

Biacore 8K/8K⁺

User Manual



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1 Introduction

About this chapter

This chapter provides an introduction to the Biacore™ 8K/8K+ system and to the terminology used in Biacore systems.

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1.1 About this manual

Purpose of this manual

The *Biacore 8K/8K* User Manual* describes how to use the Biacore 8K/8K* instrument and software to perform label-free interaction analysis experiments. Evaluation of the data obtained is described in the separate *Biacore Insight Evaluation Software Manual*.

Scope of this manual

Instrument descriptions in this *User Manual* apply to Biacore 8K systems with Product Code 29327020 and to Biacore 8K* systems. The systems differ in sample capacity (see [Sample hotel and sample compartment, on page 22](#)) but are otherwise closely similar. The two systems together are referred to as the Biacore 8K/8K* system. Unless otherwise explicitly stated, information in this User Manual applies to both systems.

For Biacore 8K systems with Product Code 29146489, refer to the User Manual provided with the system.

Descriptions of the instrument control software apply to version 2.0 or later of the Biacore 8K Control Software.

Typographical conventions

Software items are identified in the text by ***bold italic*** text.

Hardware items are identified in the text by **bold** text.

In electronic format, references in *italics* are clickable hyperlinks.

1.2 Important user information

Read this before operating the product



All users must read the entire *Operating Instructions* before installing, operating or maintaining the product.

Always keep the *Operating Instructions* at hand when operating the product.

Do not operate the product in any other way than described in the user documentation. If you do, you may be exposed to hazards that can lead to personal injury and you may cause damage to the equipment.

Intended use

The Biacore 8K/8K⁺ system, consisting of the Biacore 8K/8K⁺ instrument, Biacore 8K Control Software, and Biacore Insight Evaluation Software, supports execution and evaluation of label-free interaction analyses based on surface plasmon resonance (SPR) measurements. The system is intended for research use and quality control measurements associated with manufacturing procedures. The system may not be used for any clinical or diagnostic applications.

Prerequisites

In order to operate the Biacore 8K/8K⁺ system safely and in accordance with the intended purpose the following prerequisites must be met:

- The system should be installed according to the instructions in the *Installation* chapter of the *Operating Instructions*.
- You should have a general understanding of the use of a personal computer running Microsoft® Windows® in the version provided with your product.
- You should be acquainted with the use of general laboratory equipment and with handling of biological materials.

A system administrator familiar with management of Microsoft SQL Server® databases is required. Familiarity with database management is not required for operation of the Biacore 8K/8K⁺ system.

Safety notices

This user documentation contains safety notices (WARNING, CAUTION, and NOTICE) concerning the safe use of the product. See definitions below.



WARNING

WARNING indicates a hazardous situation which, if not avoided, could result in death or serious injury. It is important not to proceed until all stated conditions are met and clearly understood.



CAUTION

CAUTION indicates a hazardous situation which, if not avoided, could result in minor or moderate injury. It is important not to proceed until all stated conditions are met and clearly understood.



NOTICE

NOTICE indicates instructions that must be followed to avoid damage to the product or other equipment.

Notes and tips

- Note:** *A note is used to indicate information that is important for trouble-free and optimal use of the product.*
- Tip:** *A tip contains useful information that can improve or optimize your procedures.*

1.3 Associated documentation

Introduction

This section describes the user documentation that is delivered with the product, and how to find related literature that can be downloaded or ordered from Cytiva.

User documentation

The main components of the documentation for the Biacore 8K/8K⁺ system are listed in the table below.

Translations of the *Biacore 8K/8K⁺ Operating Instructions* are provided in PDF format on the documentation CD inside the back cover of the printed *Operating Instructions*. Other documentation is available for download from [cytiva.com/Biacore8K-system](https://www.cytiva.com/Biacore8K-system).

Documentation	Main contents
Biacore 8K/8K ⁺ Operating Instructions	Instructions needed to install, operate and maintain the Biacore 8K/8K ⁺ system in a safe way.
Biacore 8K/8K ⁺ User Manual	Detailed system description and instructions for preparing and running experiments.
Biacore Insight Evaluation Software User Manual	Detailed instructions for using the Biacore Insight Evaluation Software to evaluate the results of experiments with the Biacore 8K/8K ⁺ instrument.
Software help	On-screen assistance for using the Biacore 8K/8K ⁺ and Biacore Insight software.
Biacore 8K/8K ⁺ Site Preparation Guide	Requirements for space, power and other supplies, and environmental conditions for installing and running the Biacore 8K/8K ⁺ system. Required for system installation.
eLicensing Guide for Biacore Systems	Instructions for handling electronic software licenses. Required for system installation.
Biacore Insight Database Installation and Management Guide	Instructions for installing and maintaining the database used to store data from the Biacore 8K/8K ⁺ system. Intended for the database administrator.

