



## Label-free interaction analysis

# Biacore™ X100

Biacore X100 (Fig 1) is an automated and versatile system for comprehensive, label-free analysis and characterization of biomolecular interactions in real-time. Biacore X100 can be used for a broad range of applications, including structure-function studies, pathway analysis, biomarker discovery and validation, drug target identification and validation, and assay development

### Biacore X100 delivers:

- **Interaction data with an added dimension:** Real-time interaction data enables kinetic characterization, highly accurate affinity determination and detection of weak and transient binding events.
- **Versatility:** Supports many different assay formats and the study of a wide variety of biomolecules including small molecules, proteins, DNA, and cells. These interactions can be measured in buffer solutions or in crude environments such as serum.
- **Convenience and efficiency:** Workflow-oriented software provides a structured approach to assay development, minimizes hands-on time, and generates reliable data without compromising flexibility for the best assay set up. Preprogrammed workflows for supporting kits, surfaces, and reagents provide solutions for a multitude of assays.
- **Quality and sensitivity:** High quality hardware and software, together with high-sensitivity ensure reliable detection of genuine interactions and extend the application range while minimizing sample consumption.

## Biacore X100 Plus Package

The addition of Biacore X100 Plus Package extends the functionality of the system, adding capabilities that enable you to:

- Study interactions involving small molecules in organic solvents
- Measure concentration based on specific binding
- Analyze interactions over a range of temperatures



**Fig 1.** Biacore X100 is an integrated system for quickly and securely generating reliable molecular interaction data.

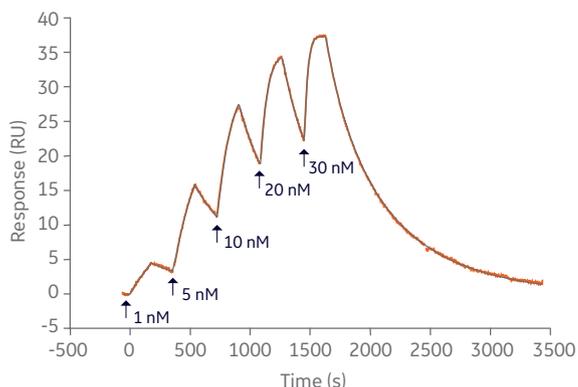
## Biacore X100 overview

### Analysis of kinetic properties

Binding affinity can be resolved into an on-rate, which is primarily driven by molecular recognition, and off-rate, which is controlled by complex stability. This enables a better understanding of molecular interactions and biological processes. Kinetic characterization of interactions can be performed using either the traditional approach, with one sample concentration at a time followed by regeneration of the surface between each analysis cycle, or the more recently developed single-cycle approach. Single-cycle kinetics is unique to Biacore systems and reduces the time to results by eliminating one step in optimizing assay conditions (Fig 2).

In conjunction with the use of a capture kit, Biacore X100 enables you to produce kinetic data in less than an hour—from assay development to final data. For capture assays, single-cycle kinetics typically reduces reagent consumption by a factor of four thus reducing cost and time. Furthermore, biomolecular investigations that were not previously feasible due to the inability to find suitable regeneration conditions can now be performed using single-cycle kinetics. Biacore X100 can measure kinetic constants over a broad range—from the fastest on-rates to the slowest off-rates:

- On-rates from  $10^3$  to  $10^7$   $M^{-1}s^{-1}$
- Off-rates from  $10^{-5}$  to  $10^{-1}$   $s^{-1}$

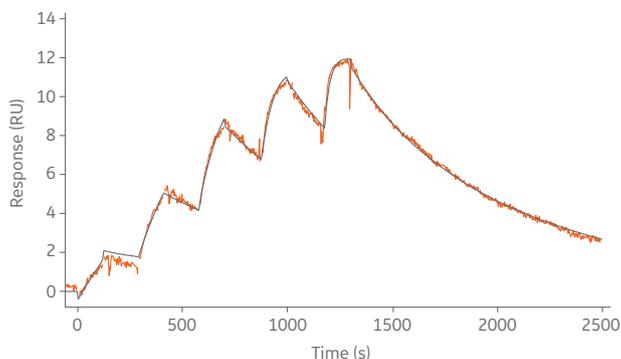


**Fig 2.** A sensorgram depicting a run in a single-cycle kinetics mode. Increasing sample concentrations were injected consecutively in the same analysis cycle without a regeneration.

