Label-free interaction analysis

Biacore™ 8K

Biacore 8K efficiently delivers binding data with the quality you expect, meeting your toughest challenges in small molecule and biotherapeutic screening and characterization.

This eight needle high-sensitivity surface plasmon resonance (SPR) system rapidly provides 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 new drug formats such as bispecific antibodies.

- A single solution for interaction analysis in both screening and characterization
- Screening of 2300 small-molecule fragments in a day
- High-quality kinetic characterization of 64 interactions in 5 h
- 60 h unattended runtime with queueing abilities and rapid multi-run evaluations
- Confident interaction analysis of small molecules binding to complex targets such as GPCRs
- Confident differentiation of high-affinity binders

One solution for small molecule and biotherapeutic screening/characterization

Biacore 8K gives you a single solution for interaction analysis in both screening and characterization for small molecule and biotherapeutic discovery. The system is well-suited to the analysis of a wide variety of samples including the smallest fragments or large multidomain proteins, even in crude matrices. Main 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

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

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 is 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. Biacore 8K also supports fast scouting of assay conditions, screening 96 buffer conditions in 80 min, optimizing your assay to deliver interaction data you can rely on.

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

Performance to meet the toughest applications

Biacore 8K comes 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

The high sensitivity of Biacore 8K provides the means to generate reliable data for rare or sensitive targets such as GPCRs (Fig 2) where only a fraction of the protein might retain its biological activity throughout the analysis. The analysis can 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.

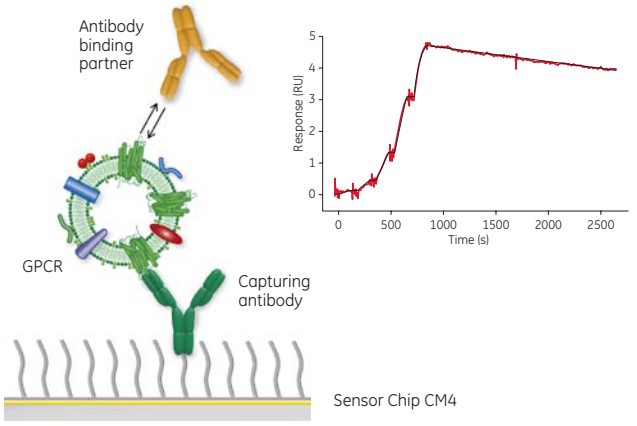


Fig 2. The high sensitivity and robustness of Biacore 8K allows the analysis of GPCRs in crude membrane preparations.

