

Biacore 8K and Biacore 8K+

LABEL-FREE INTERACTION ANALYSIS

Biacore™ 8K and Biacore 8K+ systems efficiently deliver binding data of outstanding quality, meeting your toughest challenges in screening, characterization, process optimization, and quality control (Fig 1). There are two available systems: Biacore 8K for high-throughput screening/characterization and Biacore 8K+, which offers significantly higher capacity. These eight-needle high-sensitivity surface plasmon resonance (SPR) systems rapidly provide reproducible kinetics and affinity data shortening time to results by up to eight times compared to single-needle systems. The blend of system flexibility and throughput reduces the experimental cycle time, even for complex targets and drug formats such as bispecific antibodies and offers more opportunity for screening in drug discovery.

Biacore 8K and Biacore 8K+ systems provide:

- Superb data quality while increasing your throughput by eight-fold compared with single needle SPR systems
- Interaction analysis for screening, kinetics, affinity, epitope binning, concentration, and relative potency
- Higher operational efficiency with capacity options and streamlined assay development in parallel
- Modular configuration with option for analysis in a GxP regulated environment
- Rapid optimization of assay conditions and troubleshooting

A single solution for small molecule and biotherapeutic screening/characterization

Biacore 8K and Biacore 8K+ give you a single solution for interaction analysis in screening, characterization, process optimization, and quality control and are well suited to the analysis of samples from the smallest fragments to large multidomain proteins, even in crude matrices. Applications include:

- Selection of biotherapeutic or small-molecule hits based on affinity and kinetic ranking
- Characterization and optimization of selected binders based on detailed kinetic and affinity information
- Identify and bin together antibodies even when the antigen dissociates rapidly
- Reproducible and reliable determination of protein concentrations and potency
- Obtain titer and kinetic information on your biologic candidates in a single assay cycle



Fig 1. Biacore 8K and Biacore 8K+ efficiently deliver high-quality affinity and kinetics data for small molecule and biotherapeutic screening and characterization.

High-quality data in less time, even for the toughest applications

The eight-needle parallel setup boosts efficiency regardless of the number of samples you run. With 2D kinetics methodology, detailed kinetics for a single interaction can be obtained in 35 min, without spending time on assay development. When working with multiple samples, parallel kinetic screening efficiently identifies the hits from 384 samples in less than 6 h. Both systems also support fast scouting of assay conditions, screening 96 buffer conditions in 80 min, optimizing your assay to deliver concentration and interaction data you can rely on. An 8 × 8 epitope binning array (64 interactions) can be performed in about 2 h.

To help you get the best possible data, we support you and your applications with a broad portfolio of consumables and method protocols, while providing you with access to our local application experts.

Biacore 8K and Biacore 8K+ provide the same data quality as our single-needle Biacore systems and come with the sensitivity and stability that is crucial to generate binding data with a quality that supports important decision making. The sensitivity allows screening and characterization of the smallest organic compounds and enables confident kinetic analysis over a wide kinetic range, from very fast on-rates to the slowest off-rates. High sensitivity also opens up for analysis of low-abundance molecules or sensitive, complex targets.

Interactions involving challenging targets

Analyzing GPCRs and other sensitive targets

The high sensitivity of Biacore 8K and Biacore 8K+ systems generates reliable data for rare or sensitive targets such as G protein-coupled receptors (GPCRs, Fig 2) where only a fraction of the protein might retain its biological activity throughout the analysis. Analysis may be performed directly in crude matrices such as a membrane preparation, avoiding unnecessary sample handling that risks negatively affecting the activity level. The high sensitivity also allows analysis of the smallest organic compounds even for low-affinity interactions (K_D in the millimolar range), which is important for reliable, small molecule fragment screening.

Analysis of bivalent analytes

The systems allow for full flexibility in the characterization of bivalent analytes such as antibodies or dimeric proteins. Complication from avidity is minimized by using very low

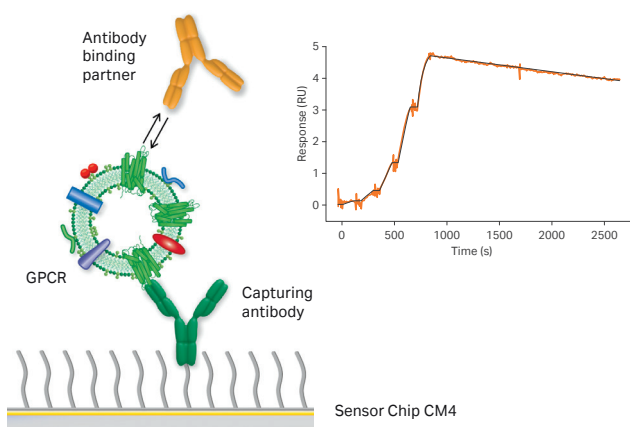


Fig 2. The high sensitivity and robustness of Biacore 8K and Biacore 8K+ systems allow the analysis of GPCRs in crude membrane preparations.

immobilization levels, which renders reliable data (Fig 3). Low immobilization levels generally give fewer secondary interactions and increase the proportion of target accessible for binding; some targets even exhibit surface aggregation at higher densities. Cleaner interaction data not only gives more accurate results but also makes analysis simpler and faster, saving time.

