illuminate your data

Belysa™ Immunoassay Curve Fitting Software is a platform developed in-house that allows for the re-curve fitting, examination and comparison of data acquired from a variety of immunoassay platforms.

Over the course of a study, scientists can run numerous single or multiplex assays to generate the data required for their statistical analyses. Each plate run has the potential to introduce variability by the end user or the manufacturer which can lead to inaccuracies in the reported data. Belysa™ software is designed to aid the end user to understand the integrity of the raw data generated from their experiment and how it compares with other similar experiments used within the study.

Belysa™ Software Features and Benefits

Belysa™ software prioritizes ease-of-use with the capability to conveniently drag-and-drop your .csv file from an xPONENT® bearing Luminex® reader or SMCxPRO™ instrument into the Belysa™ software with the data immediately displayed for analyses.
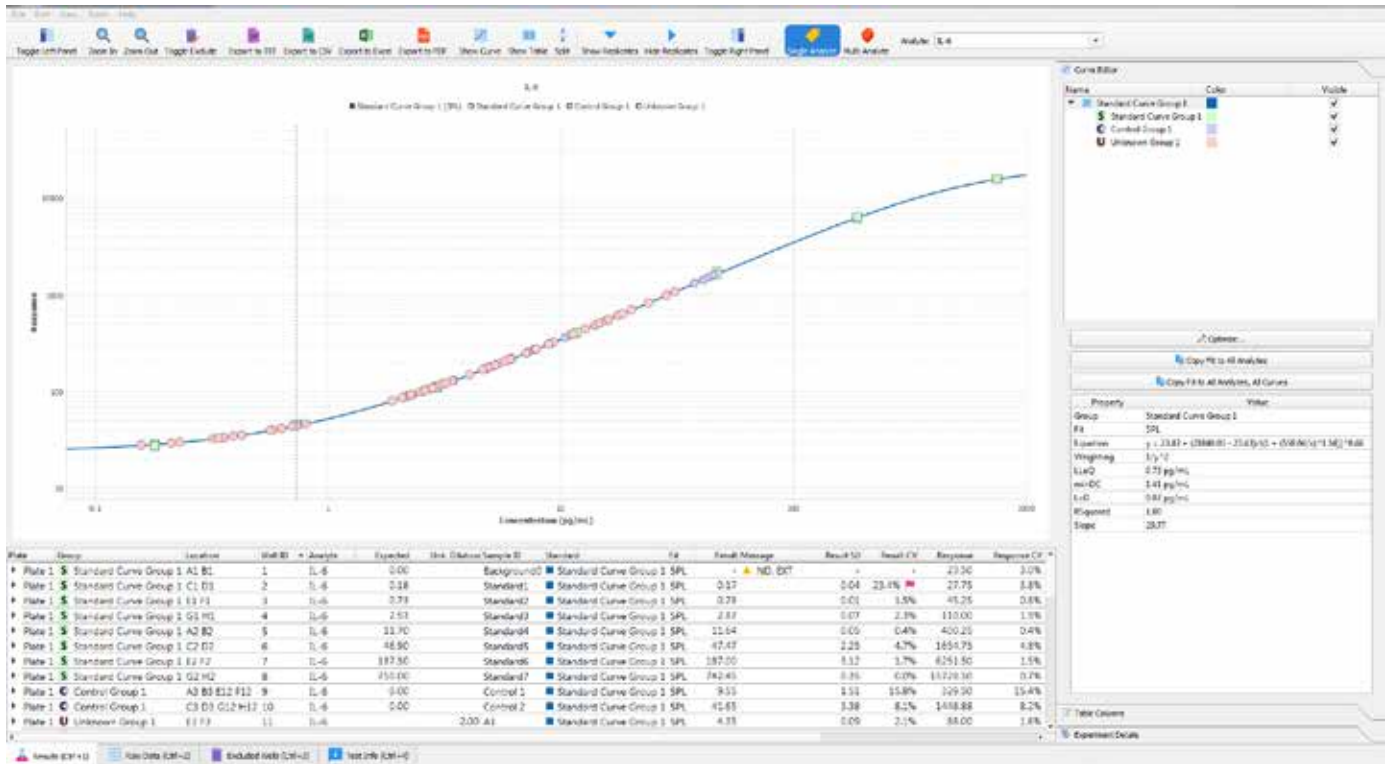

Fig 1: Belysa™ software standard curve analysis using IL-6 data (MILLIPLEX® cat. HSTCMAG-28SK).

