

Biacore™ T200 Software v3.2

LABEL-FREE INTERACTION ANALYSIS

Biacore™ T200 Software v3.2 (Fig 1) offers confident quantitation of target binding similarities even for the most complex biologics. Novel evaluation tools extend the applicability of Biacore™ T200 system into the area of comparability and biosimilarity. In addition, the software provides you with improved and simplified evaluation tools for calibration-free concentration analysis - CFCA.

- Assess comparability even for the most complex biologics
- Sensitive and reproducible determination of active concentration
- Conveniently customize and standardize export of selected data

Biacore™ T200 is an established surface plasmon resonance (SPR) instrument for which there are already validated and regulatory approved ligand-binding assays in use. By utilizing the sensitive and reproducible Biacore™ direct ligand-binding assay, you will confidently obtain the detailed characterization, comparability, and mechanism of action (MOA) data you need for new generation biologics (such as bispecific antibodies and antibody drug candidates) and that regulatory agencies require for CMC submissions.

Comparability assessment with *Sensorgram Comparison*

Comparison of binding data is an important step in late-stage development and quality control (QC) of biotherapeutics. It is essential to understand and monitor any possible effect of target binding activity on product and process changes to ensure drug safety and efficacy. Kinetic and report point analysis is typically used but becomes challenging or even insufficient when the binding data is more complex, as is the case with new generation biologics such as Fc fusion proteins, bispecific antibodies, and diabodies.

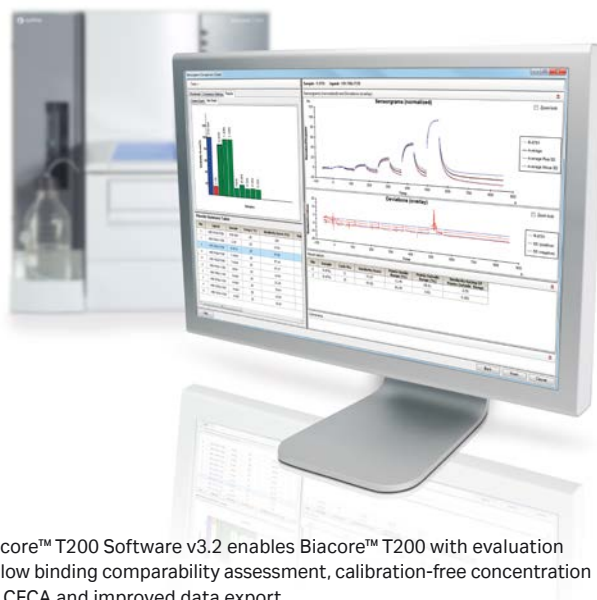


Fig 1. Biacore™ T200 Software v3.2 enables Biacore™ T200 with evaluation tools to allow binding comparability assessment, calibration-free concentration analysis - CFCA and improved data export.

Biacore™ T200 Software v3.2 makes comparability assessment easy by objectively comparing complete binding profiles of samples against that of a reference standard. With support for multiple injection data several critical quality attributes can even be compared simultaneously in a single sensorgram.

The **Sensorgram Comparison** tool enables quantitative comparisons of complex as well as simple binding data. With the resulting **Similarity Score**, up to 300 sample data sets can be rated based on relative binding similarity. Result files from different runs can be appended enabling rapid co-evaluation of, for example, historical product batches. Standard/reference sensorgrams can be saved in evaluation methods and, it is not necessary to reopen standard files for analysis of a new sample. This reduces the risk for user-mediated errors and enables seamless analysis of new samples by applying saved standards and evaluation settings.

A single display provides a holistic overview in a thumbnail pane while simultaneously giving details of the selected data series (Fig 2). Thumbnails in the **Overview Pane** enable rapid qualification of the data sets for the standard, control and samples, using color-coded frames for easy identification.

