Biacore™ Insight Evaluation Software provides you with a unified evaluation platform, designed to optimize your analysis of SPR data from Biacore™ 8K, Biacore™ 8K+, Biacore™ T200, and Biacore™ S200 systems (Fig 1). The modular design of the software allows you to maximize the application versatility of your Biacore™ systems, providing a scalable, easy to use, analysis platform that enables you to balance budget and time to result as your program or company grows.

Biacore™ Insight Evaluation Software provides:

- **Streamlined, easy to use approach to visualize and analyze kinetic and affinity data** with predefined evaluation methods and quality control tools to shorten time to decision.
- **Flexible analysis platform that grows with your project needs** via optional, add-on application specific extensions to maximize platform versatility, improve visualization and decrease time to result.
- **Evaluate your data like the experts in minutes not hours** with machine learning models pretrained by Cytiva's Biacore™ scientists and engineers for efficient and robust analysis of large data sets.
- **Simplified analysis of all your Biacore™ SPR data with a single software**: Analyze data from Biacore™ T200, Biacore™ S200, Biacore™ 8K, and Biacore™ 8K+ systems reducing the number of software licenses you need to maintain and train on.
- **Floating e-licenses** enabling large groups of users to access and benefit from software modules, while minimizing cost.
- **Easy export of data and compilation of results**: Quickly make figures for reports and presentations or transfer large data sets for aggregation and further analysis.
- **21 CFR Part 11 compliance** to enable integration into GxP-regulated workflows.

Fig 1. Biacore™ Insight Evaluation Software is a modular design software for evaluation of interaction analysis data, screening, kinetics, affinity, concentration, and relative potency data.

Biacore™ Insight Evaluation Software provides support to you, irrespective of experience (Novice vs Expert), use case (Academia vs Industry), or stage of the drug discovery workflow. This modular, toolbox approach to data analysis allows the user to pick the right tool for the right analysis job. Offering optimized solutions for key applications via optional add-on software extensions providing additional functionality and tools to further streamline your analysis and reduce time to result.
Efficient evaluation of binding level, affinity, and kinetic data for everyone

Biacore™ Insight Evaluation Software is a powerful tool for kinetic and affinity evaluation. The standard, entry level software allows you independent of level of experience to confidently evaluate SPR data and format it for reporting with a few clicks. From the analysis of large screening campaigns to deep kinetic characterization of single interactions, the generic tools scale with the size of your experiment providing results you can trust.

The flexible evaluation interface (Fig 2) is configurable to maximize the space for your most important tasks at any time:

- Get a rapid overview and qualify your data fast.
- Utilize flexible tools to customize your data analysis.
- Easily export and share your results using Microsoft® PowerPoint® format.
- Simultaneously visualize your results from thousands of samples via a Result plot.

Predefined, application-specific Evaluation methods developed by Cytiva’s Biacore™ scientists and engineers allow you to standardize and simplify data analysis. Use as is or as a template for optimized user-defined evaluation methods, combined with relevant evaluation parameters, these may be saved in Evaluation methods, avoiding repetitive tasks. When opening a run file with the selected evaluation method, the first results are obtained in seconds, shortening time to decision.

Predefined, application-specific Evaluation methods developed by Cytiva’s Biacore™ scientists and engineers allow you to standardize and simplify data analysis. Use as is or as a template for optimized user-defined evaluation methods, combined with relevant evaluation parameters, these may be saved in Evaluation methods, avoiding repetitive tasks. When opening a run file with the selected evaluation method, the first results are obtained in seconds, shortening time to decision.

Simple quality control tools for easy assessment of your kinetic data

Automated quality control tools analyze SPR data fitting quality for the magnitude of kinetic constants, parameter uniqueness, bulk refractive index, and residuals; enabling novice users to interpret results with ease and confidence via a simple and visual traffic light user interface.

Fig 2. The flat interface of Biacore™ Insight Evaluation Software provides full overview while offering flexible tools for customized data analysis. The overview allows convenient simultaneous visualization of kinetics and affinity data providing Result table, On-off rate chart, and $K_d$ chart.

Biacore™ Insight Extended Screening and Characterization Extension enables a wider range of compound-target interactions to be characterized

- Evaluate challenging interactions such as LMW and fragment binding to transmembrane G-protein-coupled receptors (GPCRs) and bivalent protein interactions with enhanced support.
- Extend your kinetic analysis via additional kinetic fitting models and evaluation settings supporting heterogenous ligand and two state reaction models.
- Dedicated software and visualization tools for screening of LMW and fragments to speed up assay setup and streamline evaluation.

