Assay Blaster!

User Manual

Table of Contents

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1. *Getting Started*

1.1. System Requirements

Assay Blaster! Data Analysis Software has been validated for use on PC computers running Windows 2000, or higher, 512 MB RAM. It has also been validated for use on Macintosh computers running 10.5 or higher.

1.2. Install Assay Blaster! Data Analysis Software

Double-click the "Assay Blaster! Data Analysis Software" icon to start the installation wizard. For PC computers, click on the .exe and for Mac computers, click on the .dmg. Start the program by double-clicking on the icon that appears.

Please contact Assay Designs Customer Support (800-833-8651 or 734-668-6113) if the Assay Blaster! Data Analysis Software fails to install correctly.

2. *Selecting a New or Existing Experiment*

Select to either begin a new experiment or to use an existing experiment template. To create a new experiment, select the top radio button and select 'Enter'. You may also select an existing experiment template saved to your hard drive during a previous analysis. To select an existing experiment template, select the bottom radio button and hit the browse button. Your navigation menu will open. Find your desired experiment template on your hard drive and select 'Enter'. At this point, the application will take you directly to the *Plate Layout* tab.

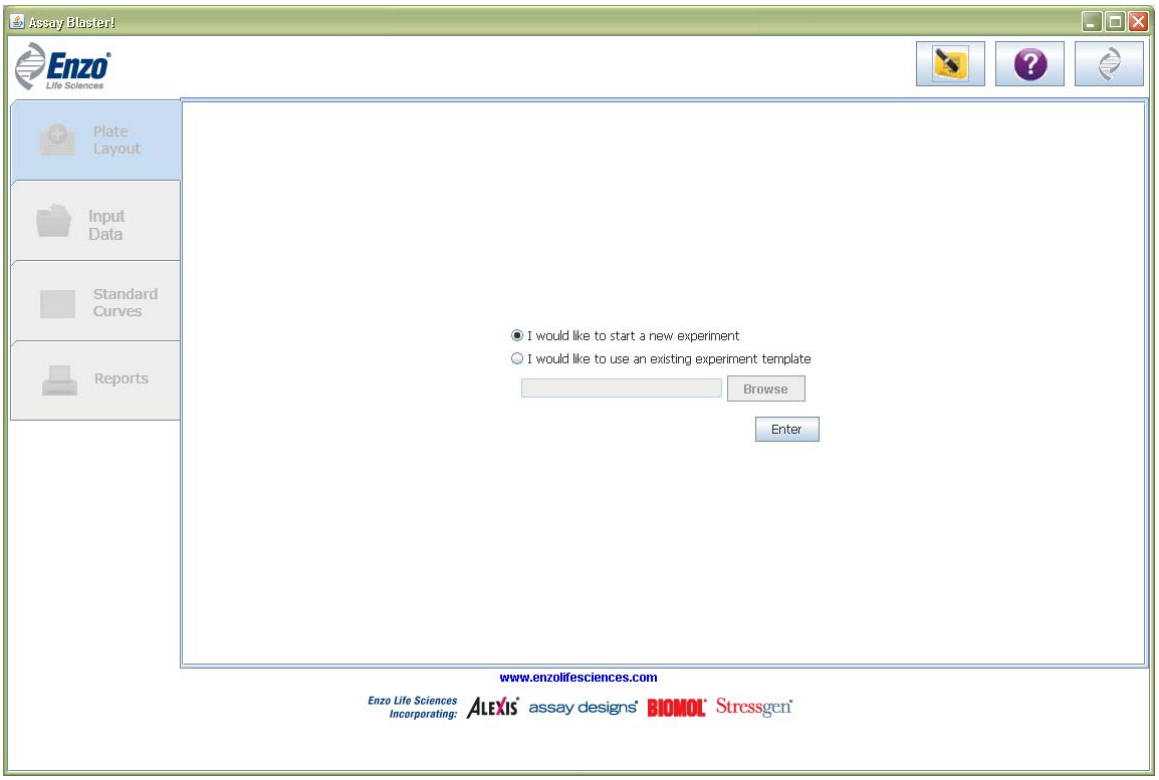


Figure 1 – Selection of the experiment

3. *Plate Layout*

This page allows you to assign specific designations to your 96-well plate layout. It also lets you alter your standard curve parameters. See Figure 2 to view the functions and buttons included on the Standard Curve Data toolbar.

You may enlarge the chart or plate section of the window by clicking on the arrows in the border between the two sections.

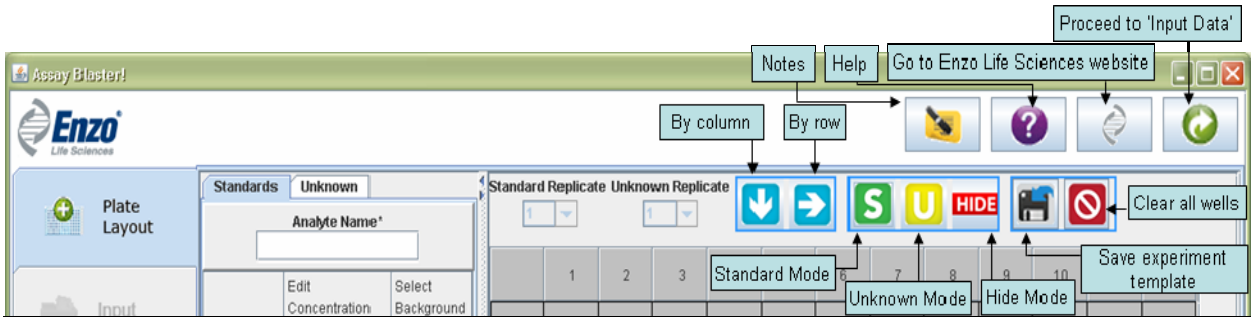


Figure 2 – Plate Layout toolbar

3.1. Assigning Standards

Turn on the Standards function by highlighting the 'Standards' button. At this point you should select whether you want to populate your standards by column (down arrow) or row (right arrow). Change your arrow selection by clicking on the correct arrow. Next, select the number of standard replicates in your analysis by using the 'Standard Replicate' drop down menu (e.g. select 1 if only one well per standard was run, select 2 if duplicate standard wells were run).

Select the wells you wish to populate as standards by clicking on a well and dragging your mouse to create the grid. You may also double-click on an individual well to either populate it or to de-populate it. The standard designation (e.g., Standard 1, Standard 2) is determined by the direction of auto-population as well as your number of standards. A minimum of 6 and a maximum of 12 standards may be selected.

Once you have populated your standards, the standard concentrations in the left margin (under the '*Standards'* tab) will become editable (Figure 3). You may enter your standard concentrations either manually, or by copying and pasting (using the keyboard command Ctrl-C and Ctrl-V for PC or Cmd-C and Cmd-V for Mac) from any spreadsheet program. You may not select a concentration of zero for more than one standard designation.

If you ran a background well, select the standard by selecting the checkbox next to that standard value. If your background is not a member of the standard curve, check the desired 'Standard' checkbox and leave the 'Edit Concentration' field blank.

If you have checked a background check box, but wish to deselect, press the *Reset Background* button.

To continue analysis, you must enter your analyte name, as well as your desired unit of measure.