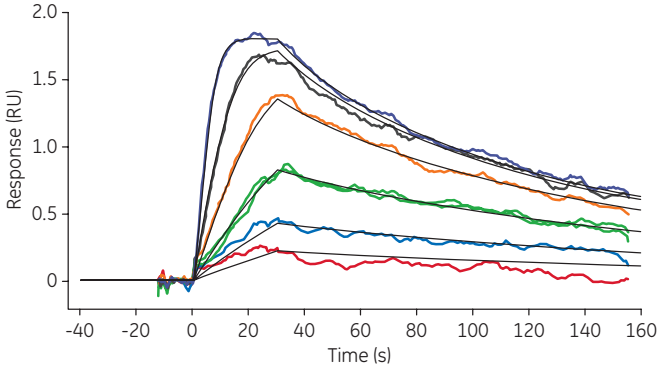
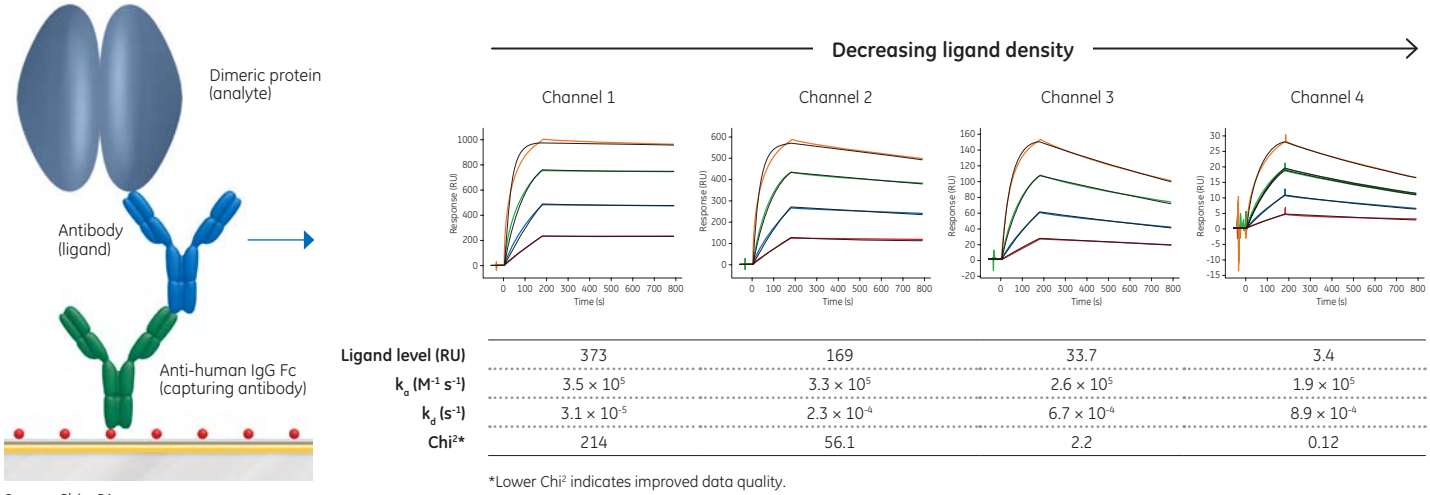


Fig 4. The high sensitivity of Biacore 8K enables confident analysis of fast on-rates. Sensorgram showing binding of melagatran to thrombin: k_a 4.0 × 10^7 M^{-1} s^{-1}; k_d 0.014 s^{-1}.

Biacore 8K allows for full flexibility in the characterization of bivalent analytes, such as antibodies or dimeric proteins. Complication from avidity is minimized by using very low immobilization levels, which renders reliable data (Fig 3). Low immobilization levels generally give fewer secondary interactions and can 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.

Biacore 8K enables measurement of really fast on-rates that allows differentiation between rapid binders. This is an important feature when studying biological processes limited by bioavailability (Fig 4).



	Channel 1	Channel 2	Channel 3	Channel 4
Ligand level (RU)	373	169	33.7	3.4
k_a (M^{-1} s^{-1})	3.5 × 10^5	3.3 × 10^5	2.6 × 10^5	1.9 × 10^5
k_d (s^{-1})	3.1 × 10^{-5}	2.3 × 10^{-4}	6.7 × 10^{-4}	8.9 × 10^{-4}
Chi²*	214	56.1	2.2	0.12

*Lower Chi² indicates improved data quality.

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 courtesy: Schräml, Biehl, von Proff, Roche Diagnostics GmbH, Centralised and Point of Care Solutions, Penzberg, Germany.

At the other end of the kinetic spectrum, today's antibody discovery often generates many very 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. The high sensitivity of 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).

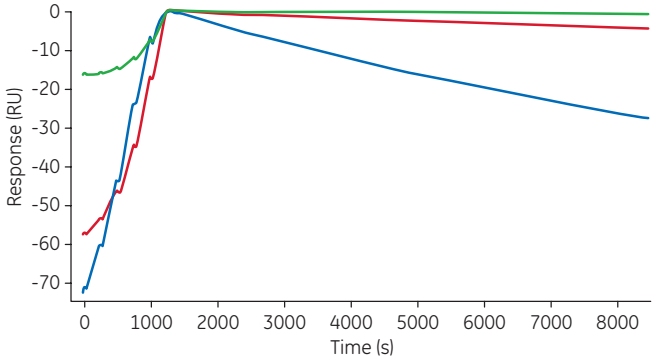


Fig 5. Biacore 8K provides sensitivity and stability that enables the differentiation of tight binders with dissociation rate constants, k_d, down to 10^{-6} s^{-1}.

