Biacore™ S200

LABEL-FREE INTERACTION ANALYSIS

The high-sensitivity Biacore™ S200 (Fig 1) delivers reliable affinity, kinetics, or fragment-screening data from a 384-well microplate in a single day, thereby supporting the need for rapid, conclusive results. With a combination of exceptional sensitivity and productivity, Biacore™ S200 raises the standard for SPR systems in fragment screening and lead optimization.

- Exceptional sensitivity that facilitates work with difficult targets where response levels are low
- 384 single-concentration fragment binding data in less than 16 h
- Competition assays for validation of binders and for binding-site mapping
- Affinity and kinetics determinations with exceptional quality for confident lead optimization

The system is designed to reduce run time and time to results. By utilizing the single-concentration ***Binding Level Screen***, you will identify binders out of a 384-well microplate within 16 h and prioritize them based on their binding behavior. Subsequent ***Affinity Screen*** of the most interesting binders will give conclusive results within two days, from start to finish.

The increased sensitivity in combination with exceptional kinetic performance enables detailed characterization and optimization of lead compounds with very fast on- and off-rates, even for difficult targets where response levels are low. Software tools for running competition assays and scouting for optimal buffers directly from microplates further broadens the applicability for low molecular weight (LMW) drug discovery applications.

Biacore™ S200 utilizes surface plasmon resonance (SPR) technology to ensure highly dependable data from small sample volumes and gives you the means to increase productivity and make informed decisions to ensure real progress with your drug discovery programs.

Fig 1. Biacore™ S200 is a high-sensitivity, label-free interaction system that delivers reliable affinity, kinetics, and fragment-screening data in a single day.

Main applications

Biacore™ S200 is a label-free interaction analysis system designed to meet the requirements of high sensitivity and short time to results for fragment screening and lead optimization in LMW drug discovery. The main analyses performed by the system are:

- Fragment screening: ***Clean Screen***, ***Binding Level Screen***, ***Affinity Screen***
- Kinetics and affinity
- Competition assays
- Focused screening
- Thermodynamics

Exceptional sensitivity pushes the limits of label-free interaction analysis

Given the trend within LMW drug discovery and fragment-based drug discovery (FBDD) of finding binders to weak, less conserved binding sites, sensitivity of the detection techniques used is becoming increasingly important. The high-sensitivity Biacore™ S200 provides potential for working with large, multidomain targets or rare/sensitive targets like G protein-coupled receptors (GPCRs), where only a fraction of the target maintains its biological activity during preparation and analysis. The higher sensitivity also allows lower surface densities to be used, which often simplifies data interpretation. A lower density surface often gives fewer secondary interactions and can also increase the proportion of the target that is accessible for binding. Some targets may even aggregate on the surface at very high densities, and as such can be a significant challenge for less sensitive instruments.

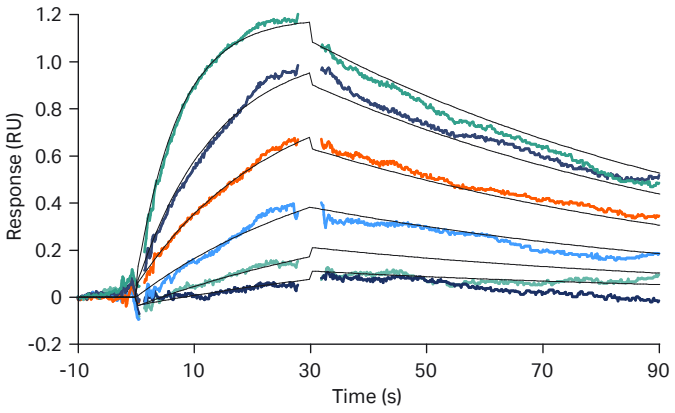

Fig 2. The high sensitivity of Biacore™ S200 enables confident analysis of data in the milli-resonance units (mRU) response range (Data: Thrombin and melagatran).

The low baseline noise in Biacore™ S200 allows sensorgrams to be clearly separated in the milli-resonance unit (mRU) range (Fig 2). This enables confident analysis of data, even if the highest concentration gives a response below 1 RU. In addition, the combination of the low baseline noise and the 40 Hz data collection rate in Biacore™ S200 increases the resolution of very rapid off-rates and enables determination of off-rates up to 6 s^{-1}. The 40 Hz data collection rate increases the number of data points that can be collected in a certain time window and improves the accuracy of rapid on- and off-rate determinations.