Figure 3 – Plate Layout

3.2. Assigning Unknowns (Samples)

Turn on the Unknowns function by highlighting the **Unknowns** button. At this point you should select whether you want to populate your unknowns by column (down arrow) or row (right arrow). Switch your arrow selection by clicking on the correct arrow. Next, select the number of unknown replicates in your analysis by using the '**Unknown Replicate**' drop down menu (e.g. select 1 if only one well per unknown was run, select 2 if duplicate unknown wells were run).

Select the wells you wish to populate as unknowns by clicking on a well and dragging your mouse to create the grid. You may also double-click on an individual well to either populate it or to un-populate it. The unknown designation (e.g., Unknown 1, Unknown 2) is determined by the direction of auto-population as well as your number of unknowns. A minimum of one unknown must be selected.

Once you have populated your unknowns, a chart listing your unknowns will appear in the left margin under the 'Unknowns' tab (Figure 4). You may alter the dilution factor for your sample as well as a Sample ID if you wish. You may enter your dilution factor or Sample ID manually or by copying and pasting (using the keyboard command Ctrl-C and Ctrl-V for PC or Cmd-C and Cmd-V for Mac). If no Sample ID is provided, your results will be listed under Y-axis value of the first replicate.

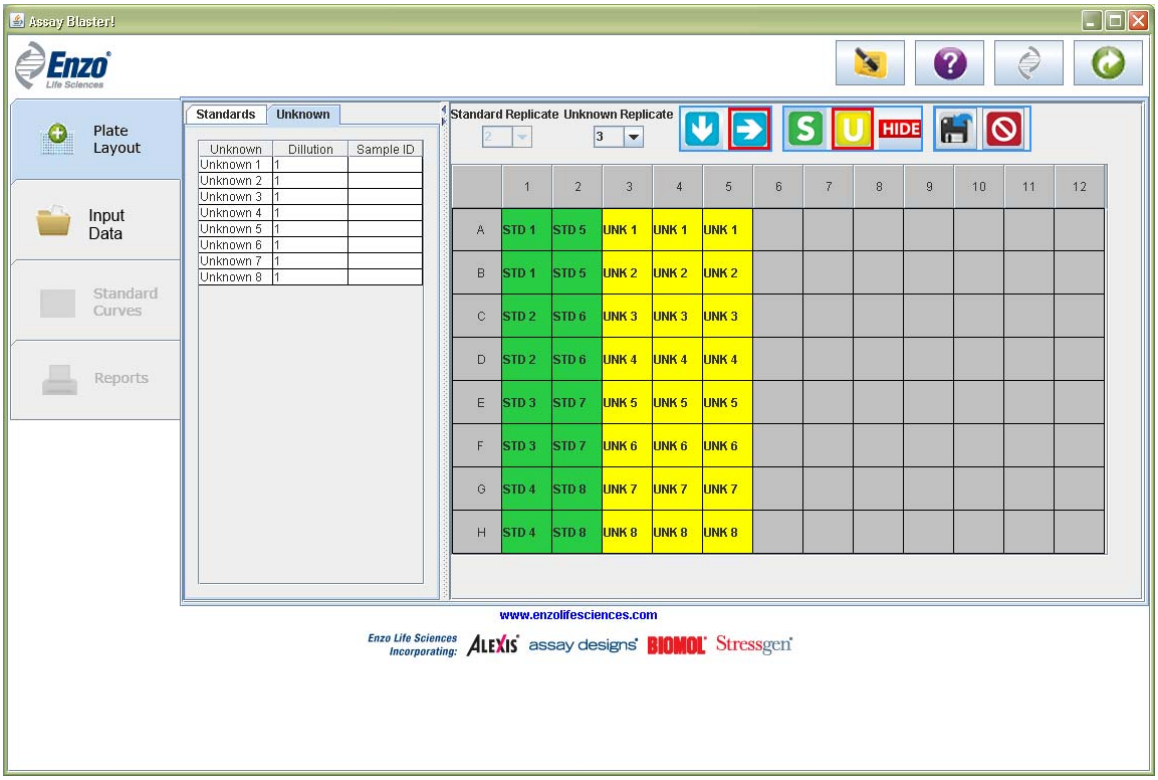


Figure 4 – Plate Layout

3.3. Hiding wells

If you wish to leave a selected well out of analysis you may use the 'Hide' function. This allows a user to skip wells that they do not wish to use in analysis. If a well is hidden, the value assigned to that well will not be used in analysis.

To hide a well, highlight the 'Hide well' button and double-click on the desired well.

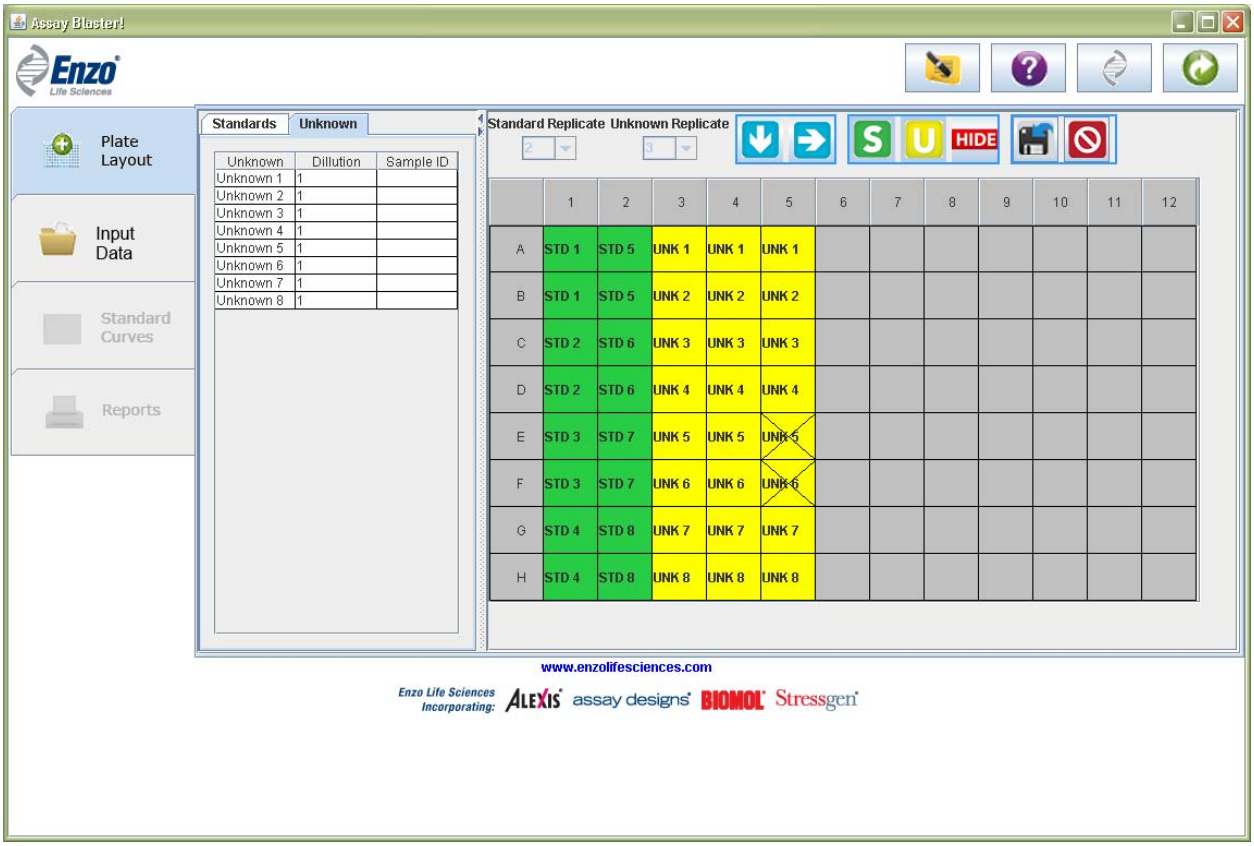


Figure 5 – Hiding wells

3.4. Clear all wells

If you wish to clear the plate layout of all designations, click the 'Clear all wells' button. A prompt will ask if you wish to proceed. If you clear the plate layout, all progress is lost and cannot be recovered.