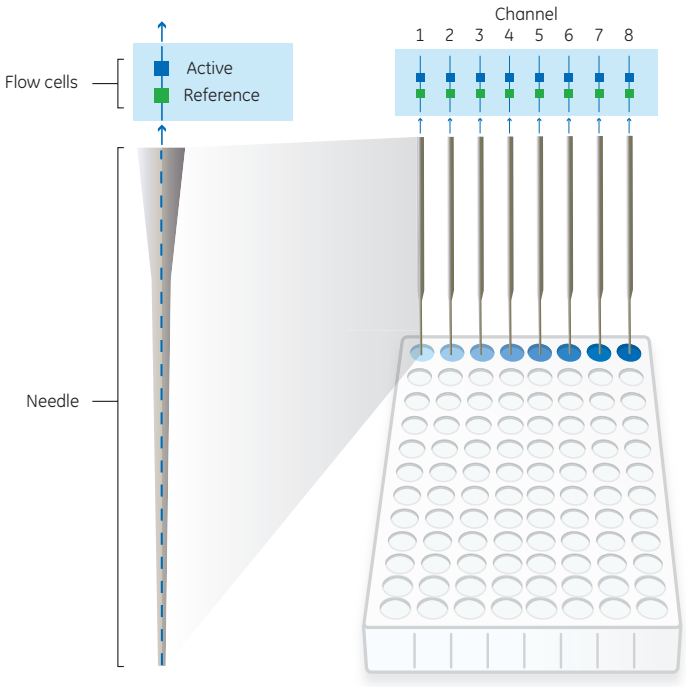


Fig 6. The simple, eight-channel concept with two flow cells per channel simplifies assay setup and operation of Biacore 8K.

Parallel setup maximizing operational efficiency

Biacore 8K is designed to maximize operational efficiency by combining rapid high-quality data with the smooth operation that comes from user-friendly software and interactive hardware.

The system features 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 of Biacore 8K has been refined to optimize stability and robustness while not compromising on performance. As other Biacore systems, Biacore 8K can provide interaction data directly from crude matrices such as hybridoma supernatants, membrane preparations, or serum samples.

Biacore 8K supports 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 accommodates two trays with two microplates and can be accessed during run to optimize operational efficiency.

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.

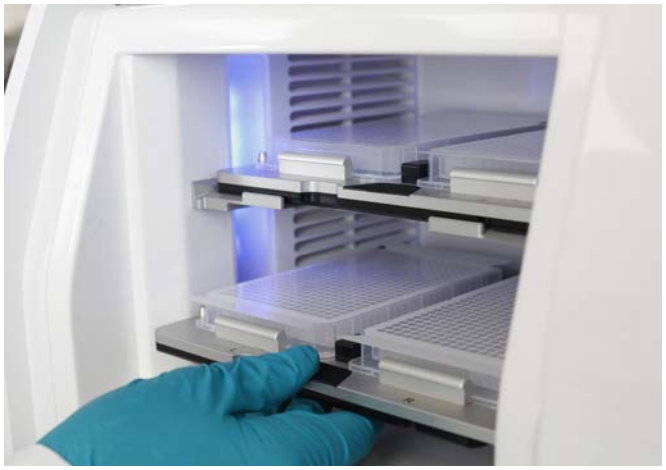


Fig 7. Biacore 8K accommodates up to four 96- and 384-well microplates simultaneously, all in a temperature-controlled environment for optimal assay performance or to ensure sample integrity in extended runs.

