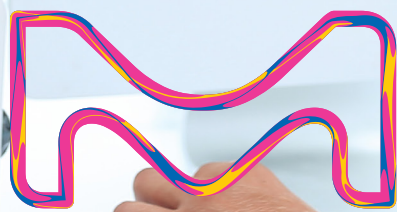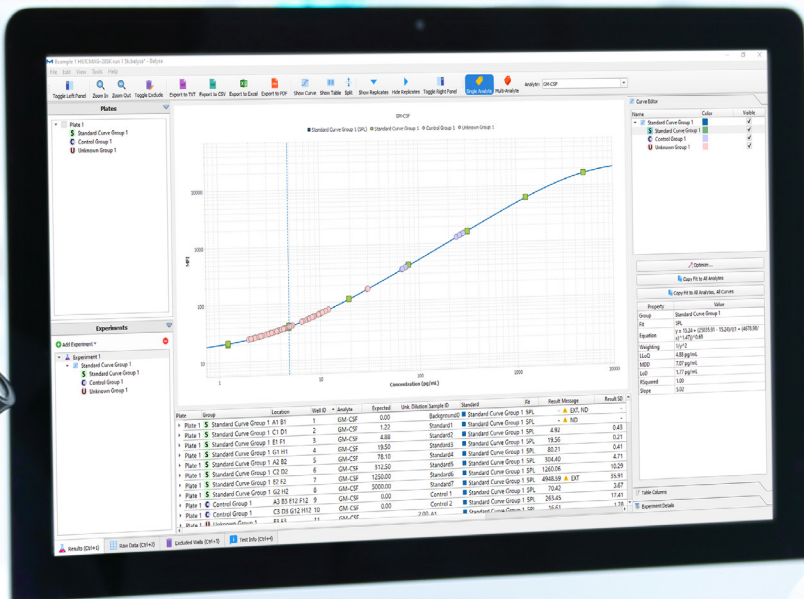# Merck

illuminate
Your Assay Performance

Belysa® Immunoassay
Curve Fitting Software

Millipore®

Preparation, Separation,
Filtration & Monitoring Products

Delve into your Data

Belysa® Immunoassay Curve Fitting Software is a platform we developed in-house that allows for the re-curve fitting, examination, and comparison of data acquired from multiple 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 helps users understand the integrity of the raw data generated from their experiment and how it compares with other similar experiments used within the study.

- Curve fitting (4pl, 5pl, Linear, Competitive, Cubic Spline)
- Rules-based data flagging
- Curve comparison tools
- User friendly with drag & drop interface
- Curve optimization wizard
- Suitable for use with Luminex®, SMCxPRO®, and ELISA data
- Compatible with Windows and macOS
- Cat. No. 40-122