3.5. Viewing well information

You may view a chart of your well information in the bottom section, below the plate layout (Figure 6). The Plate Layout chart includes information such as: well assignment, well ID, dilution (for unknowns) and sample ID (for unknowns). To select the wells you wish to display in the chart, first un-select your mode at the top of the layout. Then, either drag your mouse across the grid you wish to see, or double-click the individual well you wish to see.

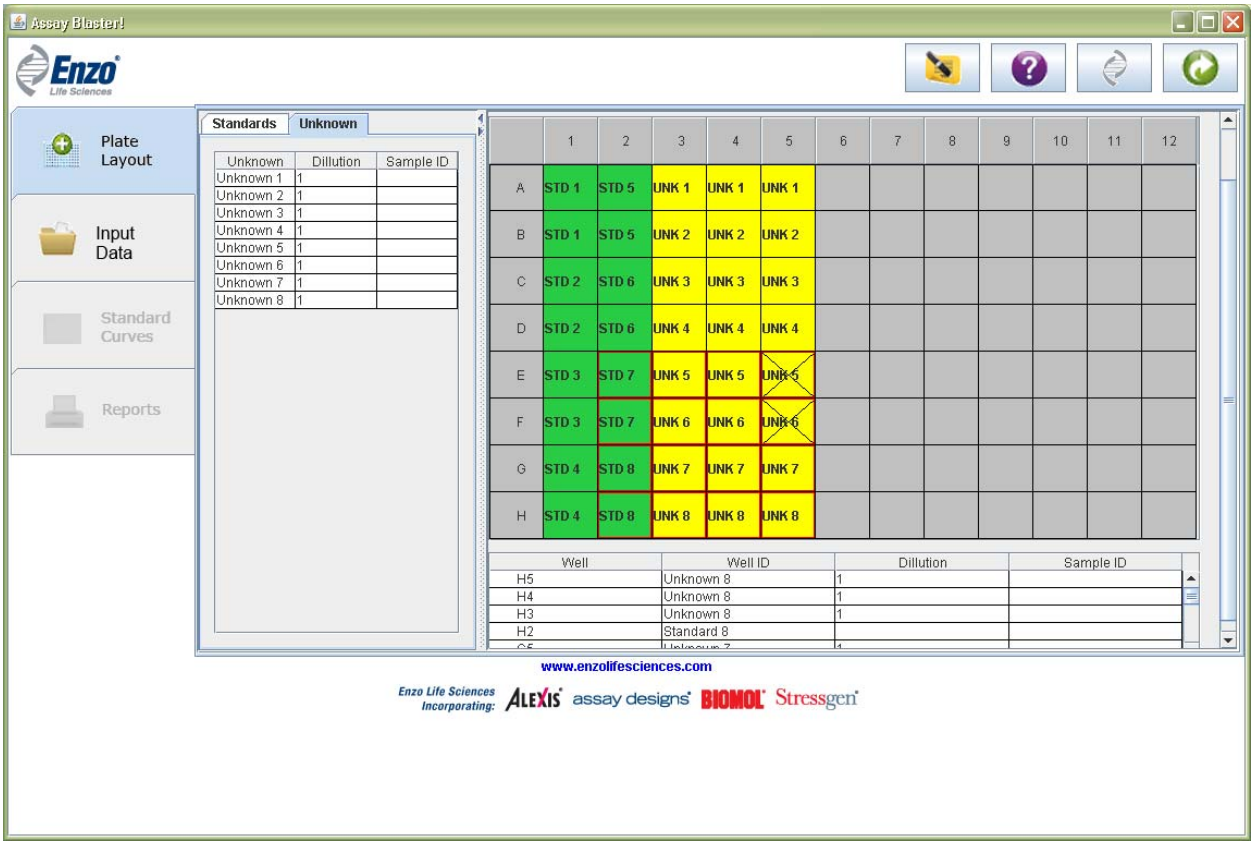


Figure 6 – Plate Layout Chart

3.6. Save plate layout

You may save your plate layout at anytime by selecting the '*Save plate layout*' button. Save the layout to your hard drive. If you would like to recall a saved plate layout, on the opening page, select 'I would like to use an existing experiment template' and find the saved file on your hard drive.

A saved layout will contain all your selections on the plate layout page, including: analyte name, unit, well designations, standard concentrations, dilutions of unknowns, sample ID's.

Proceed to the next page by selecting the *Proceed to "Input Data"* button or by selecting the '*Input Data*' tab.

4. *Input Data*

The Input Data tab allows you to assign values to each designated well. See Figure 7 to view the functions and buttons included on the Input Data toolbar. You must enter a textual description for the Y-axis label.

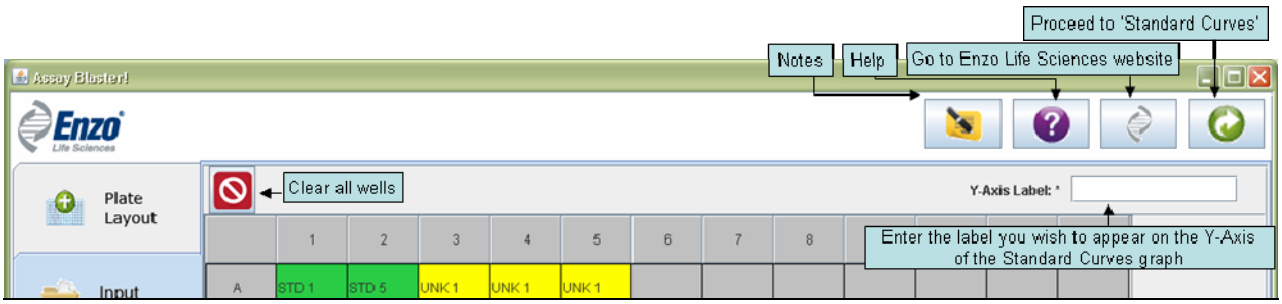


Figure 7 – Input Data

4.1. Assigning values to wells

To assign values to the wells, copy and paste your data (using only keyboard commands) from an excel spreadsheet. All copied data (within a 12x8 grid) will be pasted in the display, however, only data within the designated standard and unknown wells will be used in analysis.

Figure 8 – Input Data

4.2. Hidden wells

If your plate layout contains a hidden well you will not be able to assign a value to that well. If you try to paste into a hidden well, the value will be skipped (see Figure 9).