Fast, easy kinetic analysis with exceptional performance

In addition to the possibility to run at a 40 Hz data collection rate, which allows resolution of very fast off-rates, Biacore™ S200 software offers a range of tools for confident and reliable kinetic analyses. These can be performed using single-cycle kinetics, where several sample concentrations are injected in the same cycle. Alternatively, a multicycle approach (using one sample concentration per cycle) can be used. By eliminating the need for surface regeneration between injections, single-cycle kinetics simplifies analyses involving targets that are unstable or difficult to regenerate. It also reduces assay run time as well as assay development time. By utilizing the predefined templates for kinetics, 30 analytes can be run in 16 h.

Efficient affinity and kinetic evaluation tools

Biacore™ S200 Evaluation Software enables kinetic and affinity analysis of interaction data to be performed with a few simple clicks:

- Rapid overview and qualification of data
- Flexible tools for customized data analysis

Affinity and kinetic evaluations can be performed with up to 200 concentration series in a 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 (Fig 3).

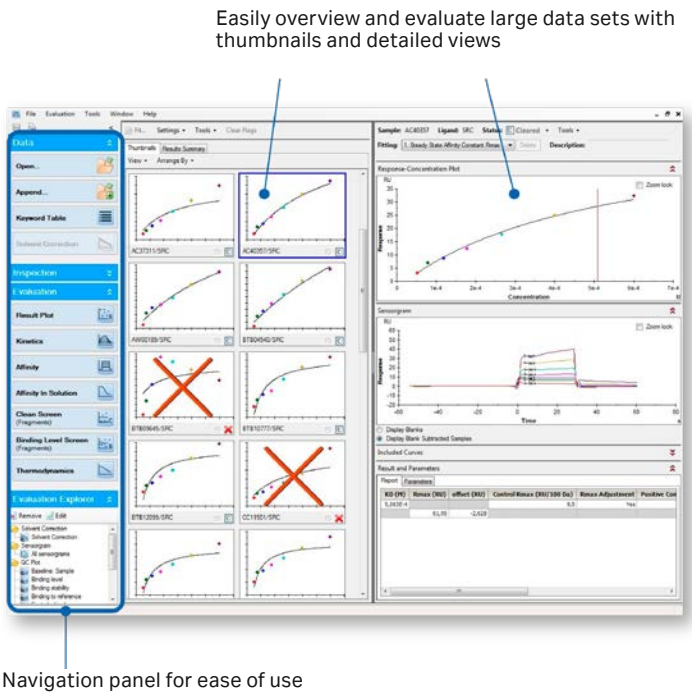

Fig 3. Good overview with easy access to detailed editing simplifies data processing giving rapid access to quality results.

Thumbnails enable rapid qualification of the data sets both pre- and post-fit. Every thumbnail displays the data of one concentration series in the sensorgram or response plot views. In the ***Overview Pane,*** each data set can be fitted with the model of choice. Data processing can be performed on individual data points, a selected subset, or all data series. In the ***Detailed Pane***, explicit operations such as outlier eliminations or detailed cutting can be performed. Any data exposing suboptimal binding behavior can be easily excluded or annotated. The fitting speed for kinetics is up to 45 times faster in Biacore™ S200 software than in Biacore™ T200 software. The software also displays the results and fit-quality assessment of the selected series. The ***Result Summary*** compiles the results of the entire evaluation in a customizable table format where the result parameters of choice, including the column order, can be changed. Resulting affinities are displayed in a ***K_D Plot*** while kinetic parameters are visualized in an ***On-off Rate Map*** (Fig 4).

Simple QC tools for easy assessment of kinetic data

Automated QC tools analyze data fitting quality for the kinetic constants, parameter uniqueness, bulk refractive index, and residuals, enabling the user to interpret results with confidence (Fig 4). These quality assessments are displayed below the sensorgrams in the ***Detailed Pane*** of the software.