High sensitivity enables accurate measurement of fast on-rates and slow off-rates

Biacore 8K and Biacore 8K+ systems enable measurement of exceptionally fast on-rates that allows differentiation between rapid binders. This is an important feature when studying biological processes limited by bioavailability (Fig 4). At the other end of the kinetic spectrum, today's antibody discovery often generates many high-affinity hits in every campaign. Differentiating these stable binders increases the challenge on the analytical system used as it requires both high sensitivity and stability over time.

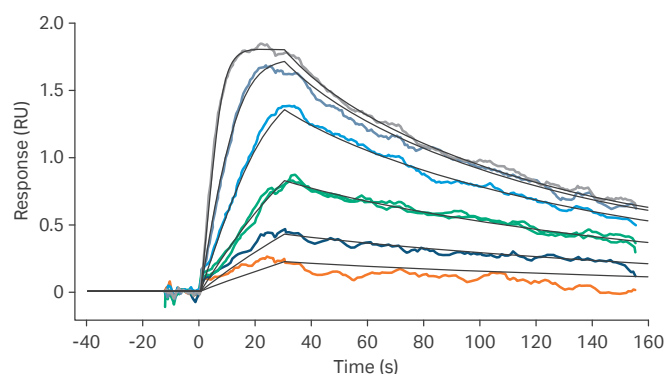


Fig 4. The high sensitivity of Biacore 8K and Biacore 8K+ enables confident analysis of fast on-rates. Sensorgram showing binding of melagatran to thrombin: association constant (k_a) $4.0 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$; k_d 0.014 s^{-1} analyzed on Biacore 8K.

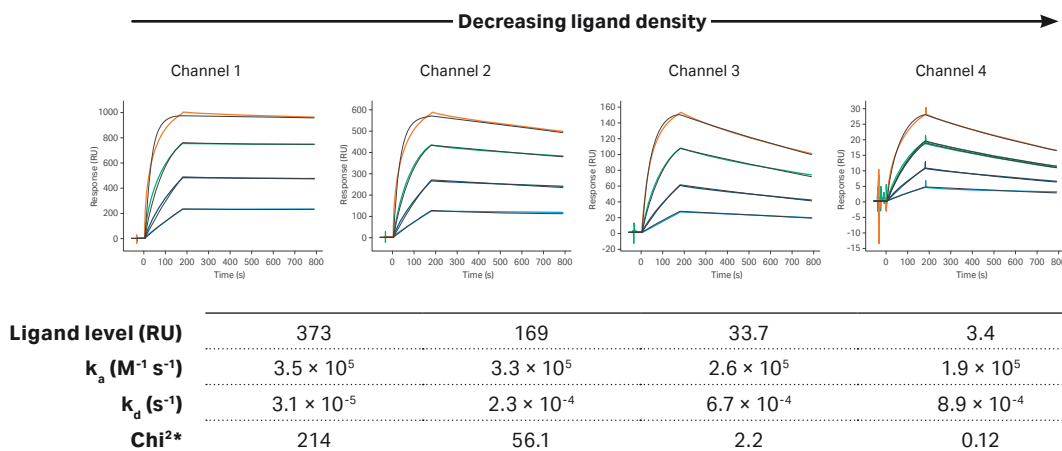
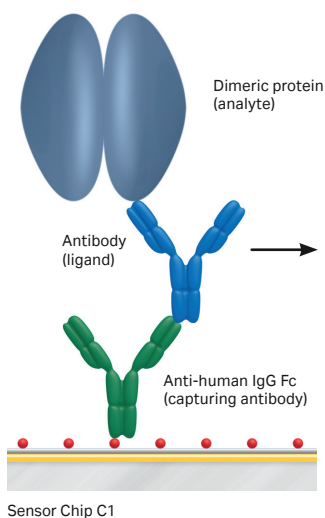


Fig 3. Analysis of a bivalent, dimeric protein with a molecular weight (M_r) of 660 000. The avidity diminishes with the ligand density revealing the true kinetics of the interaction. Data obtained using Biacore 8K, courtesy of Schraml, Biehl, von Proff, Roche Diagnostics GmbH, Centralised and Point of Care Solutions, Penzberg, Germany.