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD 1 0.194	STD 1 0.189	UNK 1 1.154	UNK 1 1.721	UNK 1 1.8							
B	STD 2 0.322	STD 2 0.291	UNK 2 1.072	UNK 2 1.064	UNK 2 1.068							
C	STD 3 0.518	STD 3 0.443	UNK 3 0.67	UNK 3 0.67	UNK 3 1.017							
D	STD 4 0.727	STD 4 0.625	UNK 4 0.48	UNK 4 0.477	UNK 4 0.441							
E	STD 5 0.943	STD 5 0.825	UNK 5 0.378	UNK 5 0.417	UNK 5							
F	STD 6 1.113	STD 6 0.924	UNK 6 0.277	UNK 6 0.267	UNK 6							
G	STD 7 1.206	STD 7 1.026	UNK 7 0.25	UNK 7 0.241	UNK 7 0.318							
H	STD 8 1.277	STD 8 1.056	UNK 8 0.226	UNK 8 0.231	UNK 8 0.3							

Figure 9 – Input Data – Hidden wells

4.3. Clear all wells

If you wish to clear the plate of all file selections, click the '*Clear all wells*' button. A prompt will ask if you wish to proceed. If you clear the values, all selections are lost and cannot be recovered.

4.4. Viewing well and file information

You may view a chart of your well and file information in the bottom section of the window (Figure 10). The File Selection chart includes information such as: well assignment, well ID, dilution, sample ID, and signal value. To select the wells you wish to display in the chart, either drag your mouse across the grid you wish to see, or double-click the individual well you wish to see.

Figure 10 – Well Information

You may increase the viewing area by selecting the arrows on the border separating the chart and plate sections.

Proceed to the next page by selecting the *Proceed to "Standard Curves"* button or by selecting the '*Standard Curves*' tab.

5. Standard Curves

The Standard Curves page displays the standard curve created using selected parameters and settings for the standard data files. Figure 11 displays the buttons and functions included on the Standard Curve page.

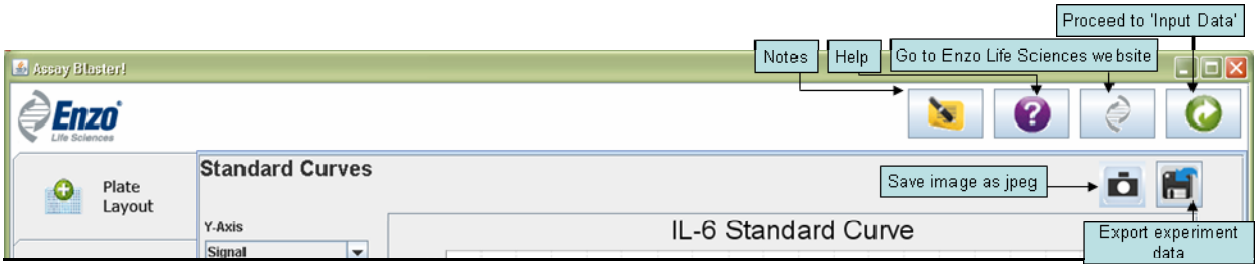


Figure 11 – Standard Curves page

Figures 12 through 17 show sample standard curve plots generated using various parameters.

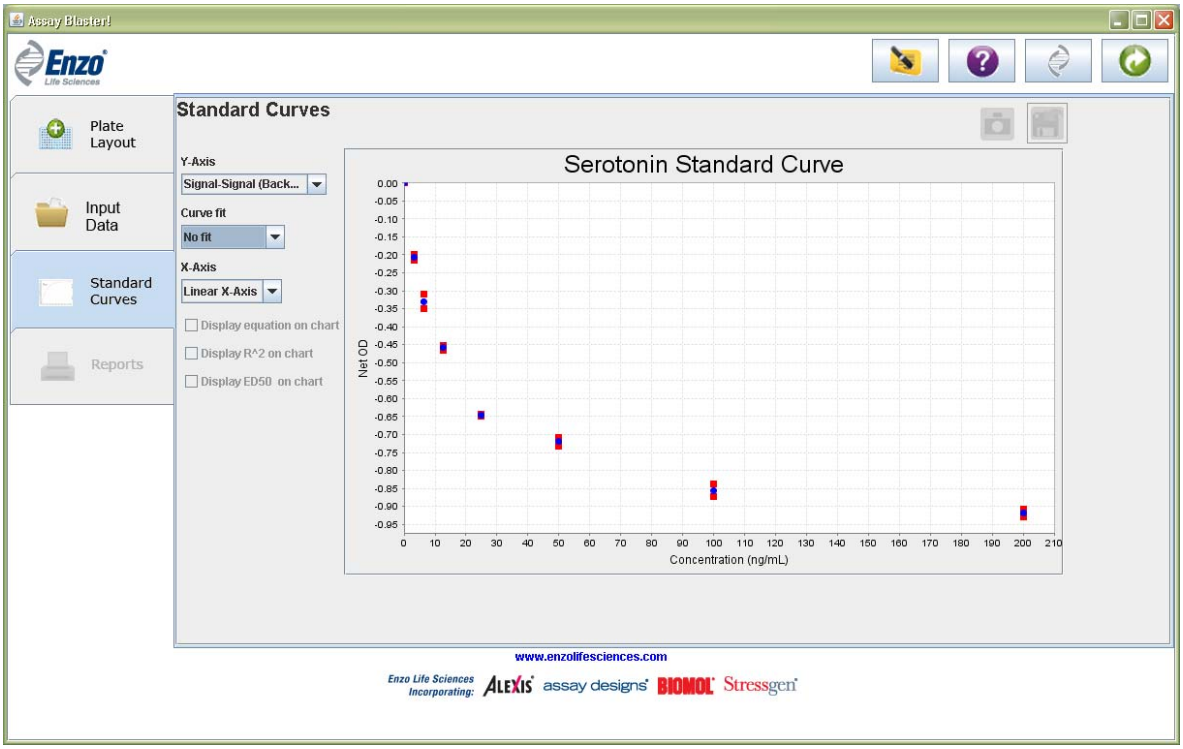


Figure 12 – Duplicate series No-fit plot using Linear X – Axis and Signal-Signal (Background) Y-axis (Competitive)