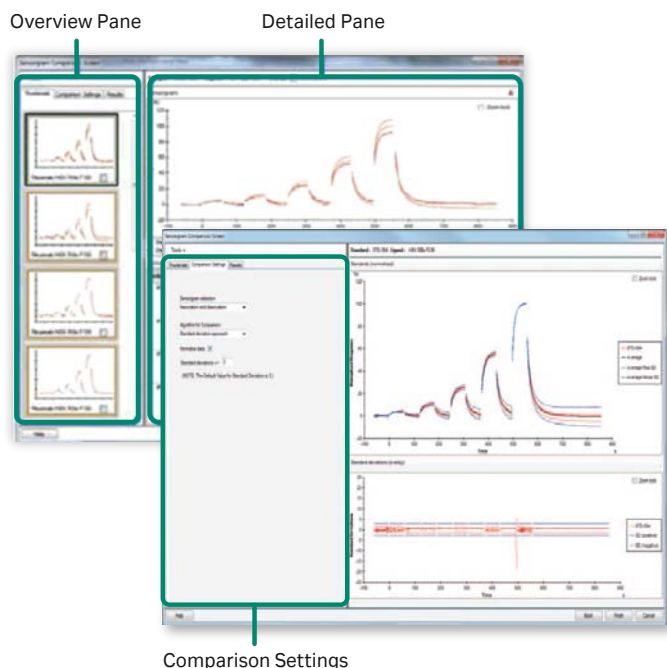
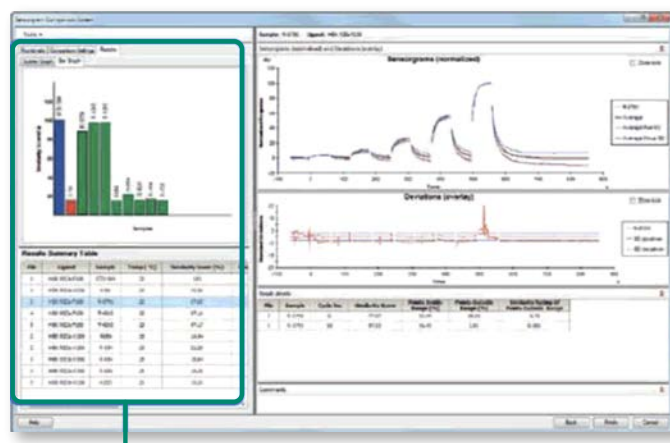


Fig 2. Good overview with fast access to detailed editing in the **Overview** and **Detailed Panes** simplifies data processing, giving rapid access to quality comparison results.

Data processing can be performed on all data series simultaneously and, any data exhibiting bad binding behavior can be easily excluded. Tools are available for cutting, rescaling of sensorgrams, or removal of wash phases (multiple injections comparison). All editing actions are clearly visualized in the **Detailed Pane**, which also provides the possibility to qualify blank subtractions.

In **Comparison Settings**, evaluation criteria are conveniently set and instantly visualized in the **Detailed Pane**. Limits for the accepted assay variation are set using simple statistical tools such as standard deviation, min./max. or % deviation algorithms. The comparisons can be performed using single cycle kinetic, multi cycle kinetic, single concentration and multiple injection data. In each case it can be split into association or dissociation phases.

Results, including the **Similarity Score**, are summarized in the **Results Summary Table** and clearly displayed in a **Bar Graph** or **Scatter Graph** (Fig 3). Detailed inspection of individual sample results in the right-hand panel is facilitated by selecting individual samples in the summary table or graphs. Data may be sorted as well as commented with free text for optimal display and reporting.



Result summary with **Bar Graph**, **Scatter Graph**, and **Similarity Score** summary table

Fig 3. A clear overview of results for easier assessment of binding comparability data is given in the **Results Summary**.

The **Sensorgram Comparison** evaluation workflow is summarized in Figure 4.

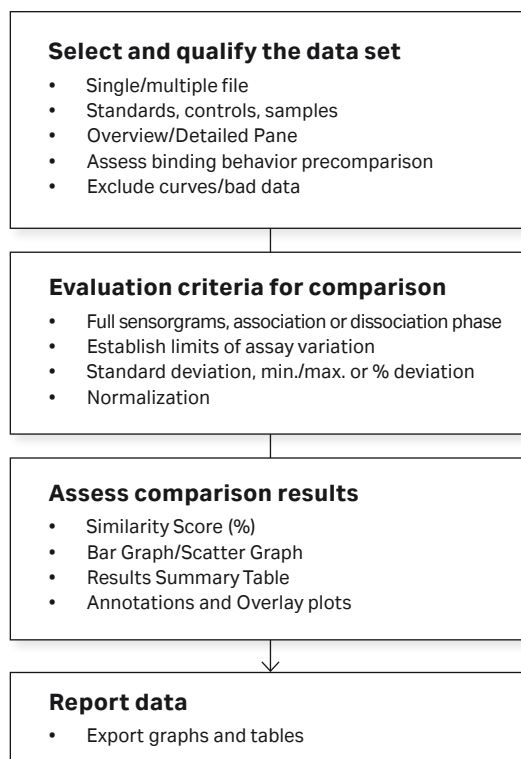


Fig 4. **Sensorgram Comparison** evaluation workflow.