### High sensitivity

Biacore X100 provides highly sensitive detection of biological interactions (Fig 3). This feature is particularly useful for the investigation of the following:

- Analysis of interactions between drug compounds and target proteins
- Biomolecules such as membrane proteins that require special conditions to remain active
- Biomolecules that are present in minute quantities, such as biomarkers in serum
- Resolution of slow off-rates, where very small decreases in signal may need to be accurately monitored during the dissociation phase

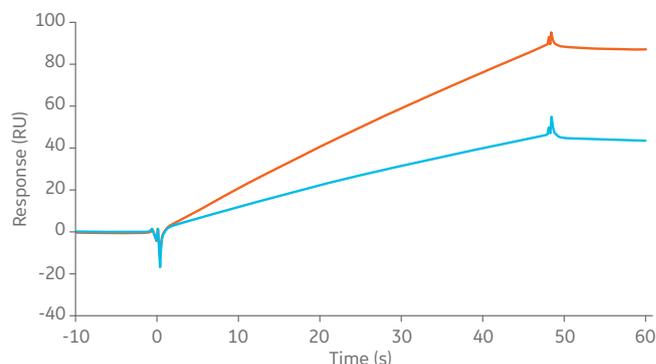


**Fig 3.** Low noise level enables reliable kinetic characterization at low signal levels. This helps ensure that signals are due to genuine interactions.

In addition, the high sensitivity of Biacore X100 facilitates improved kinetic analysis, where a low surface density of the immobilized biomolecule is generally desirable in order to avoid potential artefacts.

### Display of real-time data

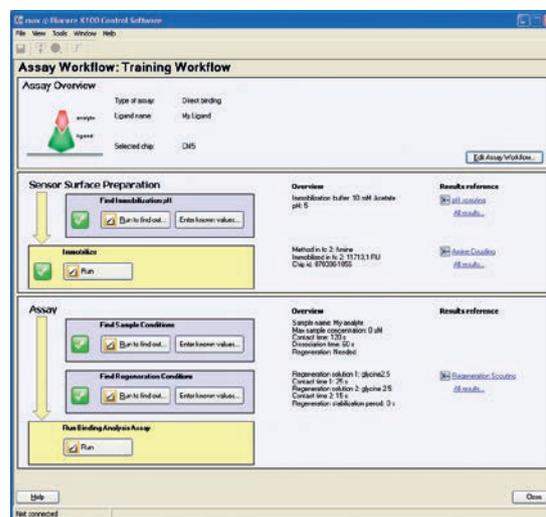
Biacore X100 provides a continuous display of the real-time data output (Fig 4), from assay development to binding analysis. This provides reassurance that the experiment is proceeding as normal, or where unexpected results are seen, enables you to decide whether the data is novel and worthy of further investigation, or represents an assay problem that requires troubleshooting.



**Fig 4.** Overlay of two runs of the same sample 16 cycles apart. The lower slope of the curve in the later cycle indicates that the immobilized protein has lost its activity; probably due to the use of regeneration conditions that were too harsh.

### A structured approach to assay development

Biacore X100 software incorporates the concept of guided workflows for specificity, affinity and kinetic analysis (Fig 5). This concept provides a structured approach to assay development and enables you to generate reliable data from day one without compromising assay flexibility.



**Fig 5.** Workflow-oriented software simplifies assay development and minimizes hands-on time.

Based on the properties of the samples, the software suggests alternative assay configurations. After you select an alternative, the software outlines the entire assay workflow from attaching one molecule to the sensor surface to measuring the interaction parameters for any number of interaction partners and evaluating the data. Each step of the workflow is supported by a specific software wizard, and guidance in the form of recommendations and explanations of concepts is provided to you at every step.

### Reliable data analysis

Biacore X100 evaluation software provides efficient data analysis in an automated and intuitive manner. Quality controls (Fig 6) are applied automatically to data fitting of kinetic parameters and if necessary, helpful suggestions or alternate models are provided for further investigation. In addition to binding profiles (i.e., sensorgram views), Biacore X100 provides a large number of customizable graph and chart tools, which you can use to generate visual representations of the data. The resulting graphics can be easily exported to other commonly used computer applications for the production of your reports and presentations.