Belysa™ software accepts files from three sources, Luminex® multiplex immunoassay readers (MAGPIX®, LX200™, FLEXMAP 3D®) using the xPONENT® or Bio-Plex® Manager acquisition software, the SMCxPRO™ platform or ELISA readers. Depending on the format, the data may require less or more annotation within the software. The displayed options and information include:

- Plate map (96 & 384 well)
- Experimental setup
- Curve with displayed points
- Curve optimization wizard
- Raw and interpolated data table
- Statistics, curve and line equations
- Single and multiple analyte views
- File exportation (.csv, .txt, Excel, and PDF).

Curve Fitting:
The first tool researchers may utilize is the curve fitting function. By clicking on "optimize", Belysa™ software will select from the following fits or the user can assign a preferred fit manually. Curve fits include:

- 4pl or Robust 4pl
- 5pl or Robust 5pl
- Linear
- Cubic spline

Once fitted, Belysa™ will adjust the information derived from the curve which includes:

- Line equation
- LLOQ (Lower Limit of Quantitation)
- MDD (Minimum Detectable Dose)
- LoD (Limit of Detection)

Should the user eliminate points from the curve, or curves in a multiplex, Belysa™ software will automatically refit the curve and back calculate all data derived from it. This tool will allow researchers to swiftly optimize their curves in an environment where they can assess the consequence of any changes almost immediately.

visualizing variance

The Raw and Interpolated Data

Examining raw data is a crucial but laborious task. Belysa™ software aids researchers by flagging wells for attention using an automatic and user defined rules-based system.

Message	Result SD	Result CV	Response	Response CV
⚠ EXT, ND	-	-	15.75	2.2%
⚠ BLOQ	0.03	3.6%	35.50	2.0%
⚠ BLOQ	0.08	10.4%	32.75	5.4%
⚠ BLOQ	0.13	16.4%	33.00	8.6%
⚠ BLOQ	0.03	6.0%	27.50	2.6%
⚠ BLOQ	0.03	3.8%	34.50	2.0%
⚠ BLOQ	0.03	4.2%	32.50	2.2%
⚠ BLOQ	0.03	3.4%	36.50	1.9%
⚠ BLOQ	0.18	25.9% 🚩	30.75	12.6%
⚠ BLOQ	0.00	0.0%	23.00	0.0%
⚠ BLOQ	0.05	13.3%	23.75	4.5%
⚠ BLOQ	0.06	27.1% 🚩	21.00	6.7%
⚠ BLOQ	0.03	10.5%	22.50	3.1%
⚠ BLOQ	0.10	10.7%	35.50	6.0%

Fig 2: Granular raw data output view including rules based (yellow) and user defined (pink) alerts. The page is selectable allowing columns to be excluded and resized as necessary.

The raw data view contains a series of reselectable columns which allow the viewer to quickly ascertain crucial information concerning their assay. Two types of alerts include:

- Yellow triangles indicate hard rules, such as data that was Non-Detectable (ND) or sample values that fell below LLOQ (BLOQ).

- The pink flag indicates where a user defined rule has been broken and will encompass physical parameters such as the bead count of a Luminex® immunoassay, as well as calculated values, including % CV of replicates or the % recovery of a control or standard point.

Using these tools, a user can easily decide and record what wells are excluded and whether the sample values derived can be trusted for onward statistical analysis.