Multiple injections in *Sensorgram Comparison*

The new multiple injection feature can be used to compare several binding events that occur in a single sensorgram. The application supports sample, dual inject, and enhancement injections. This feature can for instance be used to compare how an antibody interacts with antigen followed by Fc receptor binding (Fig 5), or how antigen 1 and antigen 2 binds to a bispecific antibody.

- Select standard
- Define comparison settings and limit sensorgrams
- View sample data (orange) in overlay plot with limit sensorgrams (blue)

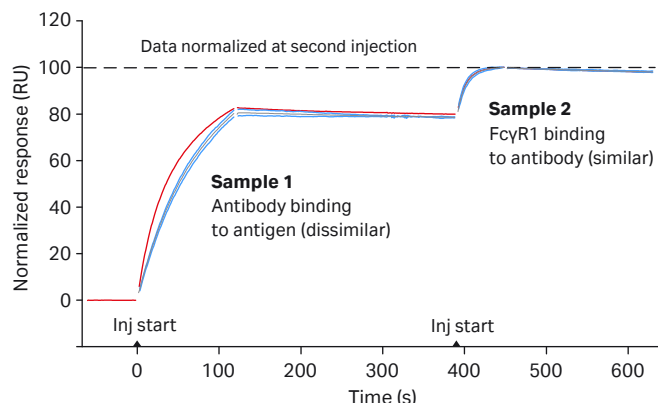


Fig 5. Multiple injection workflow applied to antibody to antigen and Fc-receptor binding.

Sensorgram Comparison for analysis of screening data

By selecting standards with preferred binding profiles (e.g., rapid on/slow off, rapid on/rapid off) sensorgram comparison can be used to evaluate screening data. The use of evaluation methods with saved profiles combined with overlay plots for rapid visualization and selection of curves and annotations, ensures consistent evaluation criteria of samples compared to reference profiles (Fig 6).

Antibody/antigen binding dissociation curves

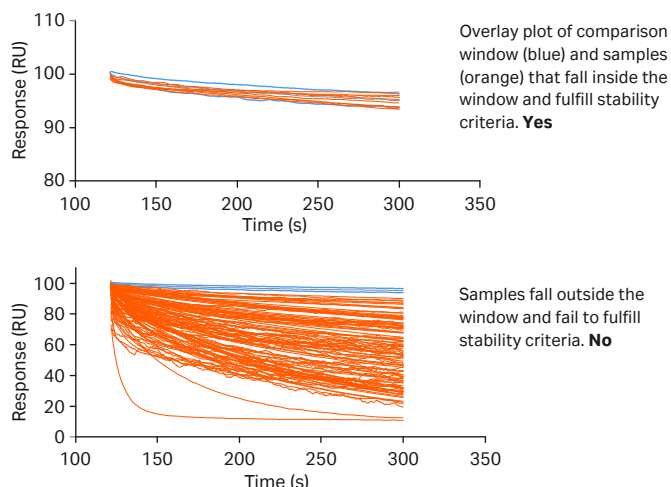


Fig 6. Antibody screening data. The comparison window is set to identify binders with slow off rate. In Annotations and Overlay plots samples can be displayed in overlay plots and rapidly annotated as Yes/No.