The optional, add-on, Biacore™ Insight Extended Screening and Characterization Extension enables a wider variety of fitting models (Fig 3) using maximum response ($R_{max}$) control and Blank subtraction. Sample responses can easily be normalized to account for differences in molecular weight of the analyte or for differences in the level of the captured ligand.

The extension also has three evaluation templates — Clean screen, Binding level screen, and Affinity screen. These evaluation methods guide the user and shorten time to results for the entire SPR workflow in fragment-based drug discovery, FBDD (Fig 4).

Clean screen (Fig 5) Enables the user to efficiently cleanup both LMW and fragment libraries and easily identify sticky, residual binders to the sensor chip surface, minimizing the risk of false negatives.

Binding level screen (Fig 6) allows users to rapidly identify, rank and prioritize primary hits for follow up analysis based off their binding response and binding behavior to the target, while excluding fragments with atypical binding/non desirable behavior.
**Fig 4.** Typical analysis workflow for FBDD and small-molecule screening using Biacore™ systems. The workflow generates reliable and conclusive results prior to structure analyses and transfer to medicinal chemistry.

**Fig 5.** LMW and/or fragment library cleanup using **Clean screen** evaluation. Sticky, residual binding is evaluated by assessing the difference in absolute baseline response between adjacent cycles in a run.

**Fig 6.** **Binding level screen** performed by assessing report point values and binding behavior based on curve shapes after reference subtraction and solvent correction. Fragments often bind very rapidly to the primary site followed by binding to secondary and tertiary sites. By using report point **Binding early** placed early during the fragment injection, the impact of such secondary and tertiary binding is reduced. An ideal fragment is expected to bind rapidly to steady state followed by rapid dissociation.

**Affinity screen** (Fig 7 and 8) enables users to verify target binding and obtain an estimate of steady-state affinities of fragments to the target. Affinities for fragments are often in the millimolar to high micromolar range. Fitting with a constant control determined $R_{\text{max}}$ facilitates determination of such low affinities. Figure 8 shows **Affinity screen** sensorgram evaluations.

**Fig 7.** With **Affinity screen**, affinity determinations are evaluated by plotting the response at equilibrium of a concentration series (A) and then fitting the data to a steady-state affinity model (B). Affinity is normally estimated by fitting the data to a one-site model, but selected data can easily be fitted with a multisite model where appropriate (B).

**Fig 8.** Evaluation of **Affinity screen** sensorgrams.
Biacore Intelligent Analysis™ — our machine learning software extension that significantly reduces the analysis bottleneck in fragment screening applications

- Reduces the time taken for you to analyze large data sets by more than 80%.
- Reduces user error and improves consistency in data analysis, driving standardization across projects and teams.
- Data annotation allows users to rapidly check and understand machine learning based decisions.
- Pre-trained prediction models by Cytiva’s Biacore™ scientists provide out-of-the-box functionality.
- Option to further train prediction model over time using your own data or start with an untrained, naive model.
- Prediction model manager allows user to import, export, track and manage prediction model versions.

Biacore Intelligent Analysis™ enables the rapid and automated analysis of large data sets by significantly reducing the manual, time-consuming steps of data curation and quality control. (Fig 9). This optional, add-on extension currently offers support for two analysis types, Binding level screen and Affinity screen for fragments. Biacore Intelligent Analysis™ comes with evaluation methods including prediction models, pretrained by Cytiva’s scientists, providing an out of the box ready solution for the analysis of fragment binding level and affinity screening data sets which have been validated to provide greater than 90% accuracy relative to human expert analysis with excellent sensitivity (> 87%) and specificity (> 90%).

Run your fragment Binding level screen or fragment Affinity screening experiments as before, then in Biacore™ Insight Evaluation Software apply Biacore Intelligent Analysis™ by choosing the relevant prediction option, selecting the Prediction model and the Model version you wish to apply, followed by Predict.

Biacore Intelligent Analysis™ for fragment Binding level screen applications, automatically analyzes and classifies your binding level data via a series of feature sets. Binder prediction quality automatically predicts the quality of the interaction (high, low, or uncertain). Binder prediction classifications are automatically provided for sensorgrams predicted to have low quality, to enable the user to understand how the Binder prediction quality was reached and trace that prediction back to the underlying sensorgram data. Binder prediction certainty is a percentage measure of how confident the model is in the prediction of Binder prediction quality (Fig 10).