The high sensitivity of Biacore 8K and Biacore 8K+, in terms of low baseline noise and drift, provides effective differentiation between stable binders, and allows reliable determination of very slow off-rates down to 10^{-6} s^{-1} (Fig 5).

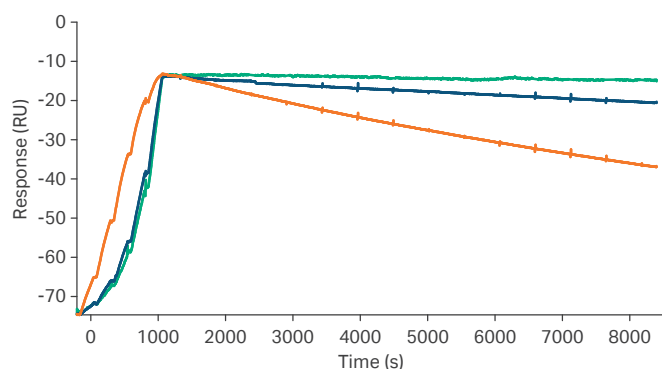


Fig 5. Biacore 8K and Biacore 8K+ provide sensitivity and stability that enables the differentiation of tight binders with dissociation rate constants (k_d), down to 10^{-6} s^{-1} .

Parallel setup maximizes operational efficiency

Biacore 8K and Biacore 8K+ systems are designed to maximize operational efficiency by combining rapid high-quality data with the smooth operation that comes from user-friendly and application-specific software modules and interactive hardware. The systems feature an eight-needle parallel setup with a novel microfluidic injection concept which enables each channel to provide high quality, reference-subtracted data (Fig 6). The simple 8×2 flow cell-setup makes planning, preparation, and operation straightforward and easy to understand. A fluidic delivery system is required for accurate kinetic determinations and the novel microfluidic system has been refined to optimize stability and robustness while not compromising on performance. As with other Biacore systems, Biacore 8K and Biacore 8K+ systems provide interaction data directly from crude matrices such as hybridoma supernatants, membrane preparations, or serum samples.

Sample capacity

Biacore 8K and Biacore 8K+ support the use of 96- and 384-well microplates in standard and deep-well formats of up to 2 mL volume (Fig 7). Both samples and reagents are taken from standard microplates with no need for special vials.

The sample hotel of Biacore 8K accommodates two trays. However, the sample hotel of Biacore 8K+ accommodates up to six trays, which increases up-times/asset utilization by up to 30% per week. Each tray can hold two microplates, which translates to a maximum unattended run capacity of 1536 and 4608 samples for Biacore 8K and Biacore 8K+ systems, respectively. This makes it possible to screen a full fragment library (with an average size of 2000 compounds) in a single experiment, without manual intervention or the need for external robotics.

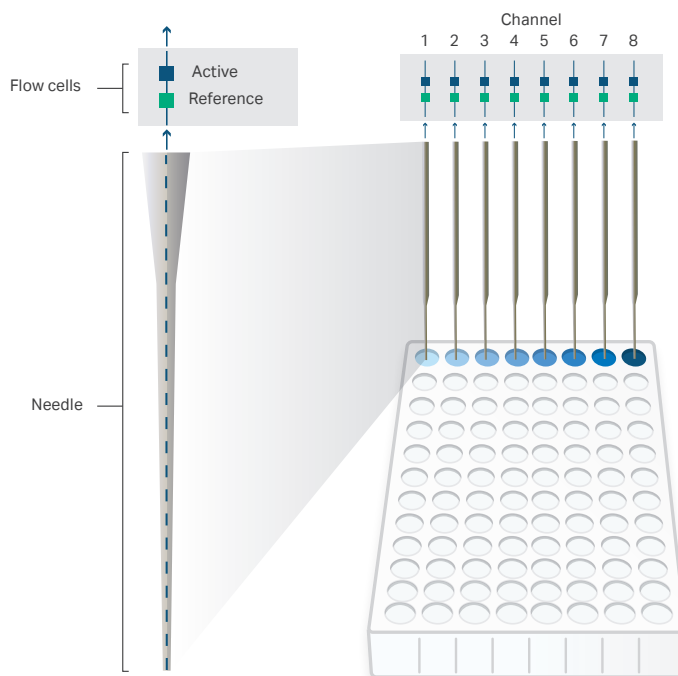


Fig 6. The simple, eight-channel concept with two flow cells per channel simplifies assay setup and operation of Biacore 8K and Biacore 8K+. This allows assay optimization of 16 samples simultaneously.