User documentation on the web

Links to laboratory guidelines, application notes, documentation and other online resources may be found on [cytiva.com/bcappsupport](https://www.cytiva.com/bcappsupport). You will need to register on the web site to access some of these links.

1.4 Glossary

Biacore terminology

Terms used in work with Biacore systems are explained in the following table.

Term	Meaning
Absolute response	The magnitude of the SPR signal measured from the detector baseline.
Active surface	The sensor surface in the flow cell used for analysis of the interaction. This is normally flow cell 2.
Adjustment for controls	Adjustment of the sample response for changes in the surface activity during the course of an experiment, by normalizing with reference to control sample responses measured at intervals.
Analysis cycle	A sequence of injections of liquid over the sensor surface, repeated as many times as required during the course of an experiment. In the Biacore 8K/8K* system, one cycle can include analysis of up to 8 interactions, one in each channel.
Analyte	<p>The analyte is the interaction partner in solution, that is injected over and interacts with the ligand on the sensor surface.</p> <p>Note:</p> <p><i>The analyte is not necessarily the object of the experimental investigation. For example, an antibody screening experiment may be set up where different antibodies are attached to the sensor surface as ligands, and challenged with antigen injected in solution as analyte. In this case, the object of the investigation is the ligand.</i></p>
Association phase	The phase of an analysis cycle where analyte is injected over the sensor surface and (potentially) binds to the ligand.
Baseline	The response level from which sample responses are measured. A baseline is automatically set before each injection in an analysis cycle: baselines may be set at other points in a sensogram if required.
Blank subtraction	Subtraction of the response from a blank sample (usually buffer) from that from a test sample, to eliminate components of the response that are common to both samples.

Term	Meaning
Capture	The term capture is used to refer to attachment of ligand to the sensor surface by high affinity binding to an immobilized capturing molecule. Attachment by capture is normally reversible.
Capturing molecule	A molecule that is permanently attached to the sensor surface with the purpose of capturing ligand by high affinity binding.
Channel	A single path for liquid flow over the sensor surface. The Biacore 8K/8K ⁺ system has 8 parallel channels, each consisting of two flow cells. For a given injection, the same flow settings apply to all 8 channels.
Detection spot	The area on the sensor surface where detection occurs. In the Biacore 8K/8K ⁺ system, there is one detection spot in each flow cell.
Dissociation phase	The phase of an analysis cycle immediately following the association phase, when buffer flows over the sensor surface and any bound analyte may dissociate spontaneously.
Enhancement molecule	A secondary analyte injected after the main analyte, intended to enhance the response and/or specificity of the first analyte binding.
Flow cell	The region of a channel where detection occurs. In the Biacore 8K/8K ⁺ system, each channel consists of two flow cells arranged in series. When both flow cells are addressed, liquid flows first over flow cell 1 (Fc1 , flow cell 1) and then over flow cell 2 (Fc2).
Flow channel	See <i>Channel</i> .
Immobilization	The term immobilization is used to refer to permanent attachment of ligand or capturing molecule to the sensor surface, normally by covalent coupling.
Ligand	<p>The ligand is the interaction partner attached to the surface. Attachment may be through covalent coupling (immobilization) or high affinity binding to an immobilized capturing molecule (capture).</p> <p>Note:</p> <p><i>Use of the term ligand in Biacore contexts does not imply that the molecule is a ligand for a cellular receptor.</i></p>

Term	Meaning
Parallel flow	A flow pattern where liquid flows through all channels in the same way and at the same time. In the Biacore 8K/8K ⁺ system, the eight channels are arranged in parallel.
Reference subtraction	Subtraction of the response from the reference surface from that from the active surface, to eliminate components of the response that are common to both surfaces.
Reference surface	The sensor surface in the flow cell used as a reference. This is normally Fc1.
Regeneration	The act of removing all non-covalently attached material from the sensor surface (usually by injection of a regeneration solution) in preparation for the next analysis cycle.
Relative response	The magnitude of the SPR signal relative to a chosen reference point (usually the baseline before sample injection).
Resonance unit (RU)	The unit of measurement for the SPR response. As a rough approximation, 1 RU is equivalent to a change in protein concentration of 1 pg/mm ² on the surface of Sensor Chip CM5. This equivalence varies with different analytes and different sensor chip types.
Report point	Median response over a short window (typically 5 s).
Running buffer	Buffer used for continuous flow during an experiment.
Sensor chip	A gold-covered glass slide to which one of the interactants (the ligand) is attached.
Sensor surface	The surface of the sensor chip on which the interaction being studied takes place.
Sensorgram	A plot of response against time during one analysis cycle. Normally, sensorgram refers to a single plot from one channel, either a single flow cell (reference or active) or the difference between flow cells (reference-subtracted).
Serial flow	A flow pattern where the same liquid flows through two or more flow cells, one after another. In the Biacore 8K/8K ⁺ system, the two flow cells in each channel can be addressed in series or separately.
SPR	Surface plasmon resonance (Surface plasmon resonance), the detection principle used in Biacore instruments.