Fig 9. Comparative example of steps in traditional user driven analysis of fragment affinity screen vs analysis steps using Biacore Intelligent Analysis™.

Fig 10. Fragment Binding level screen analyzed by Biacore Intelligent Analysis™.
Biacore Intelligent Analysis™ for fragment *Affinity screen* verifies target binding and automatically provides an estimate of steady state affinities. The prediction model automatically analyzes and assigns data into 3 categories *accepted, rejected, or uncertain* based on a series of features where each concentration series receive classifications that enable use of an appropriate steady state affinity model and provide the basis for accept and reject status. An *acceptance certainty* is calculated and shows how confident the model is in the prediction. In addition to these predictions, the prediction model will also automatically exclude deviant or outlier cycles from the affinity analysis prior to the prediction (Fig 11).

You can over-ride the predictions that any of the prediction models have made and assign your own analysis to the data by checking or unchecking the option boxes at any time. To make changes to the prediction model simply select *Train new version*. The prediction models you create (either from the blank/naive models or by retraining the pre-trained prediction models provided) can be managed, tracked, and annotated in the prediction model manager (Fig 12). You also have the option to import or export prediction models to share models between groups and collaborators to drive standardization in your analysis, projects, and programs.

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**Fig 11.** Fragment *Affinity screen* analyzed by Biacore Intelligent Analysis™ software.

**Fig 12.** Manage, track, and annotate prediction models in the *Prediction model manager*, and import or export them to share with collaborators.
Biacore™ Insight Epitope Binning Extension for rapid overview of epitope diversity

- Illustrate your protein epitope diversity quickly – from assay setup to data interpretation.
- Built in support for main binning formats – sandwich, tandem, and pre-mix assay formats.
- Overcome unstable binding of antigen to primary antibody with **Dual** injection.
- Single evaluation solution for your epitope binning experiments from Biacore™ 8K+, Biacore™ 8K, Biacore™ T200, and Biacore™ S200.

Biacore™ Insight Epitope Binning Extension enables automated identification and control to maintain unique and diverse epitopes that may broaden intellectual property protection. This add-on, application-specific extension provides support from run setup through to data evaluation, including predefined methods and an automatic sample plate layout tool to shorten your assay development. All three main assay formats for epitope binning analysis are supported; sandwich, tandem, and premix (Fig 13).

A challenge in epitope binning is that the low affinity of binding between the antigen and the first antibody can lead to the dissociation of the antigen, resulting in the underestimation of binding level of the second antibody. **Dual** command compensates for this by injecting the antigen and the second antibody solutions in sequence with no intermediate washing steps, minimizing the dissociation of antigen before the secondary antibody is injected. (**Dual** injection is also included in Biacore™ Insight Extended Screening and Characterization Extension.)

A predefined evaluation method automatically processes data generated on Biacore™ 8K+, Biacore™ 8K, Biacore™ T200, or Biacore™ S200 with the relevant method settings. Sensorgram overlay shows cut-off and read-out intervals. The sensorgrams are automatically aligned and injections are assigned according to the method. The heatmap provides a rapid overview of blocking, non-blocking and uncertain antibody pairs and allows straightforward adjustment of cut-offs in the corresponding sensorgrams. Additionally, the new bin chart provides an intuitive and easy approach to illustrate and visualize epitope diversity. Different bins containing corresponding antibodies are displayed in different colors, clusters of overlapping bins are separated by gaps, and can additionally show bin connections (i.e. unidirectional blocking behavior), curve markers and antibody tags.

The streamlined evaluation support in Biacore™ Insight Epitope Binning Extension makes data evaluation efficient through the automatic analysis and visualization of bins (Fig 14).

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**Fig 13.** **Dual** injection minimizes the dissociation of antigen before second antibody is injected, thus facilitates data interpretation. (A) Weak interaction between first antibody and antigen may lead to underestimation of second antibody level. (B) **Dual** injection enables interpretation of fast dissociating antigens for binning experiments.

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**Fig 14.** Sensorgram overlay, sorted heat map, and bin chart are central features of Biacore™ Insight Epitope Binning Extension for visualization and analysis of epitope bins.
Biacore™ Insight Concentration and Potency Extension for confident concentration and potency analysis

- Determine active protein concentration with confidence, shortening your time to results with automation and minimum hands-on time.
- Reliably determine drug product amount and activity using surrogate potency assays, reducing costs and saving time.
- Increased assay precision and reproducibility over a wide dynamic range.
- Eliminate errors in data transfer with built-in Parallel line analysis tool.