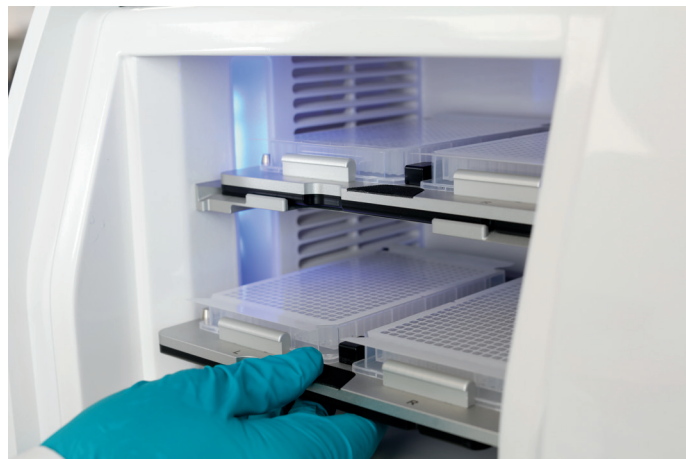


Fig 7. Biacore 8K accommodates up to four 96- and 384-well microplates simultaneously, all in a temperature-controlled environment for optimal assay performance or to ensure sample integrity in extended runs.

Trays may be accessed during run to optimize operational efficiency for both systems. The sample hotel is temperature controlled. By keeping the samples at the same temperature as the analysis temperature, optimal assay performance is obtained. To ensure the integrity of samples and reagents in extended runs, the sample hotel can be kept refrigerated.

Biacore 8K and Biacore 8K+ are equipped with a buffer selector enabling change of up to four different running buffers without the need for manual intervention, allowing for even higher efficiency.

Interaction data at physiological temperatures

Biacore 8K and Biacore 8K+ provide reliable data at physiological analysis temperature enabling better prediction of *in vivo* behavior of therapeutic candidates. Heated or cooled needles ensure that the samples have the appropriate temperature when being analyzed, even at elevated flow rates (Fig 8).

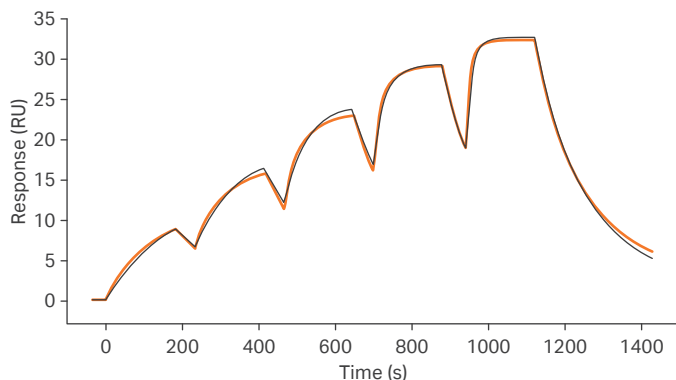


Fig 8. Biacore 8K provides high-quality data at physiologically relevant temperatures. Data shows the analysis of $\beta 2$ -microglobulin vs anti- $\beta 2$ -microglobulin at 37°C.

Software with flat interface for maximum overview

Biacore Insight Evaluation Software exhibits a software interface design that provides an overview and intuitive, rapid operation. The software offers a range of powerful tools for confident and reliable interaction analysis suitable for users of all levels of experience.

Efficient method definition and operation

Biacore 8K Control Software (Fig 9) provides graphical display of the run method, with workflow steps that guide the user from method definition to preparation of sample plates and start of analysis. Predefined methods, preloaded with application relevant default settings, are available for all major assays. Experiments using predefined methods can be started in minutes.

With the queuing capability of Biacore 8K and Biacore 8K+, operational efficiency in the lab can be significantly improved (Fig 9). Immobilization methods, analysis methods, cleaning procedures, temperature changes, and other relevant steps can all be added to the **Activity queue** in a fully flexible manner, minimizing unnecessary waiting times.