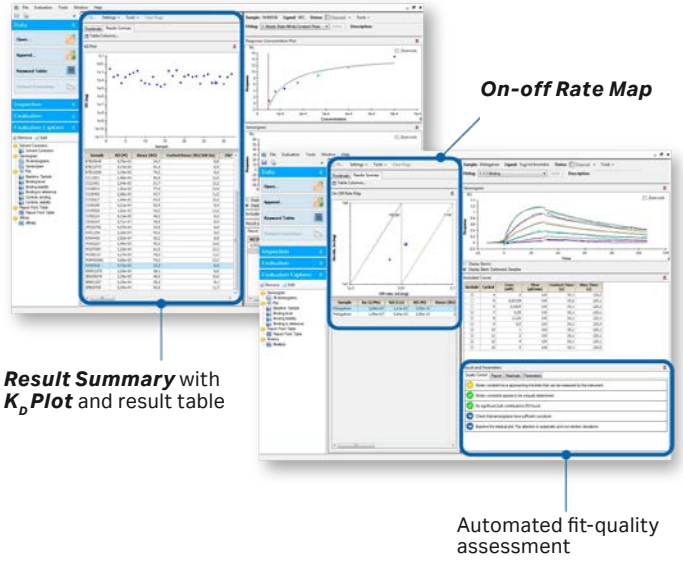

Fig 4. Results are compiled in a customizable table and visualized in ***K_D Plot*** and ***On-off Rate Map***. Automated fit-quality assessments facilitate interpretation of results.

Flexible data visualization using the *Result Plot*

Result Plot provides tools to plot the sample response versus a selection of variables, which provides flexibility to data analysis. Up to 5000 single-concentration samples from multiple runs can be co-evaluated in a single ***Result Plot*** (Fig 5). Co-evaluation of several runs provides a full overview of the data 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 performed by ranking or applying an automated control-based cutoff.

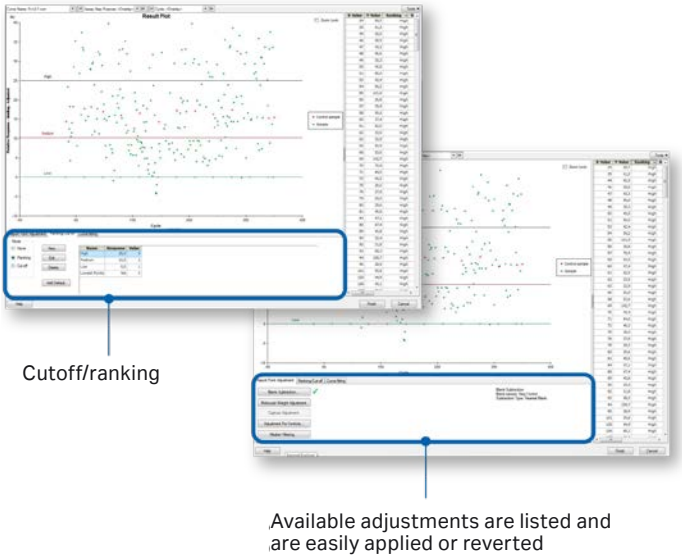

Fig 5. Using ***Result Plot***, up to 5000 samples from several runs can be co-evaluated enabling easy selection of the samples of interest.

Competition analysis for validation of binders and for binding-site mapping

Competition assays are very useful in drug discovery, giving the ability to find site-selective binders directly by identifying or confirming the location(s) of the binding site of a specific compound. Biacore™ S200 simplifies the setup of competition experiments using the new ABA-injection type (Fig 6). Competition experiments can be run with significantly lower consumption of competitor than in traditional SPR competition assays, since the competitor does not have to be present in the running buffer. Competition screens for validation of binders and comparisons of kinetic behavior in the presence and absence of a competitor are examples of assays that strongly benefit from this. A competition screen can be evaluated using the ***Result Plot*** functionality. Kinetics experiments in the presence of competitor are evaluated using the standard kinetics evaluation

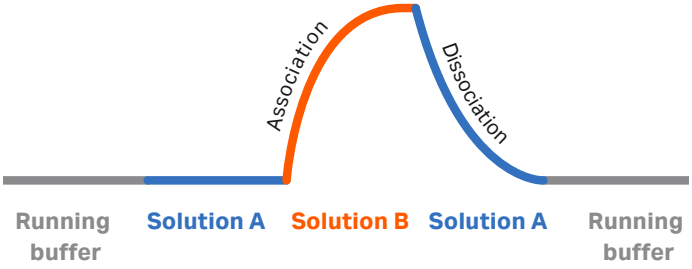

Fig 6. The ABA-injection allows two different solutions to be injected over the surface in the same cycle in the following order: solution A, solution B, and solution A. This enables new types of assays such as competition assays and buffer scouting to be run directly from a microplate.