Biacore™ Insight Concentration and Potency Extension allows you to measure the biologically active concentration, not only the total protein amount that would be obtained from an A_{280} determination. The precision and automation of the system reduces hands-on time and generates highly reproducible data over a wide dynamic range with CVs typically below 5%. The software supports both parallel and serial run modes allowing you to select the optimal assay setup based on your needs.

Calibration curves may be generated using four-parameter or linear-fitting models. The software supports evaluation of the samples using single and average calibration curves. For long runs in serial mode, interpolation of calibration curves to compensate for drift in the assay may be performed to generate highly reliable data. The possibility to include control samples allows you to ensure a rigorous quality control of your assay.

Drug product amount and activity are reliably determined using surrogate potency assays, reducing costs and saving time. Potency assays can be easily evaluated using the built-in software tools of Biacore™ Insight Concentration and Potency Extension. This allows you to determine the potency of your sample relative to a reference sample, streamlining the analysis workflow and lowering the risk for user mistakes and data manipulation when transferring data between different software. Potency analyses are performed using Parallel line analysis (PLA) or half-maximal response (EC50) determinations. The PLA functionality makes the assessment of potency based on a linear fit to the linear part of the response vs logarithmic concentration assuming a common slope (Fig 15). The EC50 determination is based on a four-parameter equation being fitted to the response versus concentration.

Work in GxP-regulated environments, compliantly

An optional Biacore™ Insight GxP Extension allows Biacore™ 8K and Biacore™ 8K+ to integrate seamlessly into GxP-regulated workflows. The software extension provides validated software supporting GLP/GCP/GMP and 21 CFR Part 11 compliance and includes validation support. For full validation support of the system, the software extension can be supplemented with Cytiva’s OptiRun™ Qualification Service. Features in the GxP Extension include:

- Data integrity – access control and enforced version handling.
- User authorization levels – administrator, developer and user levels set access rights to software functions.
- Procedures for operational control – enables assay run and evaluation settings to be locked together in routine assays.
- Audit trail – tracks record modifications and maintains complete version histories for published procedures.

The software has been developed in accordance with an accepted development model to ensure adequate validation.

Fast export of selected or comprehensive data

The flexible result export feature in Biacore™ Insight Evaluation Software lets you export selected or comprehensive data for continued data processing, result reporting, or storage in the company database. You can export data in Microsoft® Excel®, PDF, and Microsoft® PowerPoint® format. Additional export options are available in Biacore™ Insight Data Integration Extension. You can easily select evaluation items to include in the export and define detailed settings for each item. You can also include sensorgrams and plots in the export as raster images in three different sizes, and export images as vector graphics. The software can export result tables using a comprehensive format, or using the same format you defined in the evaluation data. This means you can always present your results in the format you prefer for reporting or transferring to other software.
Presentation preparation is just a click away

You can easily transfer data into Microsoft® PowerPoint® format (Fig 16). Simply select the **Presentation** option under **Export to** in the **Home** tab to select evaluation items and export them as native presentation charts and tables. From there, you can modify your data using the extensive tool set and layouts in the presentation application, making it easy to share data with your colleagues and peers.

(A) **Biacore™ T200 Evaluation Software**

*Create presentation using data export*

- Evaluation
- Data export
- Chart creation
- Chart formatting
- Chart import
- Layout formatting
- Present Biacore™ data

Biacore™ T200 Evaluation Software

Microsoft® Excel®

Microsoft® PowerPoint®

(B) **Biacore™ Insight Evaluation Software**

*Ready-to-use slides*

- Evaluation
- Presentation export
- Present Biacore™ data

Biacore™ Insight Evaluation Software

Microsoft® PowerPoint®

Fig 16. (A) Presentation preparation in Biacore™ T200 Evaluation Software (above) vs the new way of working using Biacore™ Insight Evaluation Software and the built-in **Presentation** option (below). (B) The **Presentation** option promotes a quick and easy way to share data in a presentable format.
Additional data integration options

Biacore™ Insight Data Integration Extension enables export of data from Biacore™ 8K+, Biacore™ 8K, Biacore™ T200, and Biacore™ S200 in JSON or XML format, both well-established standards for data exchange. This extension allows the most extensive export of Biacore™ SPR data including raw data, evaluated data, and data points for sensorgrams and plots. Data export as JSON or XML file is a single step operation that includes all evaluation items and makes it easy and quick to transfer data to LIMS, ELN, or other third-party software.