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® software-bearing Luminex® reader or SMCxPRO® instrument into the Belysa® software with the data immediately displayed for analysis.
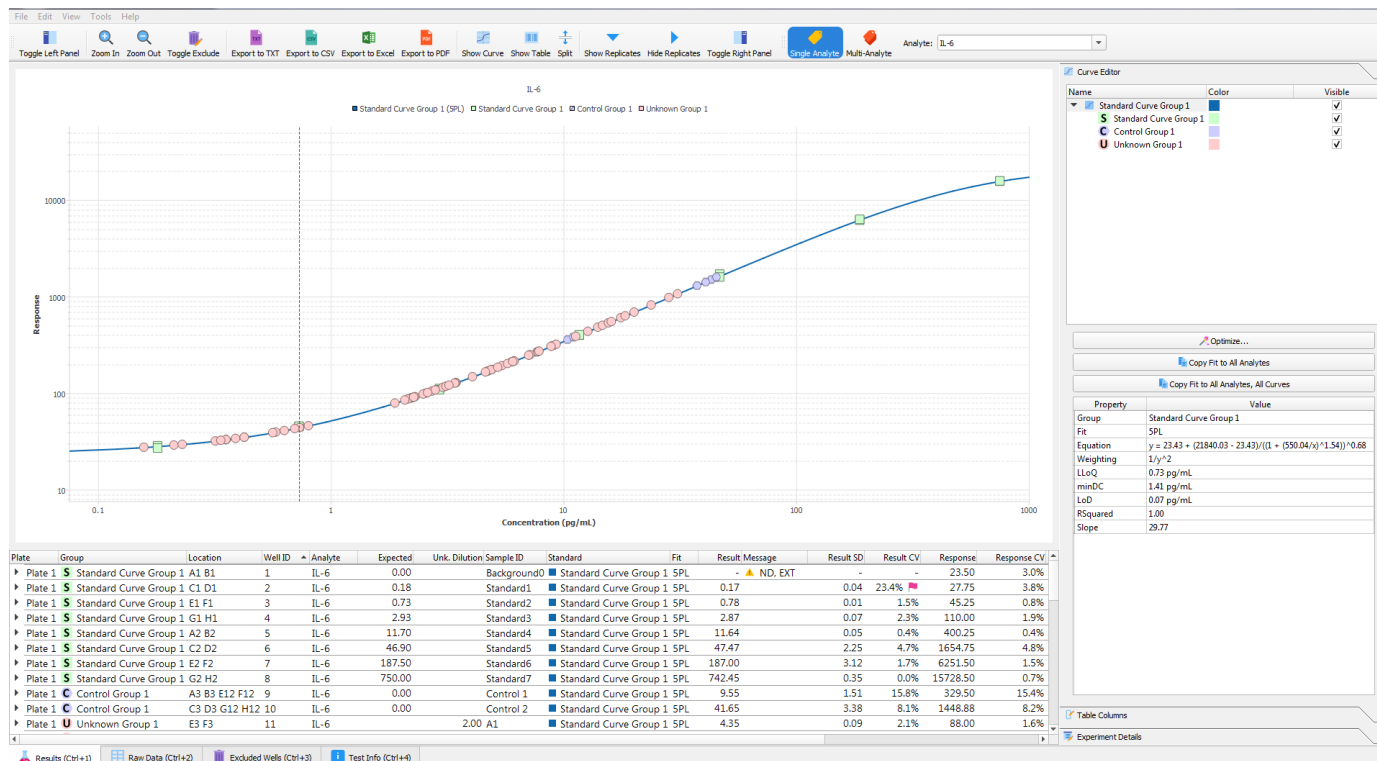

Figure 1. Belysa® software standard curve analysis using IL-6 data (MILLIPLEX® multiplex assay Cat. No. **HSTCMAG-28SK**).

Belysa® software accepts files from three sources, Luminex® multiplex immunoassay readers (xMAP® INTELLIFLEX®, Luminex® 200™, FLEXMAP 3D®, and MAGPIX® instruments) 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- and 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 use 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
- Competitive
- Cubic spline

Once fitted, Belysa® software 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%

Figure 2. Raw data output view including rules-based (yellow triangles) and user-defined (pink flags) 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 statistical analysis.