Interaction data at physiological temperatures

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

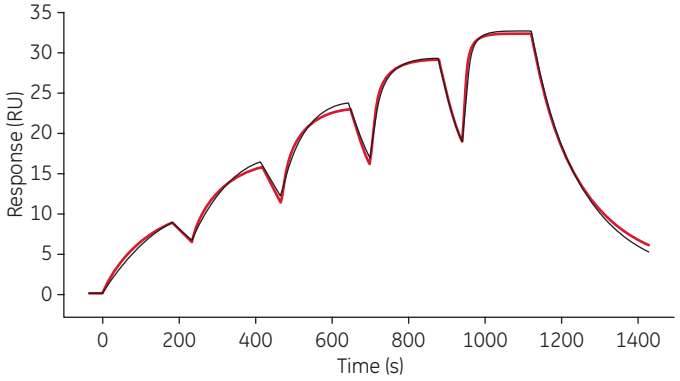


Fig 8. Biacore 8K provides high-quality data at physiologically relevant temperatures. Data shows the analysis of β2-microglobulin vs anti-β2-microglobulin at 37°C.

Software with flat interface for maximum overview

Biacore 8K software exhibits a flat interface that provides 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, operation, and evaluation

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. 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, 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.

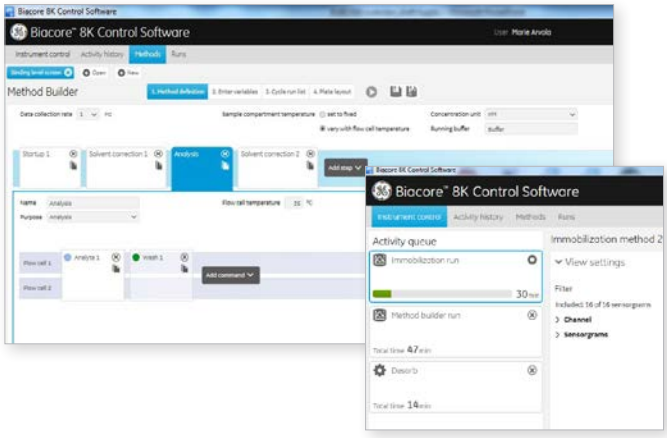


Fig 9. Biacore 8K Control Software exhibits graphical representation of the method definition, which provides intuitive run setup. Queuing capabilities maximize operational efficiency.

Biacore 8K Evaluation Software (Fig 10) enables kinetic and affinity 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. Generic tools scale with the size of your experiment rendering fast results you can trust in, irrespective of the number of samples analyzed. The flexible interface is configurable to maximize the space for your most important tasks at any time.

- Rapidly overview and qualify your data
- Utilize flexible tools for customized data analysis
- Easily export and share results

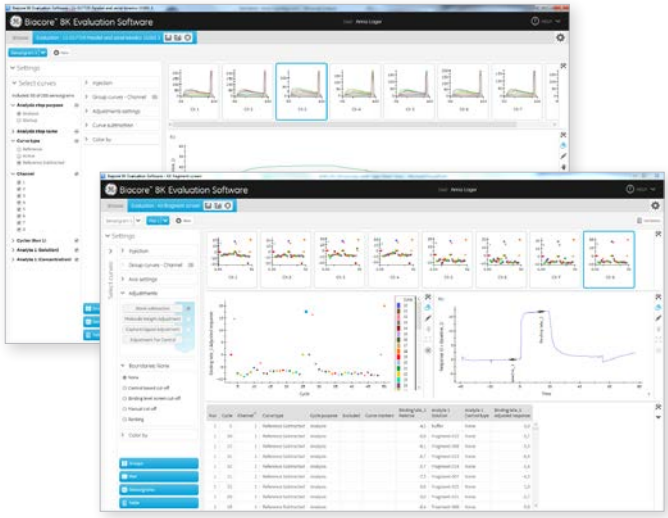


Fig 10. The flat interface of Biacore 8K Evaluation Software provides full overview while offering flexible tools for customized data analysis. In the *Result plot*, thousands of samples from multiple runs can be visualized and analyzed simultaneously.