Fast evaluation software for faster time to decision

Biacore Insight Evaluation Software (Fig 10) enables evaluations to be performed with a few simple clicks, equally suited for the rapid analysis of large screening campaigns as well as deep kinetic characterization of a single interaction, multiplexed epitope binning experiments or reproducible quantitation of

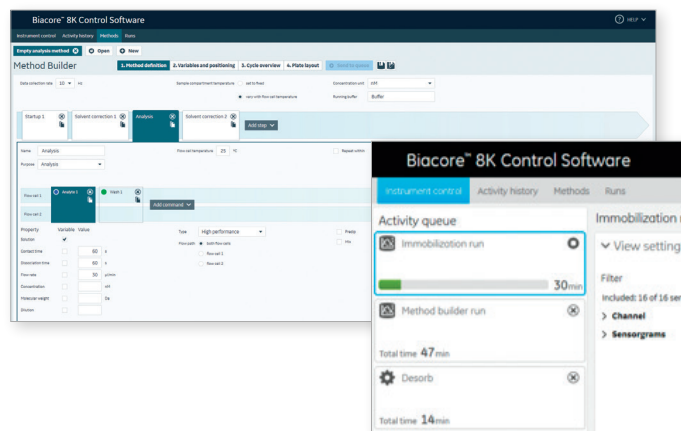


Fig 9. Biacore 8K Control Software exhibits graphical representation of the method definition, which provides intuitive run setup. Queuing capabilities maximize operational efficiency.

your valuable samples. Generic tools scale with the size of your experiment rendering fast results you can trust, regardless of the number of samples analyzed. The flexible interface is configurable to maximize the space for your most important tasks at any time.

- Shorten time to decision by applying predefined evaluation methods
- Rapidly create an overview and qualify your data
- Utilize flexible tools for customized data analysis
- Cost-efficient software—pay for basic functionality that expands to more advanced extensions as your research evolves
- Easily export large data sets to Microsoft® Excel® and rapidly share results in Microsoft PowerPoint®

See the Biacore Insight Evaluation Software data file for more information.

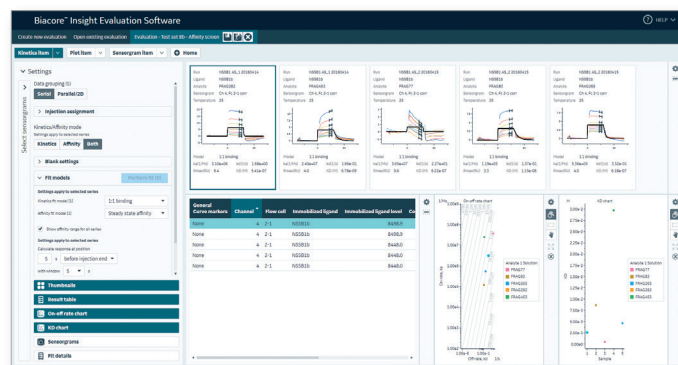
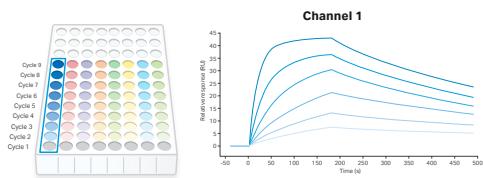


Fig 10. The interface of Biacore Insight Evaluation Software and the application-specific evaluation software extensions, provide full overview while offering flexible tools for customized data analysis. The example gives an overview that allows convenient simultaneous visualization of kinetics and affinity data providing **Result table**, **On-off rate chart**, and **K_p chart**.

Multi-cycle kinetics (MCK)

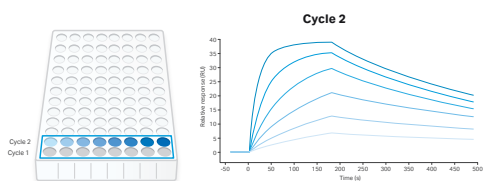
- Suitable for many samples against one ligand
- Suitable when different ligands are to be immobilized



Ex. cycle 1 to 9: sample concentrations and blanks are placed per channel

Parallel kinetics

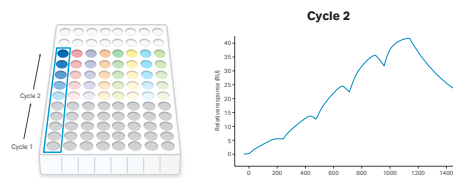
- Short run time for few samples
- Kinetic analysis in only two cycles (one blank cycle)
- Beneficial for samples with long dissociation times



Ex. cycle 2: sample in 8 concentrations (cycle 1: blank cycle)

Single-cycle kinetics (SCK)

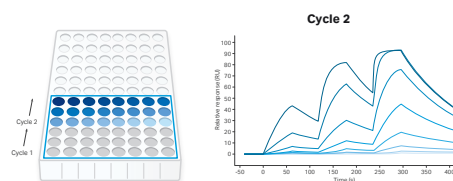
- Fast run time
- No regeneration needed
- Beneficial for long dissociation times



Ex. cycle 2: 5 × sample conc. (cycle 1: 5 × blank conc.)

