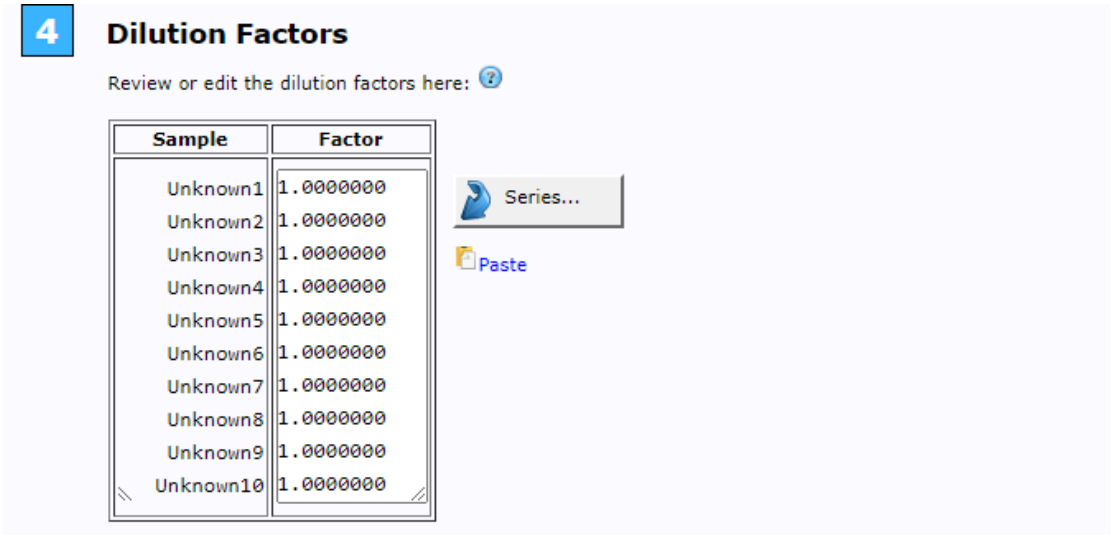
4) Adjust the Microplate so that it corresponds to the microplate set up out of your experiment.



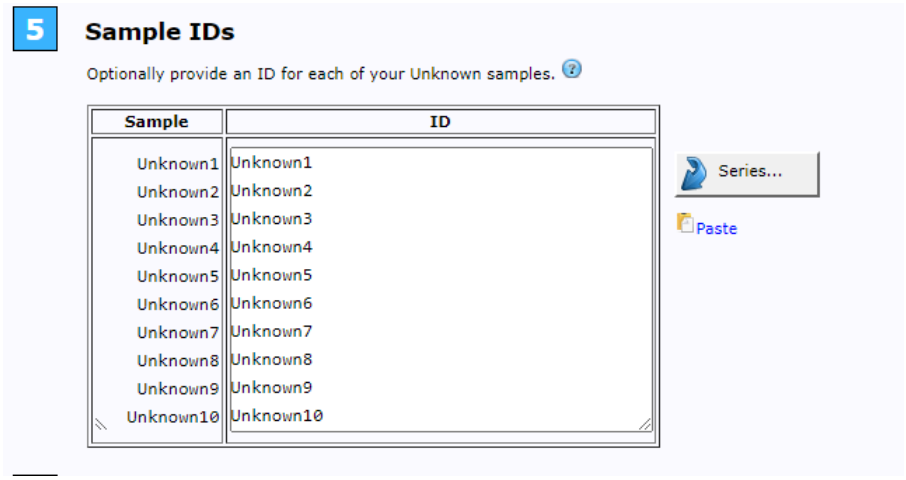
5) Fill in the standard concentrations (Calibrators). The concentrations and units should be stated on the standard vials