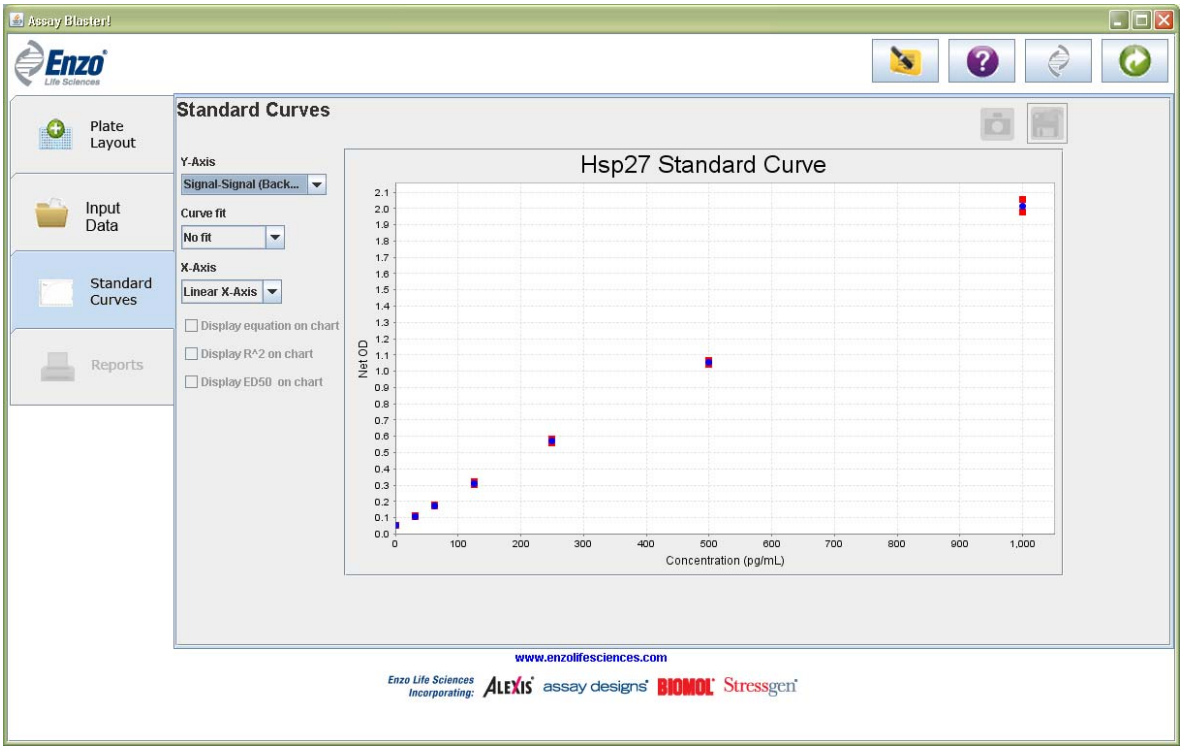


Figure 13 – Duplicate series No-fit plot using Linear X-Axis and Signal-Signal (Background) Y-axis (Immunometric)

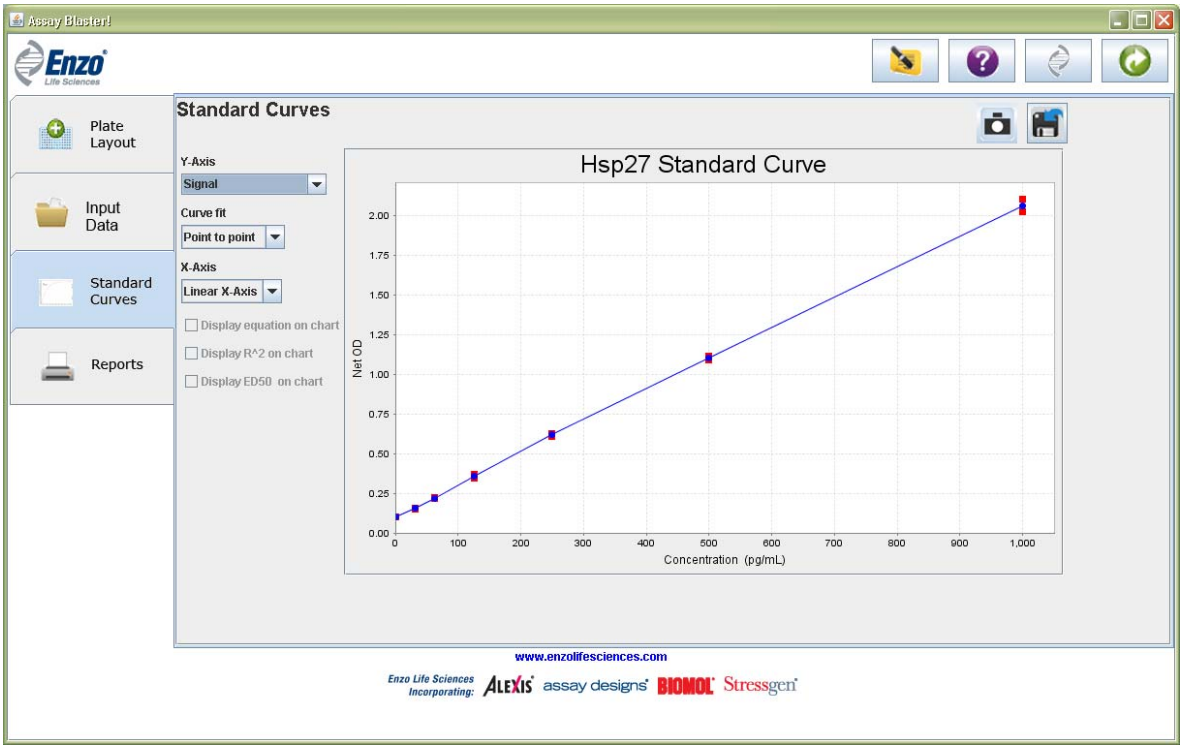


Figure 14 – Duplicate series Point-to-point plot using Linear X-Axis and Signal Y-axis (Immunometric)

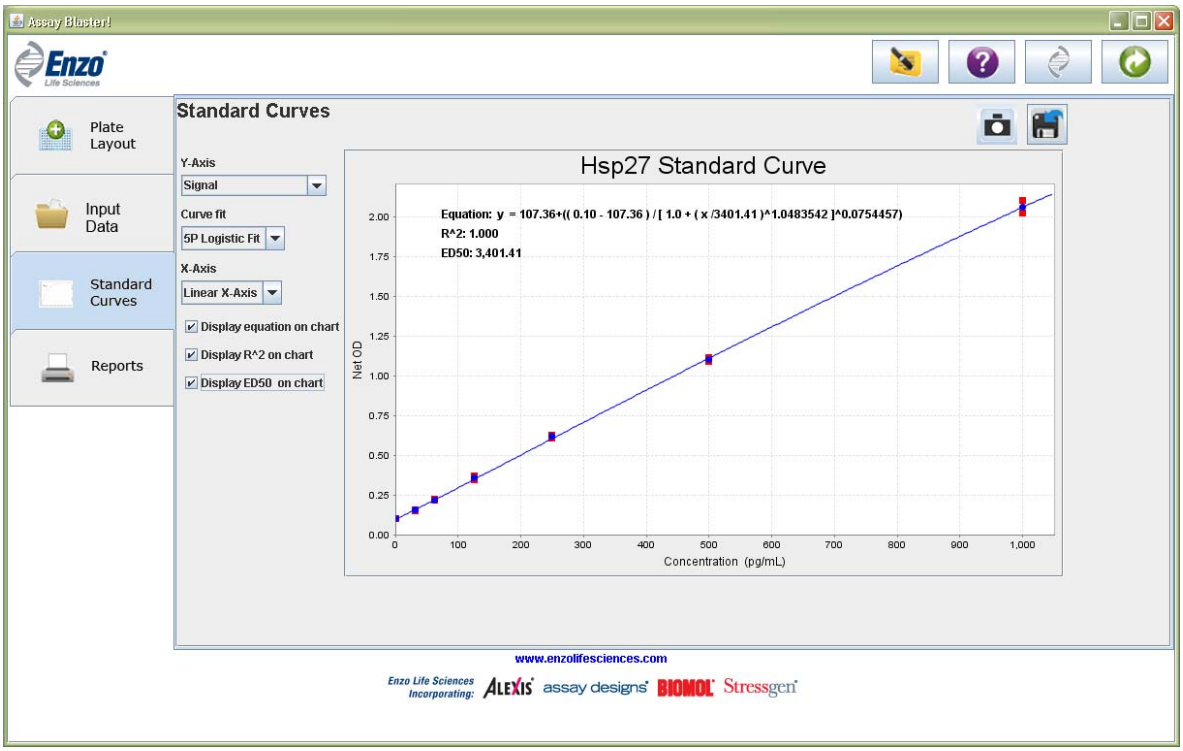


Figure 15 – Duplicate series 5P Logistic Fit plot using Linear X-Axis and Signal Y-axis (Immunometric)