Curve and Mathematical Parallelism Comparison

The standard curve of an assay can provide critical 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 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.
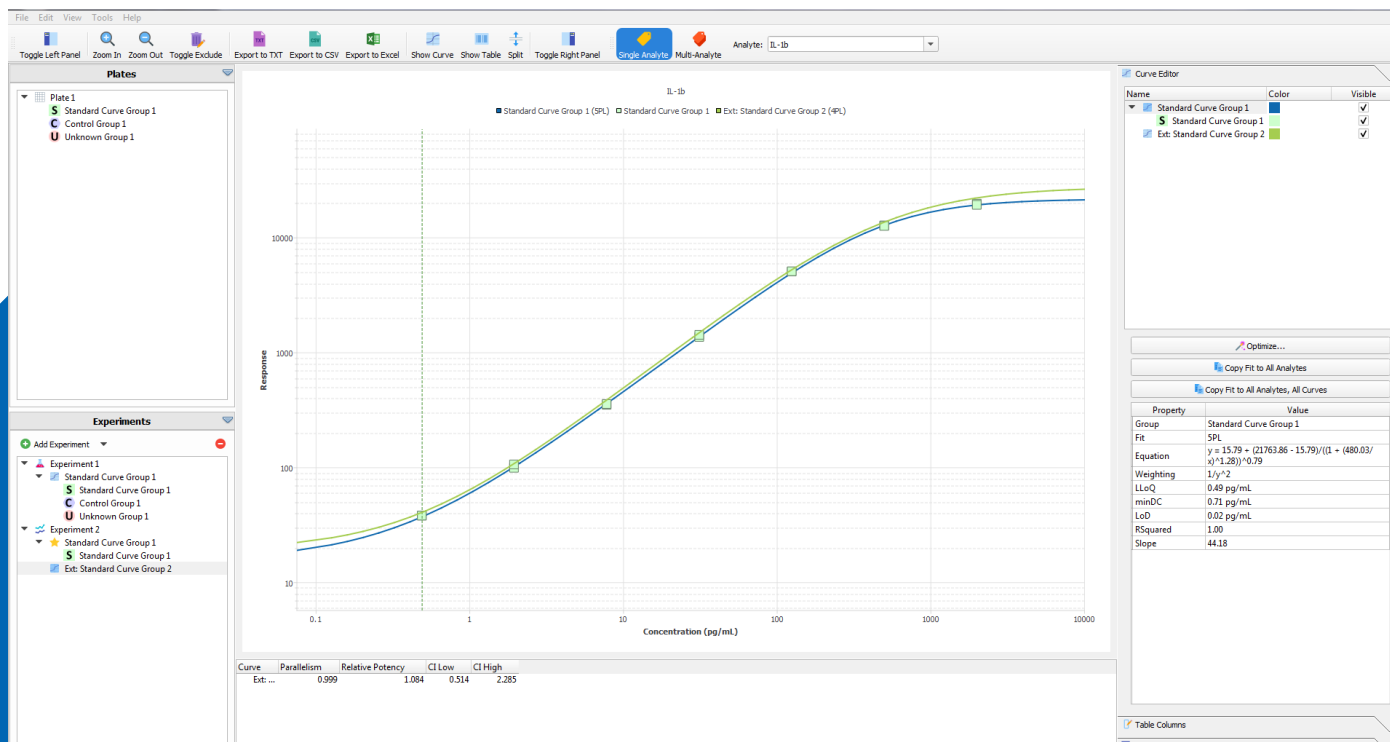

Figure 3. The mathematical parallelism of two user-generated IL-1β curves from separate lots of a MILLIPLEX® multiplex assay (Cat. No. **HSTCMAG-28SK**), with the initial run used as the reference curve.

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 helpful 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, users can confirm that the experiments were consistent with previous runs, confirming the integrity of their method.

We offer a number of platforms that are directly supported by the Belysa® Curve Fitting Software
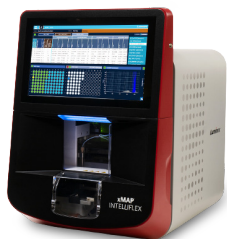
xMAP® INTELLIFLEX® System with xPONENT® Software

The xMAP® INTELLIFLEX® system is the newest instrument from Luminex® platforms. Belysa® software offers a user-friendly method to interpolate and check your plate data from this platform as well as the Luminex® 200™, FLEXMAP 3D®, and MAGPIX® instruments. Learn more at **SigmaAldrich.com/luminex**.

SMCxPRO® Platform

Ultrasensitive Single Molecule Counting (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. This platform delivers enhanced analyte quantification at both low and high levels of expression on one complete system. Learn more at **SigmaAldrich.com/smcxpro**.

BioTek® 800™ TS Absorbance Reader with Gen5™ Software

The user-friendly BioTek® 800™ TS absorbance reader is supplied with a preset configuration of four filters (450, 590, 630, and 750 nm). Explore BioTek® offerings at **SigmaAldrich.com/milliplex-washer**.

System Requirements:

Belysa® immunoassay curve fitting software requires Windows 7 SP1 or Windows 10 operating system (64-bit version) or macOS (beta version). One available USB-A port is required to attach the provided license dongle.

Discover more at **SigmaAldrich.com/belysa**
Or download updates at **SigmaAldrich.com/belysa-updates**

To place an order or receive technical assistance

Order/Customer Service: **SigmaAldrich.com/order**
Technical Service: **SigmaAldrich.com/techservice**
Safety-related Information: **SigmaAldrich.com/safetycenter**

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