

# Biacore™ T200 Upgrade Kit

## LABEL-FREE INTERACTION ANALYSIS

Biacore™ T200 Upgrade Kit allows you to take advantage of the powerful capabilities offered by Biacore™ T200 (Fig 1). An upgraded system delivers improved sensitivity and assay flexibility to users across multiple application areas enabling interactions at the extremities of the kinetic scale to be analyzed with great precision and confidence.

The upgrade includes Biacore™ T200 Software v3.2, which features workflow-based tools for improved handling of larger data sets and for confident similarity rating of biotherapeutic target-binding data. The sensorgram comparison application of v3.2 supports multiple injection data for comparison of several critical quality attributes in a single sensorgram and has also been adapted for screening purposes.

## High sensitivity for the most challenging applications

Biacore™ T200 Upgrade Kit was developed for the analysis of molecular interactions where high sensitivity is crucial. Interactions previously beyond the limits of sensitivity can be precisely evaluated so that borderline data are confidently determined.

- Obtain high-quality kinetics from really fast on-rates to very slow off-rates
- Quantitate low-abundance molecules (concentration > 1 pM)
- Analyze interactions involving the smallest low molecular weight (LMW) compounds
- Work with rare or sensitive targets such as G protein-coupled receptors
- Design assays with flexibility

Whatever the application area, the high sensitivity of Biacore™ T200 increases the number of interaction parameters that can be studied. For example, the increased range of kinetic rate constants that can be measured allows resolution and ranking of even the most strongly binding antibodies. In addition, as the system allows precise binding data to be obtained even from very low response levels, making it possible to study, for example, sensitive proteins that might lose much of their activity during sample preparation or immobilization.



**Fig 1.** Biacore™ T200 is a versatile system for high-quality characterization of molecular interactions from ions to viruses.

## Workflow-based screening, comparability, and immunogenicity tools

Biacore™ T200 Software v3.2, which is included in the upgrade kit, simplifies and speeds up evaluation workflows for performing single-concentration screens, affinity screens, and kinetic screens. Dedicated tools enable comparability assessment, immunogenicity testing, and enhanced import and export functionalities improve integration into existing information infrastructure.

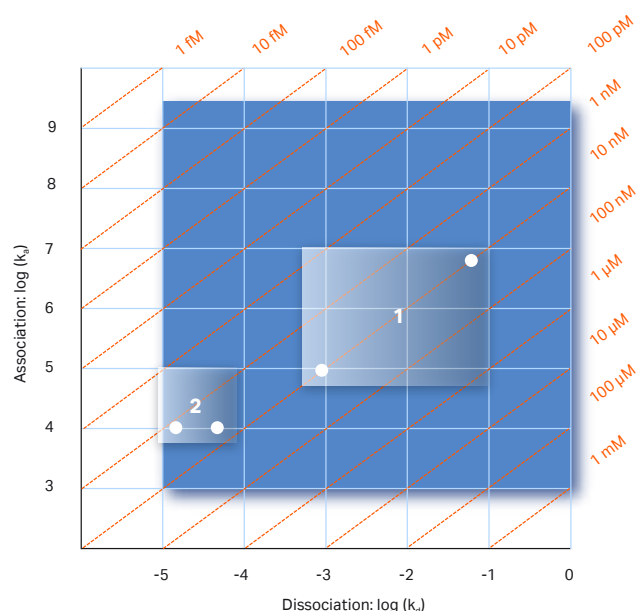
- Benefit from workflow-based screening tools supporting larger data sets
- Reliable comparability assessment of biologics
- Apply dedicated tools for confident immunogenicity testing
- Easily export and share results

# What can high sensitivity bring to your experiments?

## Excellent precision over a broad kinetic range

With Biacore™ T200 Upgrade Kit, the range of kinetic rate constants that can be confidently measured is extended to areas presently beyond reach, from really fast on-rates to very slow off-rates (Fig 2).

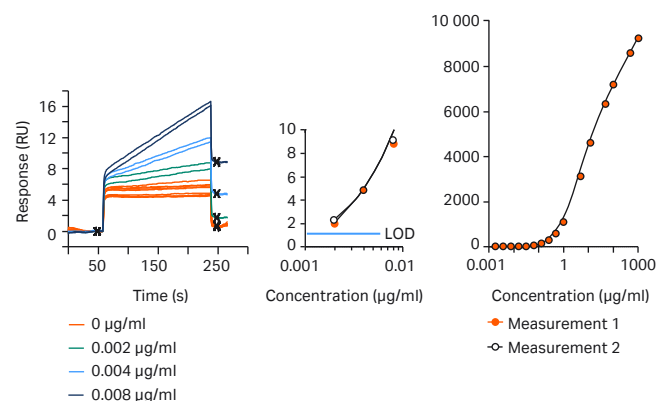
- $k_a$  from  $10^3$  to  $5 \times 10^7 \text{ M}^{-1}\text{s}^{-1}$  ( $10^3$  to  $3 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$  for macromolecular analytes)
- $k_d$  from  $10^{-5}$  to  $1 \text{ s}^{-1}$



**Fig 2.** (1) Interactions with apparently similar affinities can have very different kinetic profiles. Resolution into component on- and off-rates can improve candidate selection. (2) Even interactions at the extremes of kinetic behavior, for example, with very slow off-rates, can be detected and differentiated with confidence.