Thousands of samples from multiple runs can be visualized simultaneously and the results presented in a *Result plot*. The *Result plot* provides efficient, flexible, easy-to-use tools to process the data of interest. Absolute or referenced data can be plotted and double-referenced by applying *Blank subtraction*. Sample responses can easily be harmonized with respect to the molecular weight of the analyte or the level of captured ligand respectively, making the entire data set comparable. Adjustment for controls normalizes sample responses to the selected positive (and optional negative) controls, to correct for drift in surface activity during the course of the assay. *Cutoff/Ranking* is manually set. Alternatively, control-based automatic cutoff allows a cutoff to be set at the selected number of standard deviations in relation to the control.

Evaluation methods for ultimate speed

The data evaluation process can be standardized and speeded up by applying predefined evaluation methods. The software comes with a number of application-relevant *Evaluation methods* that can be applied as is or used as template for optimized user-defined evaluation methods. Relevant evaluation parameters can be saved in *Evaluation methods*, avoiding repetitive, non-value added tasks. When opening a run file with the selected *Evaluation method*, the first results are obtained within minutes.

Fast exporting of selected or comprehensive data

The flexible *Result export* in Biacore 8K provides the means to export selected or comprehensive data for continued data processing, result reporting, or storage in the company database (Fig 11). The data is exported in a Microsoft® Excel® format. Evaluation items to include in the export can easily be selected and detailed settings for each item defined. Sensorgrams or plots can be included in the export as images in three different sizes. Result tables are exported either using a comprehensive format or using the same format as defined in the evaluation data. Results are therefore always presented in a preferred format to facilitate reporting or adoption into other software.

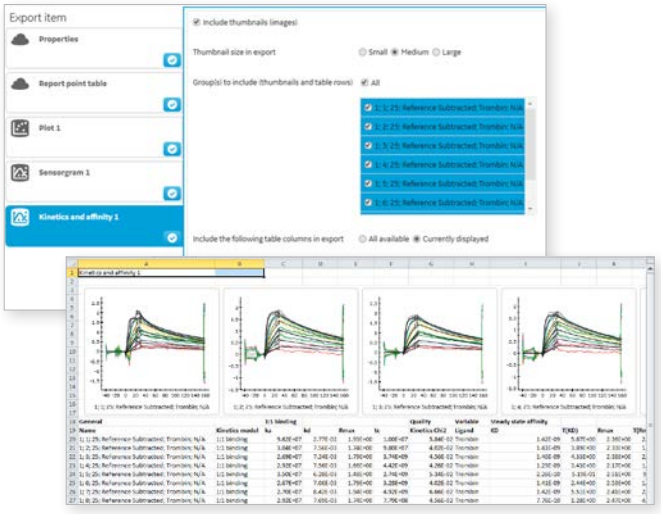


Fig 11. The *Result export* feature allows easy export of results in Microsoft Excel format.

Rapid selection of the most relevant hits

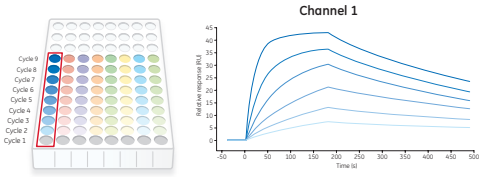
With its parallel eight-channel setup with multiple microplate capacity, Biacore 8K rapidly generates screening data for selection of the most relevant hits based on binding information. Using a binding level screen approach, more than 2300 small molecule fragments can be screened and ranked in 24 h 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.