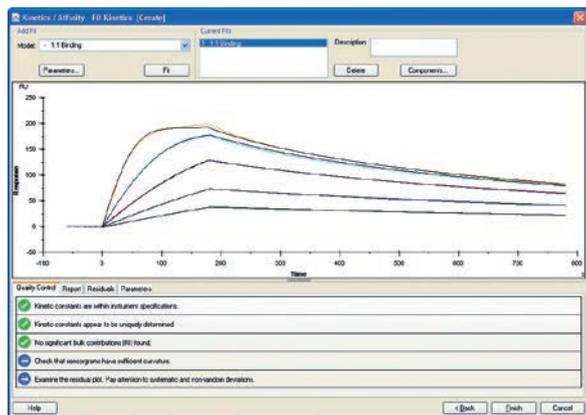


Fig 6. Easy QC tools help assess data quality and facilitate valid data interpretation.

### Support for Biacore capture kits

Biacore capture kits provide optimized assay protocols that minimize the need for assay development (Fig 7). The guided workflows include preprogrammed settings for all available capture kits and sensor chip surfaces. This further increases the convenience and ease of use, while ensuring efficient use of reagents.

Biacore capture kits save cost, time, and effort by:

- Providing pre-optimized capturing conditions for one of the biomolecules
- Providing preoptimized regeneration conditions
- Increasing the chances of success by using an immobilization strategy that maintains one of the biomolecules in a specific orientation on the sensor chip surface

Capture kits and surfaces are available for a variety of tags and molecules, including:

- GST Capture Kit for GST-fusion proteins
- Sensor Chip NTA for His-tagged proteins
- Biotin CAPture Kit for reversible capture of biotinylated molecules
- Sensor Chip SA for irreversible capture of biotinylated molecules
- Mouse Antibody Capture Kit for Mouse IgG antibodies
- Human Antibody Capture Kit for Human IgG antibodies

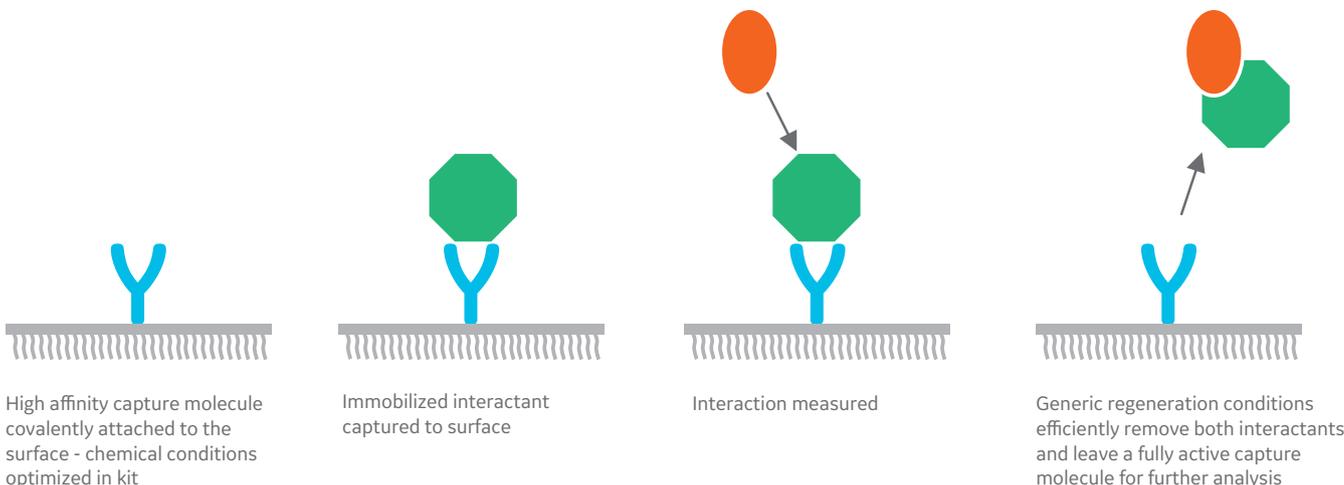


Fig 7. In a capture assay, there is no need for finding either the conditions for attachment of the immobilized interactant to the sensor chip surface or the conditions for regenerating the surface to the original state.

## Flexibility for a broad range of applications

The availability of a wide range of sensor chips ensures that you can choose the most suitable sensor surface for the nature of the molecule to be coupled and the requirements of the analysis. Covalent immobilization can be performed via reactive groups on the molecule, and special chips support irreversible capture of biotinylated molecules and capture of lipid monolayers and bilayers.

## Equipped for multiple users

Biacore X100 stores all the information about the immobilization step with the results files. In addition to increasing flexibility, this also increases accessibility to the system by simplifying interrupted usage with removal of a single chip. It also reduces risk of error by recording information on chip usage, which is an important issue if multiple users have access to the system.