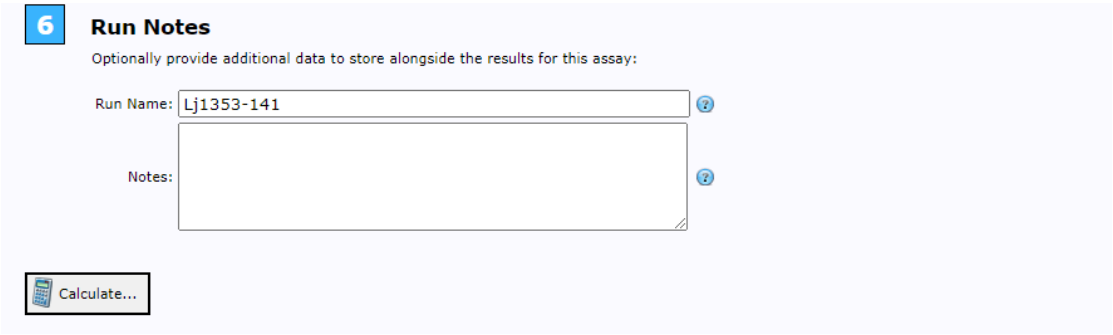
6) Add dilution factors if you have any.



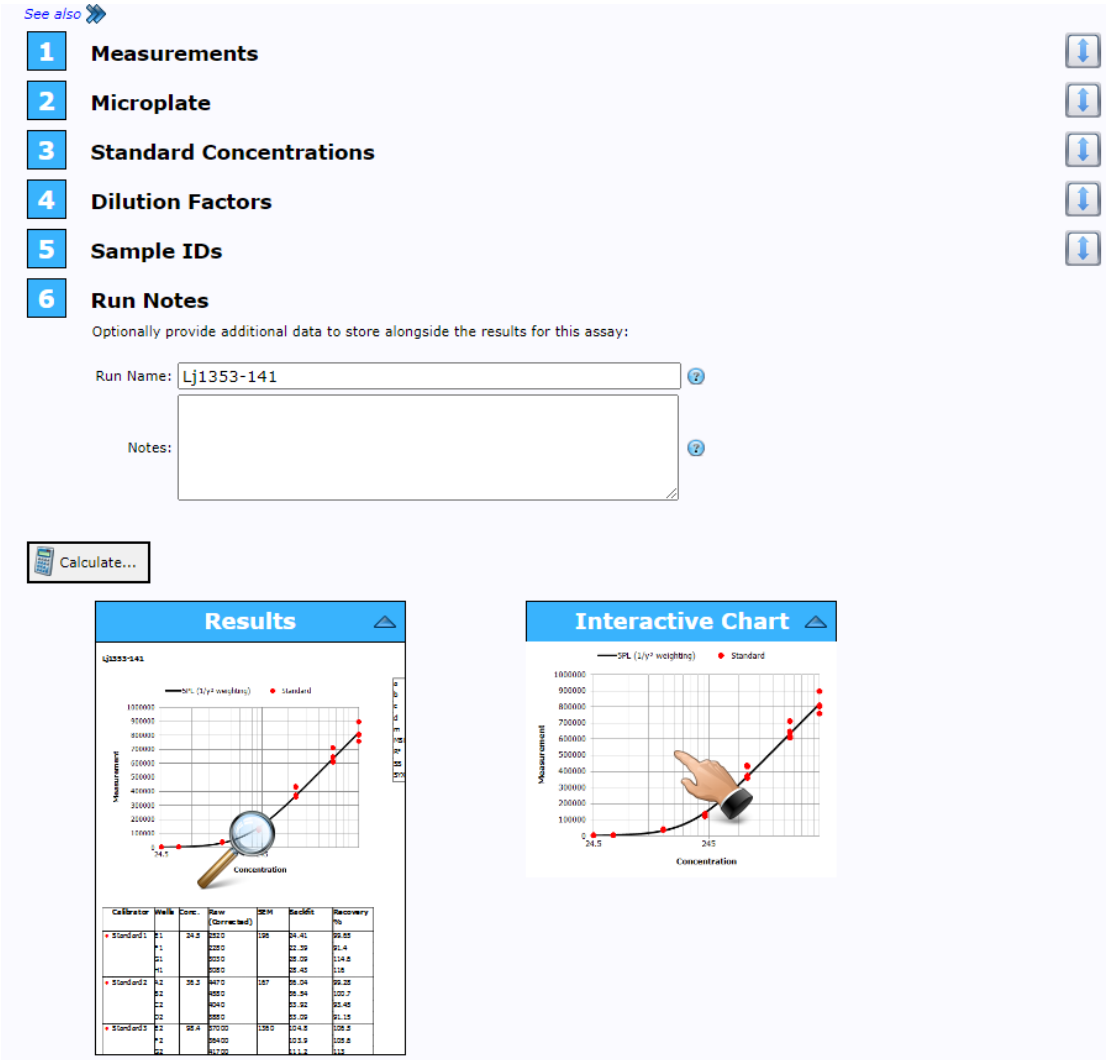
7) Add Sample IDs if you have any.