2D kinetics

- In-depth analysis in only one sample cycle
- Sample diluted in two dimensions to cover a wide concentration range
- No preknowledge of affinity or regeneration needed



Ex. cycle 2: sample in 24 concentrations (cycle 1: blank cycle)

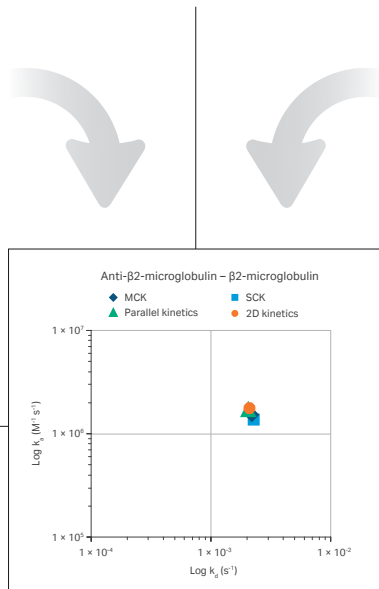


Fig 11. Biacore 8K and Biacore 8K+ approaches to kinetic determinations.

Rapid selection of the most relevant hits

With its parallel eight-channel setup with multiple microplate capacity, Biacore 8K and Biacore 8K+ rapidly generate screening data for selection of the most relevant hits based on binding information. More than 2300 small molecule fragments may be screened using **Binding level screen** and ranked in 24 h based on binding response and desired sensorgram profile.

For screening based on kinetic information, an initial single concentration screen of 384 samples is performed in less than 6 h, leaving time for setting up and starting the follow-up experiment on the samples with the best kinetic profile before going home for the day.

Characterization and optimization of the most promising binders

Regardless of your application, the systems provide efficient approaches to kinetics and affinity analysis. Affinity can be determined either with steady-state affinity analysis or via the ratio of kinetic rate constants. For small molecule fragments, specific affinity screening tools such as control based R_{max} are also available using Biacore Insight Evaluation Software and the Extended Screening and Characterization Extension.

For kinetic evaluation, the parallel setup can be utilized in several ways to ensure the shortest possible run time regardless of number of samples (Fig 11). By distributing the sample concentration series with associated blanks along the microplate, multicycle high-quality kinetic parameters can be obtained up to eight-fold faster than with single-needle systems.

For a single sample, faster determination can be obtained by distributing the concentration series across the plate without loss of accuracy. Kinetic analysis can also be performed using single-cycle kinetics (SCK). SCK simplifies analyses involving unstable targets as it can be performed without surface regeneration between concentrations. SCK also reduces assay run time and is the preferred choice for rapid kinetic characterizations of many samples, enabling analysis of 64 samples in 5 h.

For samples where prior knowledge of affinity is lacking, a novel 2D kinetics approach can be applied to deliver full kinetic characterization data within 35 min without extensive assay development. 2D kinetics combines the eight-channel parallel sampling setup of Biacore 8K with SCK. The sample is diluted in two dimensions creating a large concentration matrix. All dilutions are thereafter injected in a single cycle and globally fitted to provide reliable, high-quality kinetic data. If a capture approach is used, several consecutive samples can be analyzed using the 2D kinetics approach without the need for regeneration scouting.

Fast assay optimization for better results

Within drug discovery, an increasing amount of work is performed with more challenging targets such as membrane bound receptors, for example, GPCRs, and ion channels. These proteins are sensitive in nature and it is very important to identify the right assay conditions that retains their activity over the duration of the entire assay. Biacore 8K and Biacore 8K+ are equipped to facilitate efficient assay development and optimization. The eight-channel setup increases the number of conditions tested per unit time by up to eight fold compared with single-needle systems. Further, with the ABA-injection type, large matrices of buffer variations can be prepared in microplates and rapidly tested (Fig 12). The ABA buffer scouting approach allows testing of 96 buffer variations in less than 80 min.

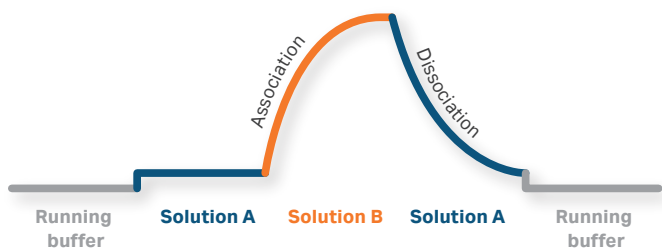


Fig 12. ABA-injection allows two different solutions to be injected over the surface in the same cycle in the following order: solution A, solution B, then solution A. This enables buffer scouting to be run directly from one microplate. The ABA injection may also be used in competition assays. In this case from left to right: Solution A = assay buffer + competitor; Solution B = assay buffer + competitor + analyte.