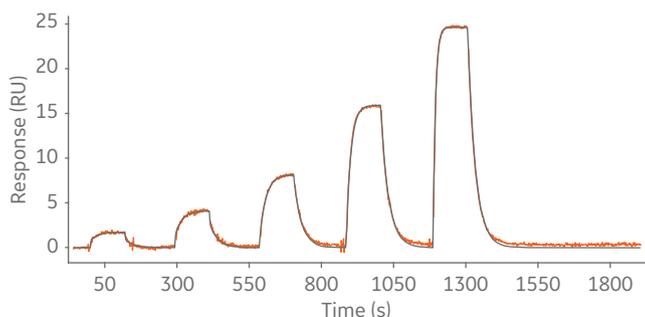
Individual user accounts can be set up for multi-user access to Biacore X100. Each user will then get a separate working area for storing methods and data.

## Biacore X100 Plus Package

Biacore X100 Plus Package extends the functionality of the system by adding features such as the ability to study biomolecular interactions involving small molecules in an organic solvent, to determine protein concentrations related to a specific binding event, and to vary and control the temperature of different assays.

## Analyze small molecules that require organic solvents for solubility

The study of how a putative drug interacts with its target protein provides crucial information for drug discovery. Putative drugs are often small organic compounds that require organic solvents like Dimethyl sulfoxide (DMSO) in order to become soluble in aqueous buffers. The analysis of an interaction in the presence of DMSO requires special care. Biacore X100 Plus Package provides all the necessary tools for studying these types of interactions (Fig 8).



**Fig 8.** Single-cycle kinetic analysis of binding of furosemide (molecular weight of 331) dissolved in 3% DMSO to carbonic anhydrase.

## Efficient and specific concentration analysis

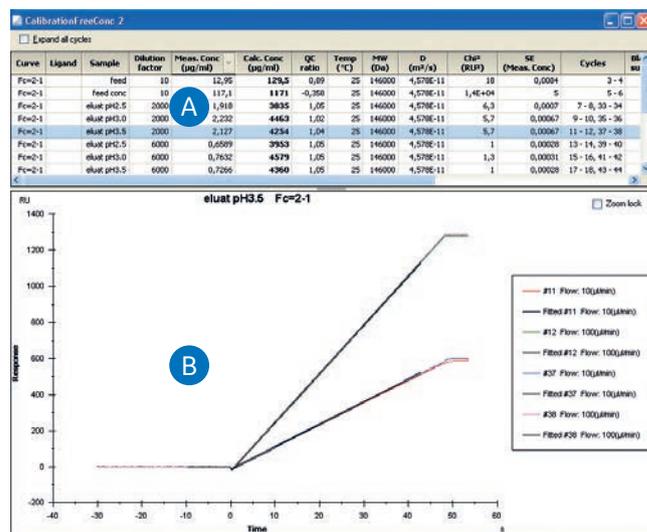
Biacore X100 Plus Package allows you to measure the concentration of a protein via its interaction with a specific binding partner. This corresponds to the concentration of the active protein in solution (unlike total protein concentration determinations from methods such as spectrophotometry). This parameter is important to many applications because it enables you to:

- Improve the accuracy of affinity and kinetic rate constants by reducing errors in the concentrations that are used for fitting the binding data
- Monitor protein stability in various sample environments
- Monitor the amount of active protein throughout a process (e.g., in a series of purification steps from cell harvest to final product)

## Concentration analysis without a standard

In addition to standard protein concentration analysis using a calibration curve, Biacore X100 Plus Package supports a novel method called Calibration-free concentration analysis (CFCA). This method also measures protein concentrations related to specific binding activity, but without the need for a protein standard. This is achieved by using the changes in binding rate that result from variations in flow rates under conditions in which the transport of molecules to the sensor surface is limited by diffusion. CFCA allows you to:

- Determine the concentration of a protein in the absence of suitable standard
- Validate the specified concentration of a protein standard



**Fig 9.** Calibration-free concentration analysis evaluation. Concentrations calculated for all samples included in the run (A). By clicking a sample, the underlying data and the fit of the model are displayed in (B).

## Temperature control provides additional information

The ability to set the temperature of an assay enables you to perform experiments at physiological temperatures, or to study how particular biomolecular interaction properties change at different temperatures. Biacore X100 Plus Package can be used to:

- Study biomolecular interactions over a temperature range of 4°C to 40°C
- Better predict *in vivo* behavior by studying interactions at physiological temperatures

An in-line degasser is included in Biacore X100 Plus Package to preserve data quality at elevated temperatures.