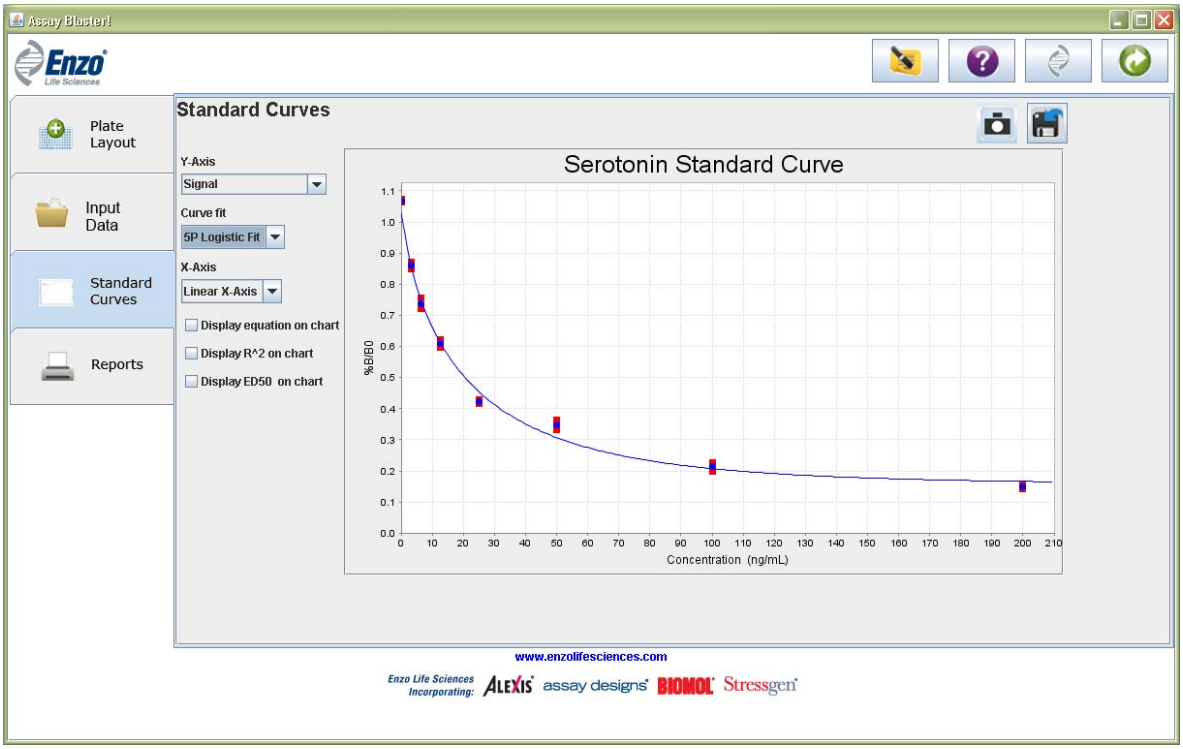


Figure 16 – Duplicate series 5P Logistic Fit plot using Linear X-Axis and %B/B0 Y-axis (Competitive)

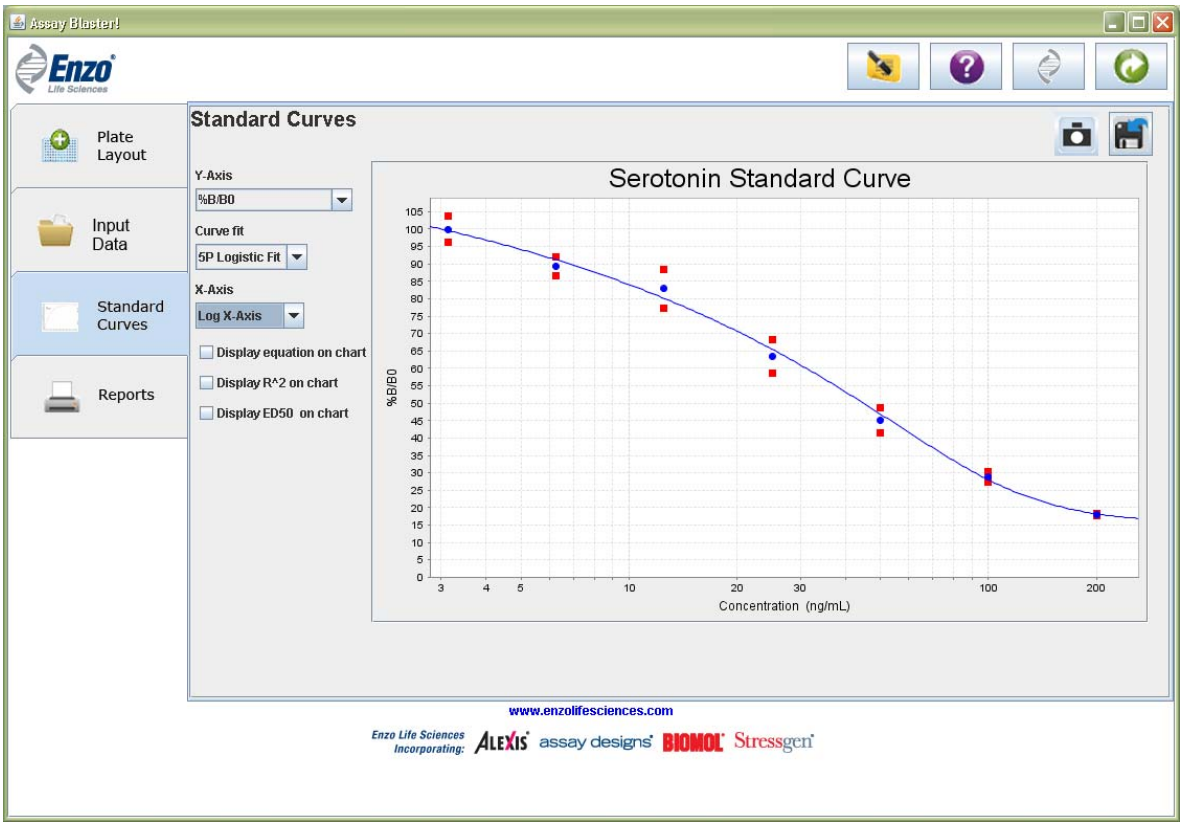


Figure 17 – Duplicate series 5P Logistic Fit plot using Log X -Axis and %B/B0 Y-axis (Competitive)

To create a more precise curve fit, you may delete outliers from your analysis by right-clicking the desired point and clicking '**Remove**' button. If you have chosen a single data series, this will remove the entire

concentration point. If you have chosen a duplicate or triplicate data series, a new mean will be determined with the remaining points. You may add these points back in by right-clicking the point and selecting '**Add**'.

There are three options you may select between for the Y-axis: Signal-Signal (Background), %B/B0, and Signal. All standard points are shown in a Linear-scale X-axis Signal or Signal-Signal (Background) plot. A zero standard will not be shown in a Log-scale X-axis or in a %B/B0 plot.

You can switch between the linear or logarithmic presentation of the standard curve by clicking the **Axis** dropdown box and then selecting either **Linear X-axis** or **Log X-axis**.

The default 'fit' is the '*no fit*' option. You must select a fit before proceeding.

The application offers three types of interpolation models:

- Point-to-Point
- 4P logistic fit
- 5P logistic fit

All curve models will show the selected unit of measure on the x-axis against your selected Y-axis.