Predefined templates provide user guidance

Biacore™ S200 provides predefined run and evaluation templates to guide the user and shorten time to results. The templates are available for all major types of assay and are preloaded with application-relevant settings. While providing guidance, the templates are fully flexible and can be easily adjusted to fit specific assay needs. Both run and evaluation templates can be saved upon customization to speed up future analyses.

Assay optimization tools to support analysis of difficult targets

Taking full advantage of high sensitivity puts demands on the general quality of the assay. Biacore™ S200 comes with several tools that support assay optimization. In addition to traditional templates such as ***Regeneration Scouting*** and ***pH Scouting***, there is also a new template for ***Buffer Scouting*** directly from a 96-well plate. Fine-tuning of assay conditions, such as additives in the running buffer, can be vital for analysis of difficult targets that require complex buffers to stay active. In the ***Buffer Scouting*** template, the ABA-injection (Fig 6) is used to analyze binding in different buffers. Up to 48 different buffers can be scouted in an overnight run, providing the means to rapidly optimize buffer conditions for maximum assay performance.

Fragment screening

The use of SPR in FBDD is increasing and SPR-based biosensors are established as powerful tools in both fragment screening and lead finding. In FBDD, high sensitivity is required to observe low-affinity binding of LMW fragments to target proteins. The trend towards using more complex and difficult targets increases the need for sensitivity even further. The pressure to deliver new drugs to market is high and shorter time to conclusive results is required to meet tight project timelines. Biacore™ S200 supports efficient drug discovery, delivering reliable data for the entire SPR workflow in FBDD (Fig 7), with focus on reducing time to conclusive results without compromising sensitivity.

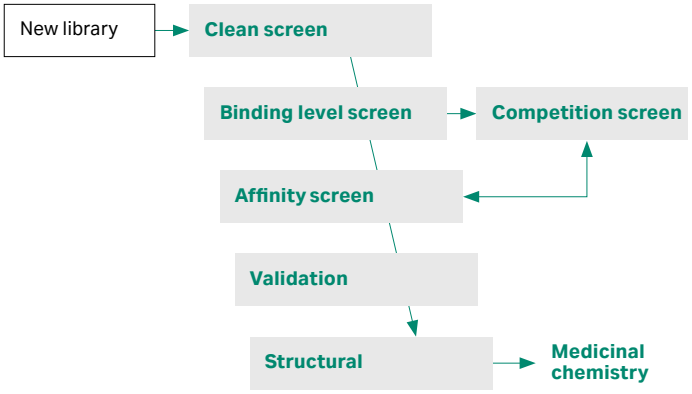

Fig 7. Typical analysis workflow for FBDD and small-molecule screening using Biacore™ S200 to generate reliable and conclusive results prior to structure analyses and transfer into medicinal chemistry.

Clean Screen *for efficient library clean up*

Clean Screen analysis identifies undesirable fragments that show persistent binding to the surface, disturbing subsequent samples. The samples are run over the target(s) and a blank dextran surface at a single concentration. By using the predefined template with an injection type specifically developed for ***Clean Screen*** assays, 384 fragments can be run in less than 6 h. ***Clean Screen*** evaluation (Fig 8) automatically identifies samples that show: (1) residual binding to all targets and surfaces (general binders); (2) residual binding to some targets/surfaces (selective binders); or (3) no residual binding at all (nonresidual binders).