## Full assay and analysis flexibility

The Custom Assay Wizard provides an interface for defining assay methods with complete control over sample injection and sample flow through the system. Pre-defined templates are available for direct use or as a starting point for modification. Biacore X100 Plus Package also allows you to define your own evaluation models for fitting affinity and kinetic equations to collected data.

## Summary

Biacore X100 is a robust and reliable system for the comprehensive characterization of biomolecular interactions. Highly sensitive, real-time detection of molecular binding allows you to carry out new types of studies such as kinetic characterization, highly accurate affinity determination and detection of weak and transient binding events. Biacore X100 Plus Package adds additional layers of functionality to the system, enabling you to perform experiments with molecules in organic solvents, to determine specific binding concentration, and to study biomolecular interactions at physiological temperatures.

# Biacore X100 specifications

## Technical specifications and characteristics

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Detection technology	Surface plasmon resonance (SPR) biosensor
Data presentation	Result tables, result plots, and real-time monitoring of sensorgrams
Working principle	2–1 detection (automatic reference subtraction)
Information provided	Kinetic and affinity data ( $K_D$ , $k_a$ , $k_d$ ) specificity, selectivity. Concentration measurements <sup>1</sup>
Sample type	LMW drug candidates to high molecular weight proteins in various sample environments (also DNA, RNA, polysaccharides, lipids, cells and viruses), e.g. in DMSO-containing buffers, plasma or serum
Kinetics:	Multi cycle or single cycle (without the need for regeneration between injections)
Association rate constant ( $k_a$ )	$10^3$ to $10^7$ M <sup>-1</sup> s <sup>-1</sup> (for typical protein-protein interactions)
Dissociation rate constant ( $k_d$ )	$10^{-5}$ to $0.1$ s <sup>-1</sup>
Dissociation constant ( $K_D$ )	100 $\mu$ M to 1 pM
Detection limit	Typically 0.1 nM for > $M_r$ 10 000 analytes Typically 1 nM for < $M_r$ 10 000 analytes
Molecular weight detection	Down to $M_r$ 100 in various sample environments
Analysis time per cycle	Typically 2 to 15 min
Sample capacity per run	Maximum 15 samples
Automation	24 h unattended operation
Injection volume	5 to 90 $\mu$ L
Required sample volume	Injection volume + 20 to 30 $\mu$ L (application dependent)
Immobilized interactant consumption	Typically 1 $\mu$ g
Concentration analysis	With standard calibration curve or Calibration-free concentration analysis (CFCA) <sup>1</sup>
Baseline noise	Typically < 0.1 RU (RMS)
Baseline drift	Typically < 0.3 RU/min
Sample refractive index range	1.33 to 1.40
In-line buffer degassing	Included <sup>1</sup>
Number of flow cells	2
Flow cell dimensions (W × H × L)	0.5 × 0.05 × 2.1 mm
In-line reference subtraction	Automatic
Flow rate range	1–100 $\mu$ L/min
Analysis temperature range	4°C to 40°C (maximum 10°C below ambient temperature)
Sample compartment temperature	Ambient
Dimensions (W × H × D)	Instrument: 596 × 563 × 593 mm
Net weight	Total 47 kg
Mains requirement	Processing unit and system controller: Autorange 100 to 240 VAC, 50 to 60 Hz, protective earthing
Power consumption	Processing unit: Max 4.0 A (at 100 VAC) System Controller: Max 7.2 A (at 100 VAC)

## Data handling and storage

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PC operating systems	Microsoft® Windows® 7 Professional, 64-bit, US English version Microsoft Windows 10 Professional, 64-bit, US English version The Biacore X100 software version 2.0.2 does not support 32-bit Windows operating system
Database storage	Oracle® Database 11g Express Edition (included)
Data export	Excel® format result data export, text file raw data export
Image export	Clipboard export

## Compliance

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Safety and EMC standards	Complies with and applies to Europe and North America (US and Can) standards
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<sup>1</sup> Included in Biacore X100 Plus Package

## Ordering information

<b>Product</b>	<b>Product code</b>
Biacore X100	BR110073
Biacore X100 Plus Package	BR100798
Biacore X100 2.0 Upgrade Kit	BR100800
Biacore X100 Plus Package 2.0 Upgrade Kit	28956516

### **Related literature**

Biacore X100 System, Brochure	28961744
A year of interaction with Biacore X100, White paper	29030218
Biacore systems, Selection guide	29007053

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