If you choose **Point-to-Point** the application will interpolate between two adjacent points using a line (y = ax + b). In the **4P logistic fit** the application creates a curve using best curve fit (y = ((a - d) / (1 + (x/c) ^ b)) + d). If you choose **5P logistic fit** the application creates a curve using best curve fit (y = ((a - d) / ((1 + (x/c) ^ b) ^ g) + d). You should choose the curve model which offers the best fit for your particular set of data.

You may save an image of the standard curve as a .jpeg file by clicking the **Save image as jpeg** button from the upper toolbar.

To save all settings (sample files used, curve fitting parameters, exclusion of data points from the analysis, etc.) for later analysis, you can click on the **Export experiment data** button on upper toolbar. A pop up window will display experiment details up to this point. Confirm with **OK** and then select the directory in which you would like to save the file. A file including all settings will be generated in .xml format.

You can import this saved setting file on the opening page. Select 'I would like to use an existing experiment template' and find the saved XML file on your hard drive. A saved experiment will contain all parameters and selections up through the '*Standard Curves*' tab.

The three check boxes at the bottom left allow you to view statistical data about your 4P and 5P Logistic Fit graphs on the standard curve plot. If you click on the **Display equation on chart** checkbox, the equation will be displayed for that plot with actual values for that curve fit. If you click on the **Display R-squared on chart** checkbox, the R-squared value will be displayed for that plot. If you click on the **Display ED50 on chart** checkbox, the ED50 value will be displayed for that plot. (See Figure 15). If selected, these options will be displayed on the graphs in the RTF report.

Proceed to the next page by selecting the *Proceed to "Reports"* button or by selecting the '*Reports*' tab.

6. *Reports*

6.1. Reports Page

The reports page allows you to customize your RTF report (Figure 16). RTF (rich text format) files may be viewed in most popular word processing software. To select components of your report, highlight the components and select the '*Include component in the report*' button. To remove a selected component, select the '*Exclude component from the report*' button. To select multiple components, hold down the control key while selecting. You may rearrange the order of report components by selecting the component and using the '*Move up*' and '*Move down*' buttons.

Once you are satisfied with your report components, select the '*Generate RTF report*' button. You will be prompted to save your report before viewing it. If you would like to alter your report, simply keep the '*Reports*' page open and re-create your RTF report.

Figure 16- Results page

7. *RTF report examples*

There are 5 report components that may be included in your RTF:

7.1. Input Data Tab Image

Displays the raw data within the assigned plate layout.

7.2. Notes

Displays any notes made during your analysis.

7.3. Sample Data Table (Values)

Displays resulting concentrations for each unknown designation.

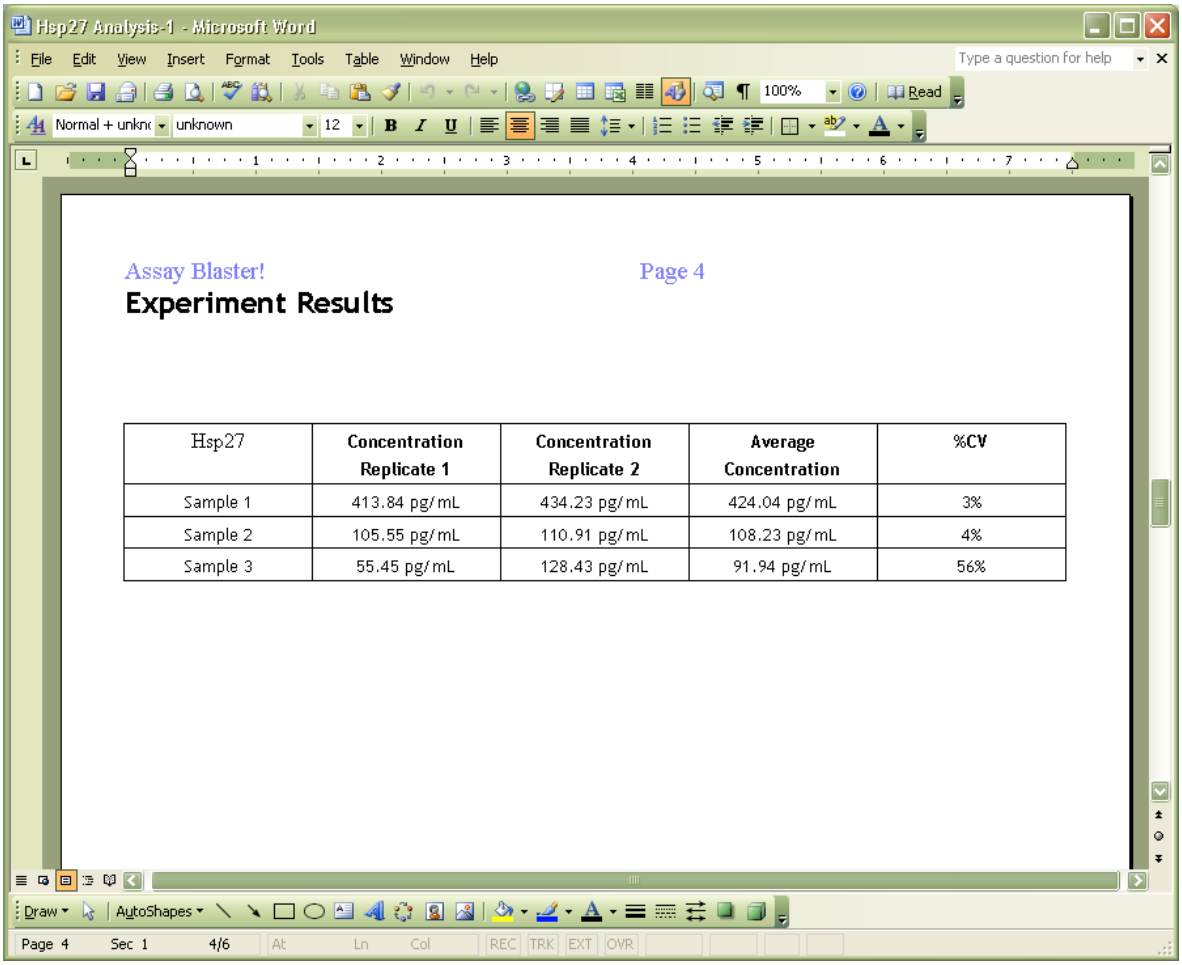


Figure 17-Sample Data Table Results

7.4. Standard Curve Graph

Displays the Standard Curve Graph as selected on the Standard Curves tabs (Figure 17).