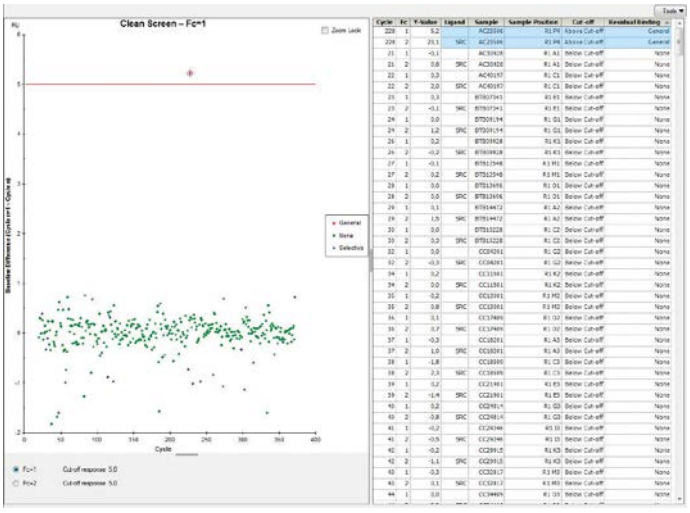

Fig 8. ***Clean Screen*** ranking plot with residual binders. General binders indicated in red, selective binders in blue, and nonresidual binders in green. Results table to the right can be sorted on binder markers to rapidly identify residual binders.

Binding Level Screen *for rapid prioritization of binders*

Binding Level Screen provides a rapid overview of the library content, automatically identifying fragments with binding levels above a defined cutoff point. Tagging fragments that display nontypical binding behavior or secondary interactions enables rapid and efficient prioritization of binders for further analysis.

In ***Binding Level Screen,*** a single concentration of each fragment is run over target(s) and reference. The possibility to use multiple targets enables selectivity information from a single sample injection. The predefined template for ***Binding Level Screen*** uses an injection type specifically developed for ***Binding Level Screen*** runs and allows a 384-well plate to be analyzed in less than 16 h. ***Binding Level Screen*** evaluation tools are tailored to address challenges associated with fragment screening assays, such as secondary interactions and nontypical binding behavior. Binding levels are plotted against cycle number and samples with binding levels above the cutoff are identified so they can be included in subsequent assays. Binding behavior markers are automatically applied to identify and classify each fragment (Fig 9) and are included in the ***Results Table*** for easy tracking.

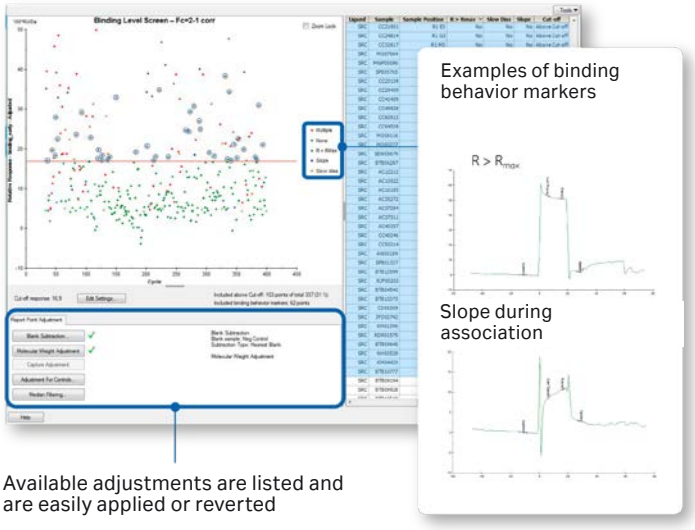

Fig 9. Ranking plot from ***Binding Level Screen*** with cutoff boundary showing nontypical binding behavior markers (color-coded) and sensorgrams as inlays. The table is rapidly sorted by columns and selected samples are highlighted in both plot and table.

Affinity Screen *for reliable affinity ranking*

Classical affinity determination requires that samples are run in several concentrations, where the highest concentration preferably should be higher than the expected affinity of the interaction. LMW fragments often exhibit low affinities in the millimolar (mM) range. When trying to dilute the fragments in millimolar concentrations, solubility issues often make it difficult to reach the concentrations needed for classical affinity determination.