Table 1. Typical run times for various applications

Application	No. of samples	Run time
Kinetic characterization	64	5 h
Kinetic screen, single conc.	384	6 h
2D kinetics of unknown	1	35 min
Clean screen	768	2 h
Binding level screen	384	2 h
Affinity screen	64	3 h

Multi-cycle kinetics (MCK)

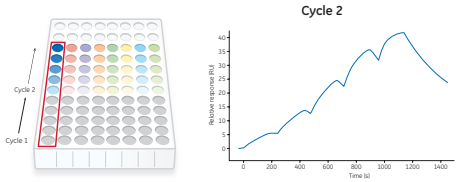
- Suitable for many samples against one ligand
- Suitable when different ligands are to be immobilized



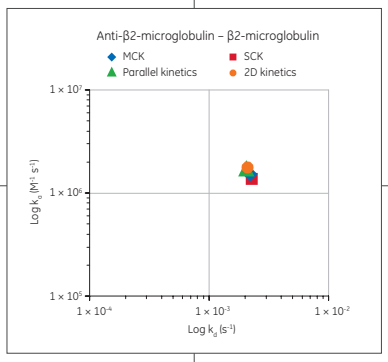
Ex. Cycle 1-9: sample concentrations and blanks are placed per channel

Single-cycle kinetics (SCK)

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

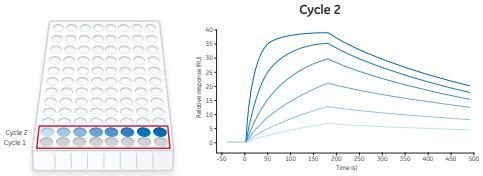


Ex. Cycle 2: 5× sample conc. (Cycle 1: 5× blank conc.)



Parallel kinetics

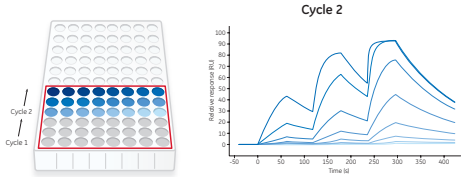
- Short run time for few samples
- Kinetic analysis in only two cycles (one blank cycle)
- Beneficial for samples with long dissociation times



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

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 12. Biacore 8K approaches to kinetic determinations.

Characterization and optimization of the most promising binders

Regardless of your application, Biacore 8K provides 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.

For kinetics, the parallel setup can be utilized in several ways to ensure shortest possible run time regardless of number of samples (Fig 12). By distributing the sample concentration series with associated blanks along the microplate, multi-cycle, 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 is 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 13). The ABA buffer scouting approach allows testing of 96 buffer variations in less than 80 min.

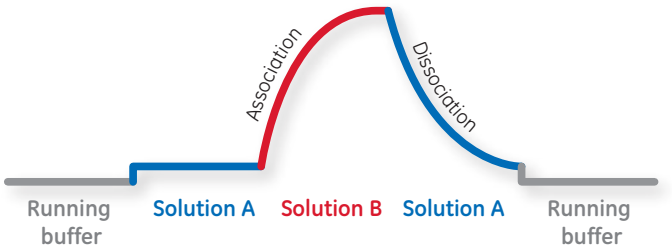


Fig 13. The ABA-injection allows two different solutions to be injected over the surface in the same cycle in the following order: solution A, solution B, and solution A. This enables buffer scouting to be run directly from one microplate.

While buffer composition can easily be varied across the plate, instrument-related parameters are varied along the plate to rapidly build up a comprehensive assay development matrix (Fig 14). Development matrices can easily be stored as run methods and reused for certain target classes, for example.