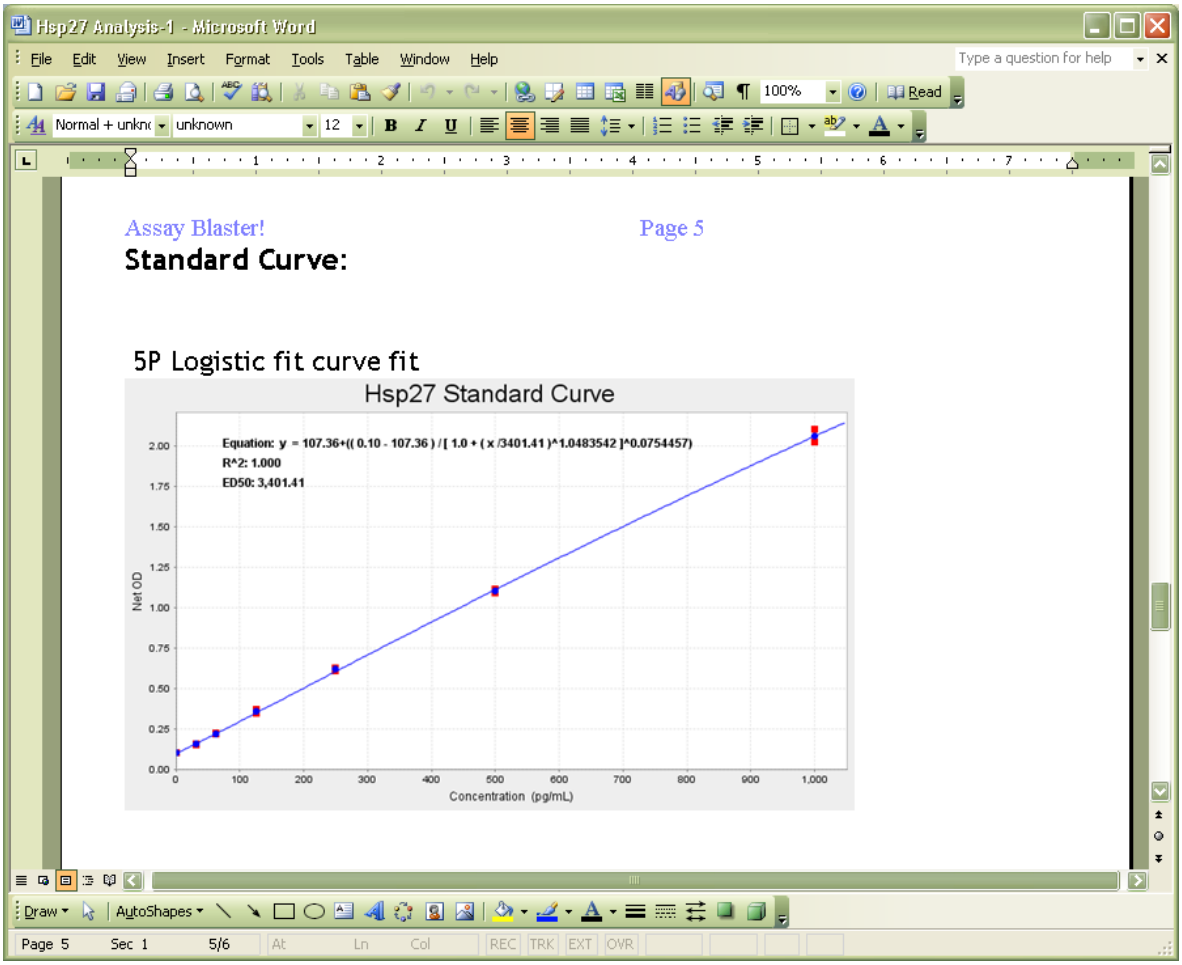
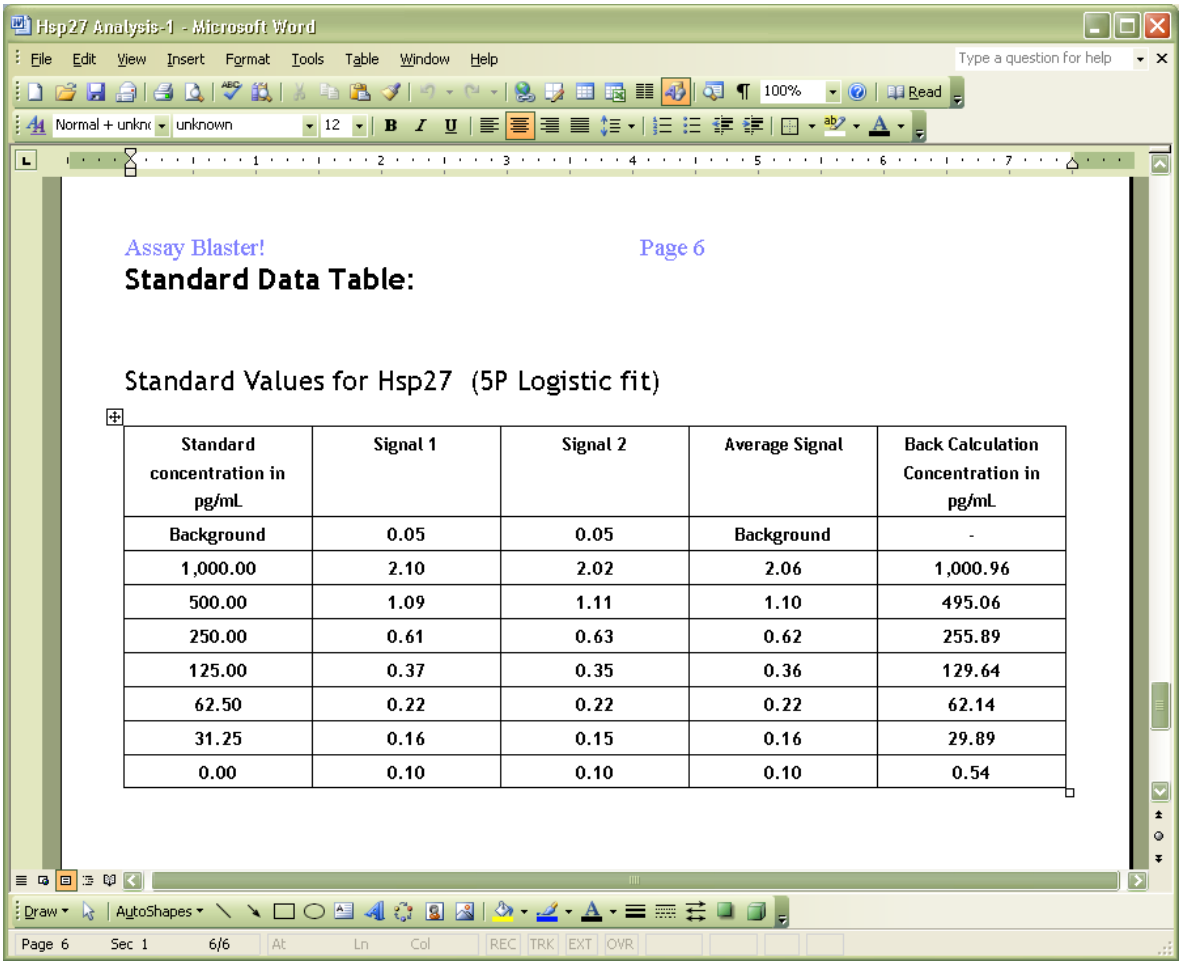


Figure 18-Standard curve graphs

7.5. Standard Data Table

Displays concentration, Y-axis value, and back-calculated value for your standard curve. (Figure 18).



Standard concentration in pg/mL	Signal 1	Signal 2	Average Signal	Back Calculation Concentration in pg/mL
Background	0.05	0.05	Background	-
1,000.00	2.10	2.02	2.06	1,000.96
500.00	1.09	1.11	1.10	495.06
250.00	0.61	0.63	0.62	255.89
125.00	0.37	0.35	0.36	129.64
62.50	0.22	0.22	0.22	62.14
31.25	0.16	0.15	0.16	29.89
0.00	0.10	0.10	0.10	0.54

Figure 19-Standard data table

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