Biacore™ S200 provides tools to circumvent solubility challenges and enables reliable affinity ranking of fragments or compounds that needs to be assayed at concentrations lower than the affinity. The assay setup guidelines and data evaluation for ***Affinity Screen*** analysis provide the possibility to use a positive control to assess the maximum response (R_{max}) of the surface. This control-based R_{max} is used to stabilize the affinity fitting and to estimate the fragment affinity, even when samples are run at low concentrations. ***Affinity Screen*** analysis uses a concentration series of samples run over target and reference, and a predefined template supports the user in assay setup. A 384-well plate with samples in multiple concentrations, as well as controls, is run in less than 25 h using the predefined run template.

To minimize disturbances caused by secondary interactions that are prominent among fragments, ***Affinity Screen*** analyses should be based on data measured early on in the binding phase. The software allows flexible placement of the data interval for the measurement and any disturbances are easily removed from individual sensorgrams (Fig 10). The affinity is normally estimated by fitting the data to a one-site model, but selected data is easily refitted to a multisite model where appropriate.

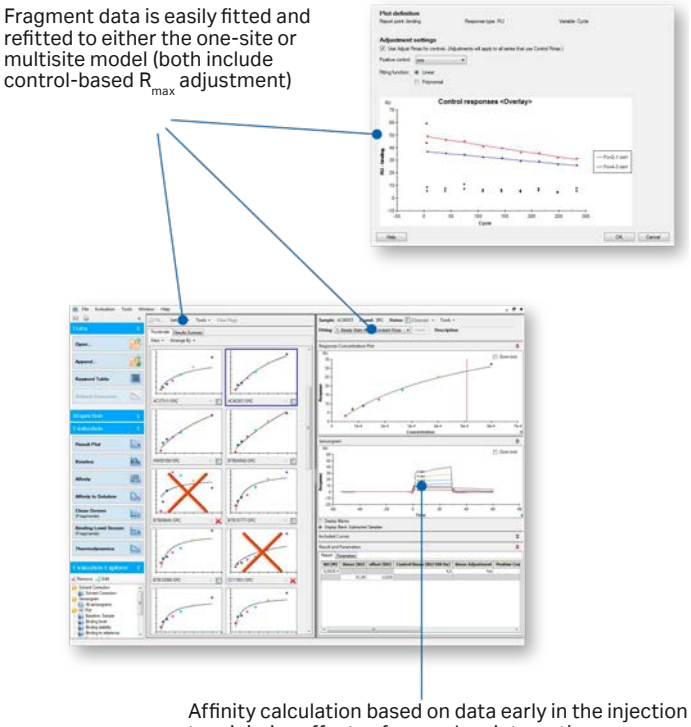

Fig 10. ***Affinity Screen*** functionality has been developed to specifically address fragment-related challenges. Data for the affinity calculation is taken shortly after injection start to minimize effects from secondary interactions; adjustment for control-based R_{max} determination stabilizes the affinity fitting when sample concentrations are much lower than needed to saturate the surface.

Transition-state thermodynamics for additional information about reaction mechanisms

Thermodynamic analysis enables prediction of binding energetics from the three-dimensional structure of protein complexes. The analysis provides deep understanding of molecular interactions, which is important for the structure-based molecular design of drugs. Dedicated software templates, built-in buffer degassing, and stringent temperature control in Biacore™ S200 enables transition-state thermodynamic analysis. Integration of kinetic rate constants measured across several temperatures into thermodynamic equations allows thermodynamic characterization of the transition state, revealing the forces driving the interaction (Fig 11).

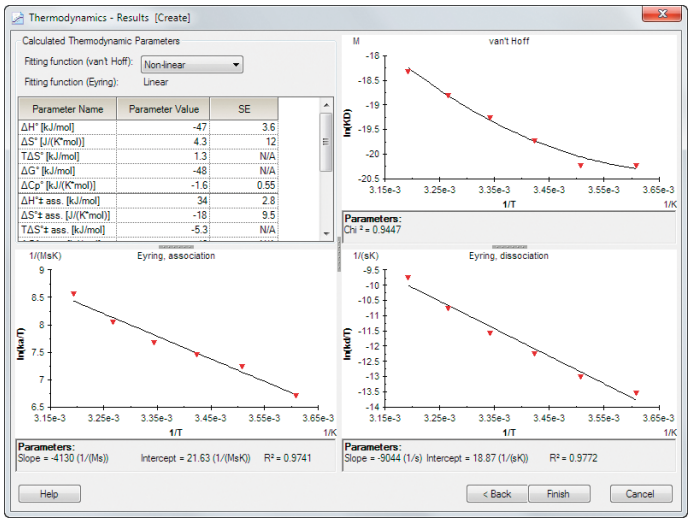

Fig 11. Automatic generation of Eyring and van't Hoff plots from kinetic data for calculation of thermodynamic parameters.