Explore epitope diversity quickly

Biacore Insight Epitope Binning Extension empowers automated identification and control to maintain unique and diverse epitopes, which contributes to broader intellectual property protection. The application-specific software provides support from run setup to data evaluation. All three major assay formats, i.e., sandwich, premix, and tandem, are supported for epitope binning analysis. Assay development time is shortened with predefined methods and automatic sample dispensing templates.

A common issue in epitope binning is low affinity of the binding between the antigen and first antibody, which results in dissociation of the antigen and underestimation of binding level of the second antibody. To at least partly overcome this problem, the antigen and second antibody may be injected using **Dual** inject. The **Dual** command injects the two solutions in sequence with no intermediate washing steps, which minimizes the dissociation of antigen before the secondary antibody is injected.

Concentration determinations and potency

Process development and quality control demands increased efficiency to allow for higher productivity without compromising data quality. Biacore 8K and Biacore 8K+ are equipped with tools that allow for efficient concentration determination regardless of sample size. The eight-channel set-up can be used to minimize time to results by running in a parallel fashion allowing eight concentrations to be determined in 30 min. A full 96-well sample plate is, with a serial setup, determined in 100 min.

See Table 1 for typical run times for applications using Biacore 8K and Biacore 8K+ systems, respectively.

Biacore Insight Evaluation Software has concentration evaluation functionality, available as an add-on extension with dedicated software tools for concentration and potency analysis and parallel line analysis (PLA). This extension allows for streamlined evaluation of concentration determinations and enables seamless determination of drug potency determinations without the need for tedious data import/export between different software.

Table 1. Typical run times for various applications using Biacore 8K and Biacore 8K+

Biacore 8K	No. of samples	Run time
Kinetic characterization	64	4 h
Kinetic screen, single conc.	384	9 h
2D kinetics of unknown	1	35 min
Clean screen	1536	3 h
Binding level screen	384	4 h
Affinity screen	64	5 h
Epitope binning	up to 30 × 30 array	33 h
Concentration analysis using serial calibration curve	96	100 min
Concentration analysis using parallel calibration curve	8	30 min
Biacore 8K+		
Kinetic characterization	64	4 h
Kinetic screen, single conc.	384	9 h
2D kinetics of unknown	1	35 min
Clean screen	4608	8.5 h
Binding level screen	3456	33.5 h
Affinity screen	64	5 h
Epitope binning	up to 40 × 40 array	59 h
Concentration analysis using serial calibration curve	96	100 min
Concentration analysis using parallel calibration curve	8	30 min

Support for working in regulated environment

An optional GxP Extension allows Biacore 8K and Biacore 8K+ to integrate seamlessly into GxP-regulated workflows. Biacore Insight GxP Extension provides validated software supporting GLP/GCP/GMP and 21 CFR Part 11 compliance, and includes validation support. Features in Biacore Insight 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
- Published 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.

For full validation support during the lifetime of the system, Biacore Insight GxP Extension can be supplemented with Cytiva Qualification Services.

For more details on the GxP support and Qualification Services provided, please see the *Biacore Insight GxP Extension data file*.

Biacore consumables for reproducible data with minimum time and effort

Biacore 8K and Biacore 8K+ operate using the extensive range of Biacore Series S sensor chips, which offer support for analysis of a wide range of interactions. A variety of capture kits offer a number of options for capturing the most common antibodies and tags to significantly reduce the time and effort you need to spend on developing your assay.

The range of Biacore consumables also includes coupling kits, with selected reagents for stable, covalent attachment of the ligand to the surface. Convenient, ready-made buffers and solutions developed and verified to work in Biacore systems are also available to further enhance analysis efficiency.

Join our family—Biacore community

As an owner of a Biacore system, you are connected to a world of knowledge and experience in interaction analysis. A Biacore system comes with professional local application support from highly skilled, experienced application scientists. These scientists are able to help you to get the most out of your Biacore system for all applications.

Thousands of Biacore systems are installed globally and over 50 000 scientific articles are published in peer-reviewed journals. All Biacore users are invited to share their experiences and learn more at regional user days and DiPIA conferences. Our instrument service is performed by specially trained service experts available close to you. They can help improve efficiency by minimizing system downtime. Streamlined maintenance of your equipment and fast response times let you focus on your work to deliver reliable binding analysis results.