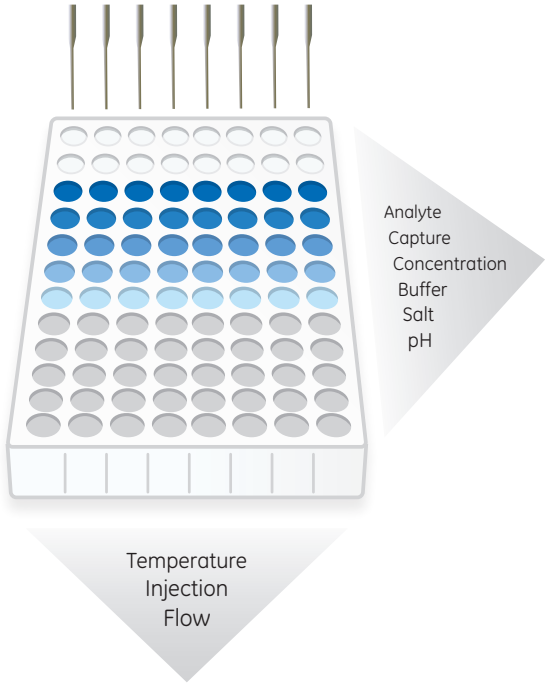


Fig 14. The eight-channel setup makes Biacore 8K highly amendable for comprehensive assay development. Analytes or buffer composition can easily be varied across the plate while instrument-related parameters can be varied along the plate.

Biacore consumables for reproducible data with minimum time and effort

Biacore 8K operates using the extensive range of Biacore Series S sensor chips, which offers 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 – the Biacore Network

As an owner of a Biacore system you get 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 30 000 scientific articles are published in peer-reviewed journals. All Biacore users are invited to share their experiences and learn more at regional user days. Furthermore, the DiPIA community, gelifesciences.com/dipiadigital, has become an international internet forum for fast information sharing on interaction analysis.

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 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 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	60 h unattended operation
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 µL (application dependent)
Injection volume	1 to 200 µL
Flow rate range	1 to 100 µL/min
Flow cell volume	40 nL
Flow cell height	70 µm
Data collection rate	1 or 10 Hz
Sample/reagent capacity	4 × 96- or 384-well microplates, normal and deep-well.

Technical specifications and characteristics

Typical run time	Clean screen (384-well plate): 1 h Binding level screen (384-well plate): 2 h Affinity screen (64 samples): 3 h Kinetic analysis (64 samples): 5 h Kinetic screen, single concentration (384-well plate): 5.5 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 × H × D)	902 × 860 × 622 mm
Net weight total	127 kg
Mains requirements	Processing unit: Autorange voltage 100 to 240 V~, Frequency 50/60 Hz
Power consumption	Processing unit: maximum 350 VA

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

DVD drive for software installation

Typical working ranges

Association rate constant (k_a)	Proteins: up to 10^9 M^{-1} s^{-1} LMW molecules: up to 10^7 M^{-1} s^{-1}
Dissociation rate constant (k_d)	10^{-6} to 0.5 s^{-1}
Sample concentration	≥ 1 pM
Molecular weight detection	No lower limit for organic molecules
Baseline noise	Typically < 0.02 RU (RMS)
Baseline drift	Typically < 0.3 RU/min
Blank subtracted drift	< +/-0.03 RU/min
Immobilized interactant consumption	Typically 0.03 to 3 µg/flow cell

Data handling and storage

PC operating systems	Windows® 7 Professional SP1, 64-bit Windows 10 Professional 64-bit
Interfacing	Possibilities for import of sample data and export of results

Compliance

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

Product	Product code
Biacore 8K System	29215379

gelifesciences.com/biacore

GE Healthcare Bio-Sciences AB, Björkgatan 30, 751 84 Uppsala, Sweden
GE Healthcare UK Ltd., Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK
GE Healthcare Europe GmbH, Munzinger Strasse 5, D-79111 Freiburg, Germany
GE Healthcare Dharmacon Inc., 2650 Crescent Dr, Lafayette, CO 80026, USA
HyClone Laboratories Inc., 925 W 1800 S, Logan, UT 84321, USA
GE Healthcare Japan Corp., Sanken Bldg., 3-25-1, Hyakunincho Shinjuku-ku, Tokyo 169-0073, Japan
For local office contact information, visit gelifesciences.com/contact.

29205902 AA 06/2016

GE Healthcare Bio-Sciences Corp.
100 Results Way
Marlborough, MA 01752
USA