Biacore™ S200 specifications

Technical specifications and characteristics

Detection technology	Surface plasmon resonance (SPR) biosensor
Information provided	Kinetic and affinity data (K_D, k_a, and k_d), specificity, selectivity, thermodynamics and screening data
Data presentation	Result tables, result plots, and real-time monitoring of sensorgrams
Analysis time per cycle	Typically 2 to 15 min
Automation	48 h unattended operation
Sample type	LMW drug candidates to high molecular weight proteins (also DNA, RNA, polysaccharides, lipids, cells, and viruses) in various sample environments (e.g., in DMSO-containing buffers, plasma, and serum)
Required sample volume	Injection volume plus 20 to 50 µL (application dependent)
Injection volume	2 to 350 µL
Flow rate range	From 1 to 100 µL/min
Flow cell volume	0.06 µL
Data collection rate	1, 10, or 40 Hz
Flow cell height	40 µm
Sample/reagent capacity	1 × 96- or 384-well microplate and up to 33 reagent vials or 78 vials for samples and reagents
Typical run time	Clean screen (384-well plate): 6 h Binding level screen (384-well plate): 15 h Affinity screen (384-well plate): 25 h Kinetic analysis: 30 samples in 16 h
Analysis temperature range	4°C to 45°C (maximum 20°C below ambient temperature)
Sample storage	4°C to 45°C (maximum 15°C below ambient temperature)
Sample refractive index range	1.33 to 1.40
Buffer selector	Automatic switching between four buffers
In-line reference subtraction	Automatic

Minimum computer requirements

3.0 GHz processor
RAM > 1 GB free
Hard disk drive > 2 GB free
Graphics resolution at least 1280 × 1024
Serial communication: 2 RS-232 ports configured as standard PC COM1 and COM2

Typical working ranges

Association rate constant (k_a)	Proteins: 10^3 to 3×10^9 $M^{-1}s^{-1}$ LMW molecules: 10^3 to 5×10^7 $M^{-1}s^{-1}$
Dissociation rate constant (k_d)	10^{-5} to 6 s^{-1}
Sample concentration	≥ 1 picomolar (pM)
Molecular weight detection	No lower limit for organic molecules
Number of flow cells	Four
Baseline noise	Typically < 0.015 RU (RMS)
Baseline drift	Typically < 0.3 RU/min
Blank subtracted drift	< ± 0.003 RU/min
Immobilized interactant consumption	Typically 0.03 to 3 µg/flow cell
Dimensions (W × H × D)	600 × 690 × 615 mm
Net weight total	60 kg
Mains requirements	Processing Unit Autorange 100 to 240 VAC (± 10%), 50 to 60 Hz, Class 1 equipment (protective earthing)
Power consumption	Processing Unit: max. 4 A (at 100 VAC) Max. power consumption (including PC and printer) 800 VA

Data handling and storage

PC operating systems	Windows® 10 Professional, 64-bit Windows® 10 Enterprise, 64-bit
Interfacing	Possibilities for import of sample data and export of results (e.g., to and from LIMS)

Compliance

Compliant with	CE, cETLus, EAC, FCC, ICES-001, RCM
Safety	EN/IEC 61010-1, EN/IEC 61010-2-081, UL 61010-1, CAN/CSA-C22.2 No. 61010-1 EN ISO 12100
Electromagnetic compatibility (EMC)	EN/IEC 61326-1, FCC Part 15 B, ICES-001
Environmental RoHS	China RoHS

On-site requirements: Contact your local representative for the latest information regarding on-site requirements.

Ordering information

Processing unit[1]	Product code
Biacore™ S200	29136649

[1] Includes a processing unit, control and evaluation software, and Windows® operating system. Computer, monitor, printer, keyboard, and cables should be ordered separately.

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