Belysa™ Software Curve and Mathematical Parallelism Comparison

The standard curve of an assay can provide crucial information to an end user concerning the reproducibility of a method over multiple plates within specific runs or over the course of a longitudinal study. In the context of the Belysa™ software, parallelism is the similarity of the curves at the mathematical level and not the biological definition where an endogenous and recombinant version are compared in a like matrix across a dilution series.
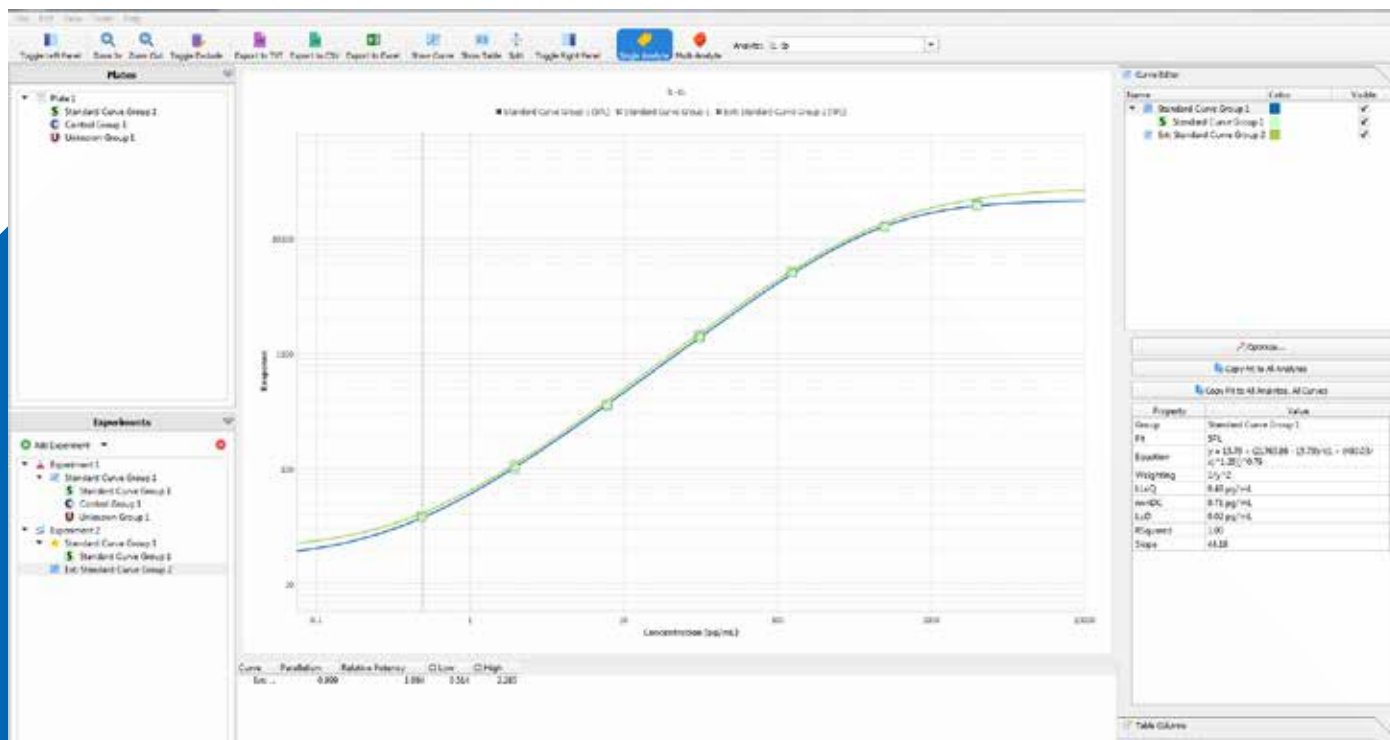

Fig 3: The mathematical parallelism of two user generated IL-1b curves from separate lots of HSTCMAG-28SK (MILLIPLEX®), with the initial run used as the reference curve.

Belysa™ software provides researchers with a curve comparison tool which allows a user to compare multiple curves against each other by simply importing the curve from a previously saved Belysa™ output file. The first curve is used as a reference curve to which each subsequent curve is compared. Two tests are currently available.

1. Parallelism value
Compares the statistical similarity of the curves and is of value when comparing completed immunoassays against each other. This test ensures that sample values will be consistent, irrespective of the assay from which they are derived.

2. Relative potency
Relative potency is primarily used to show the consistency of individual reagents in biopharmaceutical products. This test is useful for assay developers looking to compare the activity of individual components as they build their protocol.

By comparing the curves after an assay run the user can confirm that the experiments were consistent with those run previously, confirming the integrity of their method.

We offer a number of platforms that are directly supported by the Belysa™ Curve Fitting Software

Luminex® MAGPIX® with xPONENT® Software
A benchtop unit designed specifically for multiple analyte analysis, the MAGPIX® is a flexible and cost-effective instrument which can also provide access to over 600 soluble protein biomarkers through the MILLIPLEX® range of assays. The LX200™ and FLEXMAP 3D® systems are also available, each with enhanced and unique capabilities.

SMCxPRO™ Platform
SMC™ technology provides maximum immunoassay performance while following a workflow similar to traditional ELISA technology. By combining a unique assay elution step and robust digital counting, SMC™ technology achieves improved signal-to-noise ratios over traditional immunoassay technologies. The SMC™ platform delivers enhanced analyte quantification at both low and high levels of expression on one complete system.

800™ TS Absorbance Reader
Representing the most recent addition to the BioTek® product range, the user-friendly 800™ TS absorbance reader is supplied with a preset configuration of four filters (450, 590, 630 & 750nm). The platform is also available in a variety of options, ranging from the basic reader to a higher-end model with Gen 5 BioTek® software.

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System Requirements:
Belysa™ immunoassay curve fitting software requires Windows 7 SP1 or Windows 10 operating system (32-bit or 64-bit versions). One available USB-A port is required to attach the provided license dongle.

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