Multisystem support and easy data handling

Biacore™ Insight Evaluation Software analyzes data derived from Biacore™ 8K+, Biacore™ 8K, Biacore™ T200, and Biacore™ S200 systems for the supported applications (Table 1). Users analyze data from different sources using the same familiar interfaces, features, workflows, and graphical presentation of data. This reduces training needs if multiple and different Biacore™ instruments are used.

A detailed list of features included in Biacore™ Insight Evaluation Software versus Biacore™ Insight Extended Screening and Characterization Extension are listed in Table 2.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Biacore™ Insight Evaluation Software</th>
<th>Extended Screening and Characterization Extension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fitting models</td>
<td>• 1:1 binding • Steady-state affinity</td>
<td>• 1:1 dissociation • Bivalent analyte • Heterogeneous ligand • Two-state reaction • 1:1 dissociation Dual B</td>
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<tr>
<td>Max. response ($R_{max}$)</td>
<td>-</td>
<td>• Purpose $R_{max}$ control • Adjust $R_{max}$ for ligand decay</td>
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<tr>
<td>Grouping</td>
<td>• Channel • Analyte solution • Analyte concentration • Analyte molecular weight • Cycle</td>
<td>• Analysis step purpose • Analysis step name • Flow cell • Immobilized ligand • Sensorgram type</td>
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<tr>
<td>Adjustments</td>
<td>• Molecular weight adjustment • Capture/ligand adjustment • Adjustment for controls</td>
<td>• Channel vs channel</td>
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<tr>
<td>Sensorgrams</td>
<td>• Sensorgram subtraction</td>
<td>• Sensorgram normalization</td>
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<td>Evaluation methods</td>
<td>• LMW screen • Antibody/general screen</td>
<td>• LMW kinetics and affinity • Antibody/general kinetics</td>
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<tr>
<td></td>
<td></td>
<td>• Clean screen • Affinity screen</td>
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<tr>
<td>Color by</td>
<td>• Cycle • Channel • Sensorgram ID • Immobilized ligand level</td>
<td>• Analyte solution • Analyte concentration • Analyte molecular weight • Curve markers</td>
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<tr>
<td>Injection commands (enabled in Biacore™ 8K Control Software)</td>
<td>• Analyte (High performance, Low sample consumption) • Single Cycle Kinetics • A-B-A</td>
<td>• Dual • Fast injection</td>
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Table 1. Biacore™ Insight Evaluation Software compatibility for different Biacore™ instrument models

<table>
<thead>
<tr>
<th>Software and extensions</th>
<th>Biacore™ 8K</th>
<th>Biacore™ 8K+</th>
<th>Biacore™ T200</th>
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<td>Extended Screening and Characterization</td>
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<td>Biacore Intelligent Analysis™</td>
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<td>GxP</td>
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*Biacore™ Insight Evaluation Software and/or extensions support the evaluation, but the instrument-specific control software does not contain dedicated application support.

Table 2. Features of the available screening and characterization extensions available for Biacore™ Insight Evaluation Software
Networking capabilities

You have several ways to deploy Biacore™ Insight Evaluation Software and take advantage of the functionality available in a networked configuration (Fig 17).

Advantages of network setup include:

- Data sharing
- Floating licenses for optimized usage
- Centralized administration

![Diagram](image)

Fig 17. (A) Software installed on system controller (computer connected to Biacore™ 8K) and additional computers that all connect to a shared network database and/or network e-licensing server. (B) Installation on local database and e-licensing server on a standalone/portable computer.

E-licensing

Biacore™ Insight Evaluation Software introduces floating licenses that allow sharing of access rights between users and computers and removes restrictions on the number of simultaneously installed copies of the software. You can electronically secure your machine license for one computer, or a concurrent license for networks. In addition, time-limited licenses are available, allowing you to scale the number of licenses needed for more flexibility.

Technical specifications

**Operating system:** Windows® 10 (Professional or Enterprise, 64-bit)

**Database:** Biacore™ Insight Evaluation Software includes SQL Server Express 2019. Performance improvements are seen with SQL Server Standard, SQL Server Enterprise, or SQL Data Warehouse version 2017 or 2019 (available separately from Microsoft®).

Contact your local Cytiva representative for full technical specifications.

Ordering information

<table>
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<th>Product</th>
<th>Software</th>
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For local office contact information, visit cytiva.com/contact

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