Sensorgram Comparison features

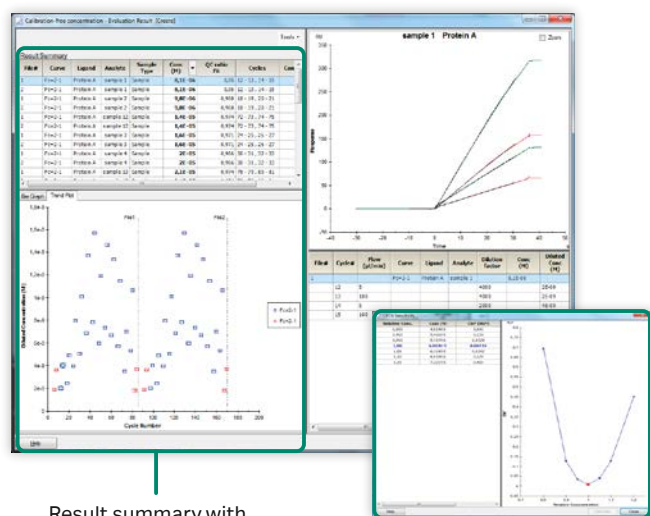
Feature	v3.0	v3.1/v3.2
Top menu item	No	Yes
Data structure		
Single cycle kinetics	Yes	Yes
Multicycle kinetics	Yes	Yes
Single concentration	Yes	Yes
Multiple injections	No	Yes
Comparisons		
Association/dissociation	Yes	Yes
Association	Yes	Yes
Dissociation	Yes	Yes
Algorithms		
Standard deviations	Yes	Yes
Max/min	Yes	Yes
% Deviation or RU difference	No	Yes
Normalization yes/no	Yes	Yes
Normalization level input	No	Yes
Standard info and settings		
File name shown in settings	No	Yes
File path shown in settings	No	Yes
Save sensorgrams in evaluation methods	No	Yes
Tools		
Remove data	Yes	Yes
Annotations	No	Yes
Overlay plots	No	Yes
Apparent stoichiometry	No	Yes

Reproducible concentration analysis without standards

In development and production of biotherapeutics, it is important to determine the concentration of functionally active molecules in a given sample as opposed to total concentration. In addition to traditional active protein concentration analysis using a calibration curve of known standards, the direct ligand-binding assay applied in Biacore™ T200 system supports determination of active concentration without standards by means of calibration-free concentration analysis - CFCA. Using calibration-free concentration analysis - CFCA allows determination of the protein concentration in unknown samples based on the diffusion properties of the protein, measured as the rate of binding to a target immobilized on the sensor surface under conditions of mass transport limitation.

Biacore™ T200 Software v3.2 facilitates reproducible and simple co-evaluation of more than 300 sample data sets from single or multiple runs. Rapid qualification and prefit selection of only relevant data speeds up the evaluation by minimizing the fitting time. **Global Fitting** of sample data improves the data fitting procedure and generates a single concentration value valid for a dilution series.

In **Results Summary**, fitted results are summarized in a table and simultaneously visualized in **Bar Graph** or **Trend Plot** overviews (Fig 7), for easy assessment of results. Data can be conveniently sorted and commented on with free text.



Result summary with **Bar Graph**, **Trend Plot**, and **Summary Table**

Sensitivity Check

Fig 7. Results Summary, Bar Graph, and Trend Plot overviews allow easy assessment of CFCA results. Several dilutions of the same sample are globally fitted for robust evaluation.

The **Trend Plot** provides full overview of samples and control samples against cycle number for better assessment of assay performance. Results from multiple runs are automatically separated by vertical lines.

In **Sensitivity Check**, the reliability of both the fitting procedure and the reported concentration can be assessed. **Sensitivity Check** complements other available QC tools (such as QC ratio, χ^2 , and standard error) for confident evaluation of generated results.

The calibration-free concentration analysis - CFCA algorithm relies on a known value for the diffusion coefficient of the protein, and direct access to a web-based Diffusion Coefficient Calculator tool is available in Biacore™ T200 Software v3.0 and higher.

The calibration-free concentration analysis - CFCA evaluation workflow is summarized in Figure 8.

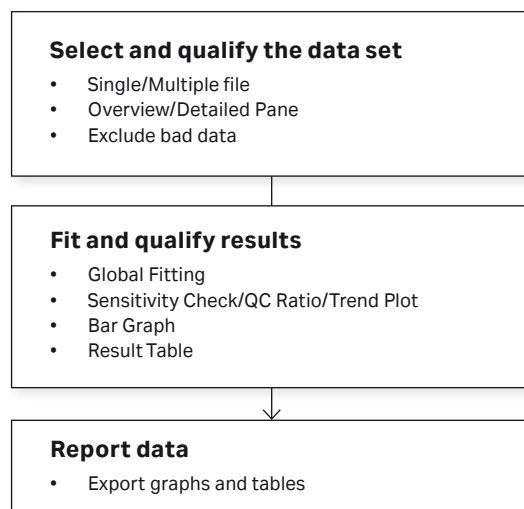


Fig 8. Calibration-free concentration analysis - CFCA evaluation workflow.