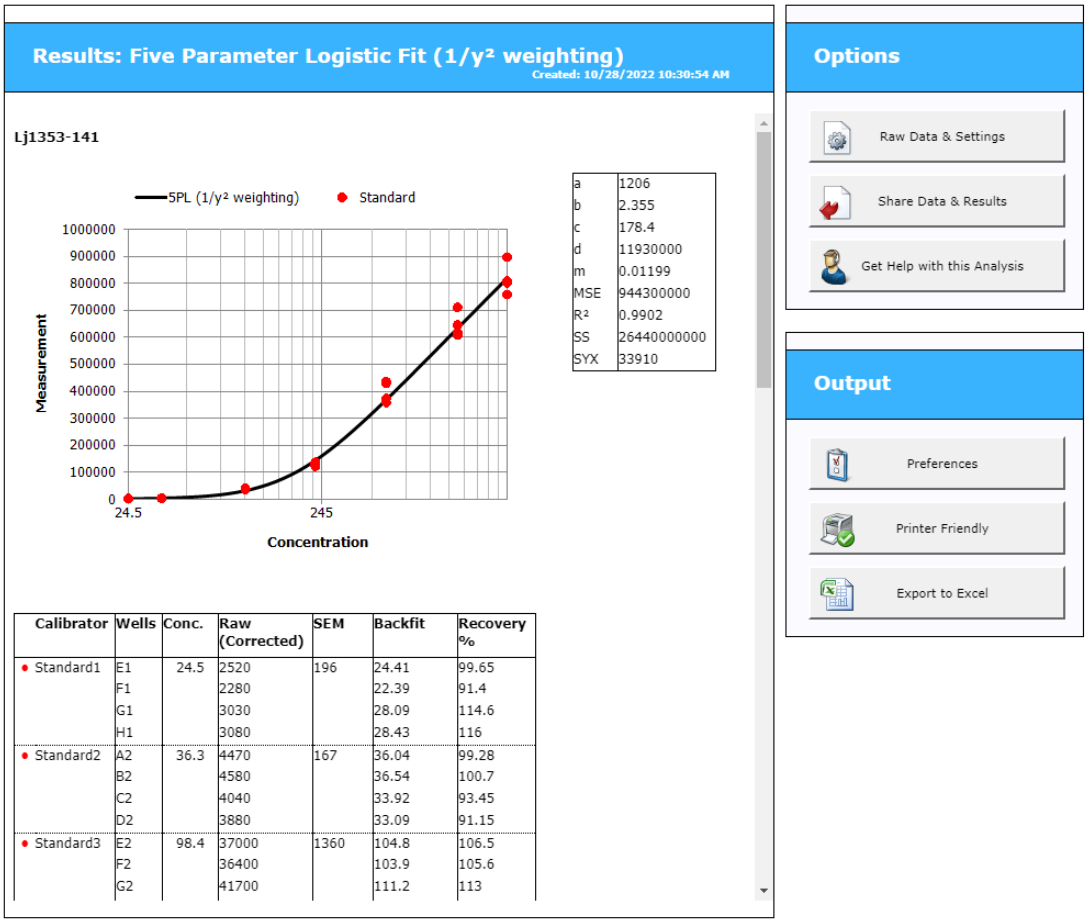
8) Add run notes if needed and press “Calculate”.



9) Click on the results file.



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.



Calibrator	Wells	Conc.	Raw (Corrected)	SEM	Backfit	Recovery %
Standard1	E1	24.5	2520	196	24.41	99.65
	F1		2280		22.39	91.4
	G1		3030		28.09	114.6
	H1		3080		28.43	116
Standard2	A2	36.3	4470	167	36.04	99.28
	B2		4580		36.54	100.7
	C2		4040		33.92	93.45
	D2		3880		33.09	91.15
Standard3	E2	98.4	37000	1360	104.8	106.5
	F2		36400		103.9	105.6
	G2		41700		111.2	113