## Quantitate low-abundance molecules

High sensitivity gives lower limit of detection (LOD) and excellent precision in concentration measurements. As an example, Xolair™, a human antibody from Novartis, was precisely quantitated at all concentrations tested down to 2 ng/ml (Fig 3).



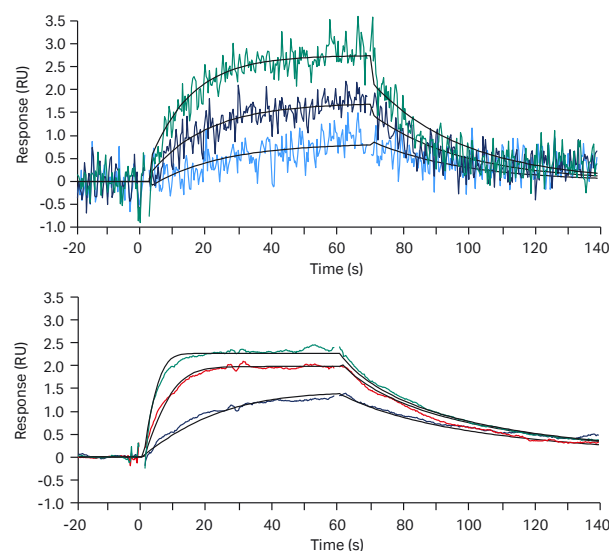
**Fig 3.** Concentration analysis of the therapeutic human antibody, Xolair™. The LOD for Xolair™ was determined from the mean of 10 blanks + 3 SD (0.8 RU).

## Precise analysis of the smallest compounds

The sensitivity of an upgraded system allows researchers to detect and precisely characterize any organic compound, regardless of its molecular weight, enabling confident analysis of the simplest analytes.

## More success with sensitive proteins

The possibility to derive high-quality data from low levels of immobilized interaction partners is advantageous in the analysis of sensitive proteins. The three-dimensional shape of G protein-coupled receptors, for example, is strongly coupled to the microenvironment of the plasma membrane and these proteins tend to partly denature in isolation. Even if only a fraction of the total immobilized targets retain their structural integrity after sample preparation and immobilization, and hence the capacity to interact with binding partners in solution, the improved sensitivity of upgraded systems will be sufficient to obtain high-quality data from an extremely low frequency of interactions (Fig 4).



**Fig 4.** Binding of a small molecule, xanthine amine congener (XAC), to stabilized histidine-tagged GPCR (StaR™) A2. Data of higher quality is generated by Biacore™ T200 (lower sensorgram) compared to Biacore™ T100 (upper sensorgram) when using low levels of immobilized GPCR StaR™. Data courtesy of Dr. Andrei Zhukov, Heptares Therapeutics Ltd, Welwyn Garden City, UK.

## Increased flexibility in assay design

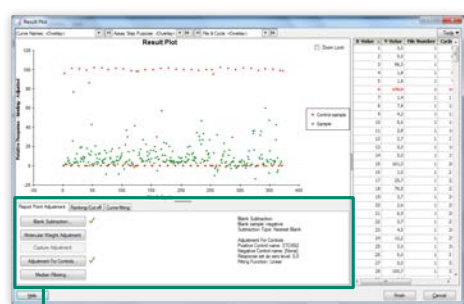
To avoid avidity effects in Biacore™ assays involving antibodies, it has been recommended to immobilize the antibody (rather than the antigen) on the sensor surface.

The increased sensitivity achieved with the upgrade allows antigen immobilization levels so low that avidity is no longer a complicating factor and thus makes assay design more flexible.

# Get to final results faster using efficient screening evaluation tools

## Visualize and make the right selections using the Result Plot

**Result Plot** provides tools to plot the sample response versus a selection of variables. Up to 5000 single-concentration samples from multiple runs can be co-evaluated in a single **Result Plot** (Fig 5). Co-evaluation provides full overview of the entire data set and improves the quality of results by enabling the same adjustments and normalizations to be applied. Repetitive operations are removed saving time and reducing the risk of user-mediated errors. Selections in the data set can be done by ranking or applying an automated control-based cutoff.