Biacore 8K and Biacore 8K+ specifications

Technical specifications and characteristics

Detection technology	Surface plasmon resonance (SPR) biosensor
Information provided	Kinetic and affinity data (k_a , k_d , K_D), specificity, selectivity, screening data, epitope binning, concentration and relative potency data
Data presentation	Monitoring of real-time sensorgrams or evaluation data for result tables and result plots
Analysis time per cycle	Typically 2 to 15 min
Automation	60 h unattended run time for Biacore 8K 72 h unattended run time for Biacore 8K+
Sample type	Small molecule 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 μ L (application-dependent)
Injection volume	1 to 200 μ L
Flow rate range	1 to 100 μ L/min
Flow cell volume	40 nL
Flow cell height	70 μ m
Data collection rate	1 or 10 Hz
Sample/reagent capacity	4 \times 96- or 384-well microplates, normal, and deep-well (Biacore 8K) 12 \times 96- or 384-well microplates, normal, and deep-well (Biacore 8K+)
Typical run times	Clean screen (384-well plate): 45 min Binding level screen (384-well plate): 4 h Affinity screen (64 samples): 5 h Kinetic analysis (64 samples): 4 h Kinetic screen, single concentration (384-well plate): 9 h Epitope binning, 8 \times 8 array (64 samples): 2 h
Analysis temperature range	4°C to 40°C (maximum 20°C below ambient temperature)
Sample storage	4°C to 40°C (maximum 18°C below ambient temperature)
Sample refractive index range	1.33 to 1.39
In-line reference subtraction	Automatic
Number of flow cells	16 in 8 channels
Dimensions (W \times H \times D)	902 \times 875 \times 616 mm
Net weight total	127 kg (Biacore 8K); 141 kg (Biacore 8K+)
Mains requirements	Processing unit: Autorange voltage 100 to 240 V~, frequency 50/60 Hz
Power consumption	Processing unit: max. 350 VA (Biacore 8K); max. 550 VA (Biacore 8K+)

Minimum computer requirements

3.0 GHz processor, at least two cores

RAM > 2 GB free

Hard-disk drive > 40 GB free

Graphics resolution at least 1920 × 1080

Minimum network SQL Server requirements

CPU: Intel™ Xeon™ Processor E5-2408L v3 (10M Cache, 1.80 GHz)

RAM > 12 GB

Hard-disk drive > 400 GB hard disk

Windows® Server 2012

Uninterrupted power supply

Typical working ranges

Association rate constant (k_a) Proteins: up to $10^9 \text{ M}^{-1} \text{ s}^{-1}$
LMW molecules: up to $10^7 \text{ M}^{-1} \text{ s}^{-1}$

Dissociation rate constant (k_d) 10^{-6} to 0.5 s^{-1}

Sample concentration $\geq 1 \text{ pM}$

Molecular weight detection No lower limit for organic molecules

Baseline noise typically $< 0.02 \text{ RU (RMS)}$

Baseline drift typically $< 0.3 \text{ RU/min}$

Blank subtracted drift $< +/-0.03 \text{ RU/min}$

Immobilized interactant consumption Typically 0.03 to 3 $\mu\text{g}/\text{flow cell}$

Data handling and storage

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

Interfacing Import of sample data and export of results possible

Licenses Multiple licences available

Server requirements Includes SQL Server Express 2014 Release 2. Performance improvements are seen with SQL Server Standard, SQL Server Enterprise, or SQL Data Warehouse version 2016, 2017, or 2019 (available separately from Microsoft)

Compliance

Compliant with CE, cETLus, EAC, FCC, ICES-001, KC, RCM

Safety IEC/EN/UL/CSA-C22.2 61010-1,
IEC/EN/UL/CSA-C22.2 61010-2-081,
EN ISO 12100

Electromagnetic compatibility (EMC) EN/IEC 61326-1, FCC Part 15 B, ICES-001

Environmental EN 50581, China RoHS

Notes: The server is supplied by the end user. The system should be installed on the trolley included. Contact your local representative for the latest information regarding on-site requirements.

Ordering information

Product	Product code
Biacore 8K Includes: Biacore 8K instrument only	29327020
Biacore 8K and Instrument Kit Includes: Biacore 8K instrument (29327020); Table (29307354); Waste container (29308541); 2 × Licenses for Biacore Insight Evaluation Software (including Biacore 8K Control Software, 29310602); 2 × Licenses for Extended Screening and Characterization (29310610)	29337763
Biacore 8K+ Includes: Biacore 8K+ instrument only	29283382
Biacore 8K+ and Instrument Kit Includes: Biacore 8K+ instrument (29283382); Table (29307354); Waste container (29308541); 2 × Licenses for Biacore Insight Evaluation Software (including Biacore 8K Control Software, 29310602); 2 × Licenses for Extended Screening and Characterization Software (29310610)	29344964
Biacore Insight Evaluation Software	Various licenses ¹

¹ See cytiva.com for details of the various e-licenses available.

cytiva.com/biacore

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