Data export

Biacore™ T200 Software v3.2 provides means to customize and standardize the export of the **Report Point Table** and the **Kinetic/Affinity** result table. The standardized export reduces the risk for user-mediated errors upon export to third-party software and saves time since it can be re-applied to new results files.

Customization, reuse, and export of the Report Point Table

The **Report Point Table** lists numerical values for all report points in the current result set. Selection, sorting and filtering of the **Report Point Table** allows the user to focus on relevant results (Fig 9). All user-defined settings can be conveniently saved into an evaluation method and reapplied. The customized report point table can be exported to Microsoft® Excel® (XLS) and Excel® macro (XLM) formats.

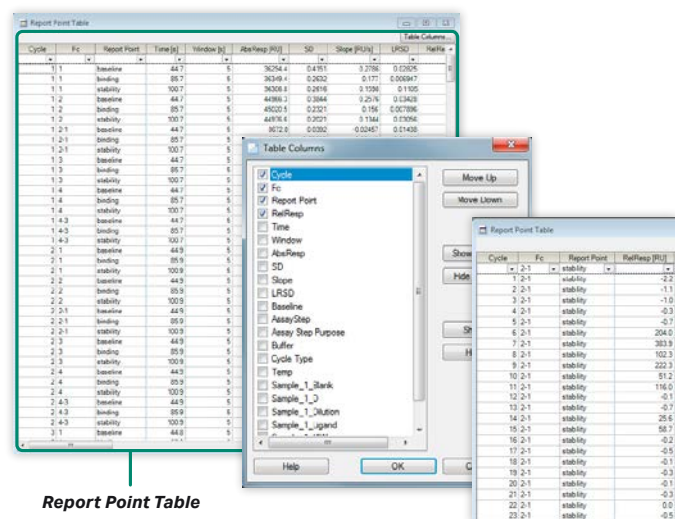


Fig 9. Selection, sorting, and filtering of a **Report Point Table** allows a user to focus on relevant results.

Export of results from Kinetic/Affinity

Results for **Kinetic/Affinity** are automatically displayed in a graph and a preset **Result Table**, ready for export. If needed, the user can sort and order results into a customized table, **Data for Export**, for storing of relevant results only. The customized table setting can be saved as a template and will be part of an evaluation method for the kinetic affinity item enabling a standardized export format.

Copy of result graphs from Kinetics Affinity and Kinetic Screen

Results from **Kinetic Screen** consists of graphs and tables. In Biacore™ T200 Software v3.2 it is possible to change color and thickness of sensorgrams in graphs and to copy graphs into three sizes (small, medium, and large) (Fig 10).

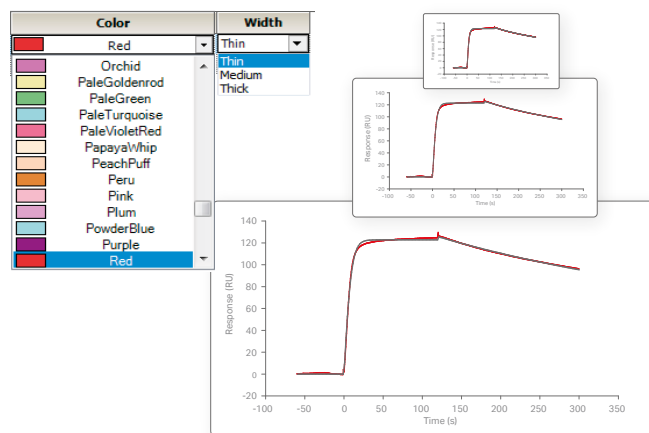


Fig 10. Change of curve appearances and copy small, medium, or large.

The resulting graphs can be pasted into PowerPoint® and Word documents.

Data handling and storage

Compatible PC operating systems

Windows® 10 Professional, 64-bit

Windows® 10 Enterprise, 64-bit

Minimum computer requirements

3.0 GHz processor

RAM > 1 GB free

Hard disk drive > 2 GB free

Graphics resolution at least 1280 × 1024

Ordering information

Product	Product code
Biacore™ T200 Software v3.2*	29148695
Biacore™ T200 GxP Software v3.2*	29148699

*Includes software set, product key, and handbooks

Related products	Product code
Biacore™ T200 Processing Unit	28975001
Biacore™ T200 GxP Package	28977954
Biacore™ T200 Upgrade Kit	28977963
Biacore™ T200 GxP Upgrade Kit	28977964
Biacore™ T200 Software v3.2, additional license	28981619

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