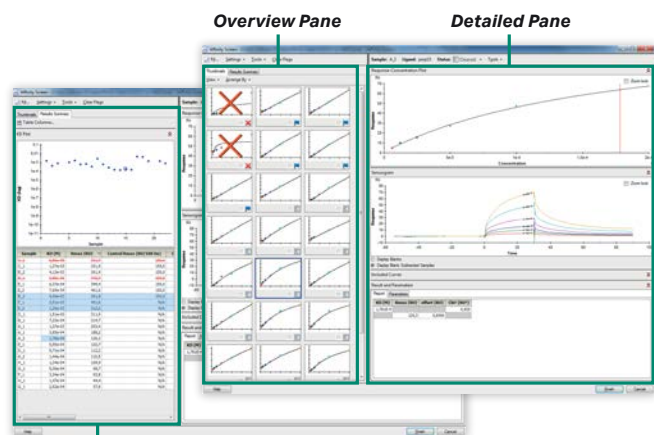


Available adjustments are listed and are easily applied or reverted.

**Fig 5.** Biacore™ T200 Software v3.2 offers tools that enable efficient selection of samples of interest.

## Rapidly retrieve high-quality affinity and kinetic results

Affinity and kinetic evaluations of up to 200 concentration series can be performed in one single evaluation with data from one or several runs. A single display provides a holistic overview in a thumbnail pane while simultaneously giving details of the selected data series. Data processing can readily be performed on individual data points, a selected subset, or all data series.



Result summary with  $K_d$  plot and result table

**Fig 6.** Good overview, flexible analysis, and comprehensive result summaries enable kinetic and affinity evaluations to be performed faster.

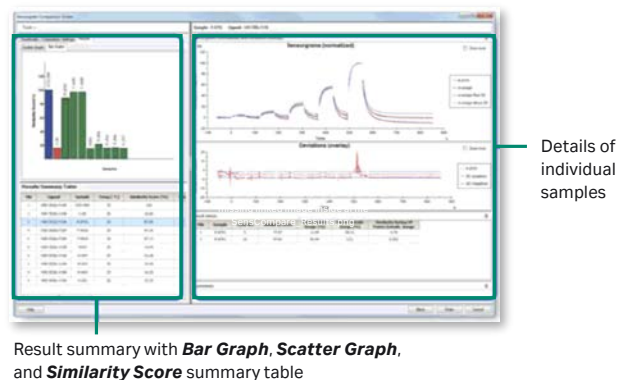
The results of the entire evaluation are neatly compiled in a sortable and customizable table format. Resulting affinities are displayed in a  $K_d$  plot while kinetic parameters are visualized in an On-off rate map (Fig 6).

This flexible setup enables simple and powerful processing of data, streamlining the evaluation process and getting to final results faster.

## Comparability assessment with Sensorgram Comparison

It is essential to understand and monitor any possible effect on target binding activity upon 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.

Biacore™ T200 Software v3.2 makes comparability assessment easy by objectively comparing complete binding profiles of samples against that of a reference standard. Sensorgrams obtained with the reference standard can be saved in an evaluation method and, and the comparison criteria can seamlessly be applied to new data. The **Sensorgram Comparison** tool enables quantitative similarity rating using the **Similarity Score** for both complex and simple binding data. Up to 300 data sets from different runs can be appended enabling rapid co-evaluation of, for example, historical product batches. Results, including the **Similarity Score**, are summarized in a **Results Summary Table** and clearly displayed in a **Scatter Graph** or sortable **Bar Graph** (Fig 7). Sensorgram comparison can be applied to single cycle kinetic, multicycle kinetic, single concentration, and multiple injection data.



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

**Fig 7.** With the **Sensorgram Comparison** tool, up to 300 sample data sets can be rated based on relative binding similarity.

## Tools for immunogenicity testing

Biacore™ T200 software provides dedicated tools for confident detection and characterization of antidrug antibodies (ADA). Low-affinity ADA, which are easily missed in endpoint assays due to losses during washing steps can be readily detected. ADA can also be detected in the presence of drug, avoiding false negative results. For even more comprehensive characterization, Biacore™ T200 enables sample screening, antibody isotyping, and epitope mapping.

## Product specifications

### Biacore™ T200 system

Association rate constant ( $k_a$ )	Proteins: $10^3$ to $3 \times 10^9$ M <sup>-1</sup> s <sup>-1</sup> LMW molecules: $10^3$ to $5 \times 10^7$ M <sup>-1</sup> s <sup>-1</sup>
Dissociation rate constant ( $k_d$ )	$10^{-5}$ to $1$ s <sup>-1</sup>
Sample concentration	> 1 pM
Molecular weight detection	No lower limit for organic molecules
Baseline noise	Typically < 0.03 RU (RMS)
Baseline drift	Typically < 0.3 RU/min
Immobilized interactant consumption	Typically 0.03 to 3 µg/flow cell

### Biacore™ T200 Upgrade Kit

Biacore™ T200 Upgrade Kit	Includes Biacore™ T200 Software v3.2
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

Products	Product code
Biacore™ T200 Upgrade Kit	28977963
Biacore™ T200 GxP Upgrade Kit	28977963
Desktop package	Product code
Computer, screen, and printer - 110 V	28922726
Computer, screen, and printer - 220 V	28922725
Optional packages†	Product code
Biacore™ T200 GxP Package	28977954
Cytiva Validation GxP Services	BR-2001-06

† For more information, contact your local Cytiva representative.

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