1.5 Data storage and management

Introduction

All data from the Biacore 8K/8K⁺ system (method definitions, runs, and evaluations) is stored in a Microsoft SQL Server database. A local installation of SQL Server Express is provided with the system, but installation of the database on a network server is recommended. Instructions for installing and managing the database are given in the separate *Biacore Insight Database Installation and Management Guide*.

Data stored on a network is accessible to all users of the Biacore 8K/8K⁺ software according to their membership in database roles. Data stored locally may be accessible from other computers depending on local IT policies.

A Biacore 8K/8K⁺ system can be set up with multiple separate databases. You select the database to be used when you log in to the software.

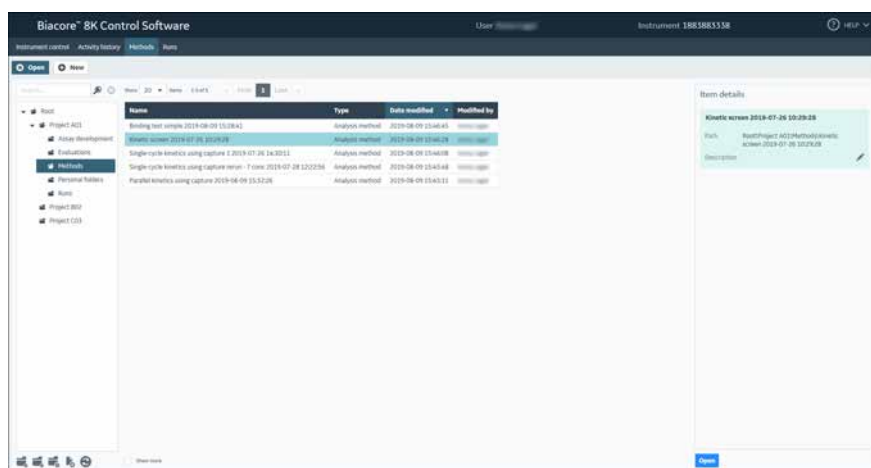
Note: *If multiple users work on the same database object at the same time, the user who first saves changes can save the object with the original name. Other users must save their changes as a new object, using **Save as**.*

Data organization

Data is organized in folders in the database. User data (methods, runs, evaluation methods, and evaluations, collectively referred to as *database objects*) are stored in user folders, under a top-level **Assay** folder. Storing user data directly in the **Assay** folder is not recommended.

The folder structure is displayed in the left-hand panel in workspaces where required, with the contents of the selected folder in the right-hand panel.

Tip: *To find a folder easy, select any row in the left-hand panel and type the first letter(s) of the folder. Type again to go to next.*



Select **Show more** in the contents panel to show additional details including action buttons for exporting and removing objects from the database.

Note: All folders and objects in the database are accessible to all users. See the *Biacore Insight Database Installation and Management Guide* for security management options.

Opening database objects



To open a database item in the Biacore 8K Control Software, double-click the item in the workspace or select the item and click **Open**. Methods and runs are opened in the **Methods** and **Runs** workspaces respectively. See [Section 7.1 Managing methods, on page 73](#) and [Section 8.1 Opening a run, on page 111](#) for more details.

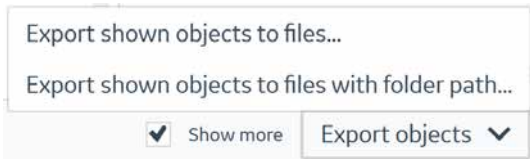

Runs imported from Biacore T200 and Biacore S200 systems cannot be opened in the Biacore 8K Control Software.

Evaluations cannot be opened in the Biacore 8K Control Software.

Managing database objects






Manage objects in the database according to the instructions in the following table.

Operation	Instruction
Move object	Drag the object from the list in the right-hand panel of the workspace to a different folder in the left-hand panel.
Rename object	<p>Click twice on the object name in the right-hand panel of the workspace to highlight the name and enter a new name.</p> <p>Note: <i>Click twice with a longer interval than double-clicking.</i></p>
Remove object	<p>1. Choose Show more in the right-hand panel of the workspace.</p> <p>2. Scroll to the right if necessary.</p> <p>3. Choose  Remove in the Action column.</p> <p>Note: <i>The Remove button may not be visible depending on the database security settings. See the <i>Biacore Insight Database Installation and Management Guide</i> for details.</i></p>
Export a single object	<p>1. Choose Show more in the right-hand panel of the workspace.</p> <p>2. Scroll to the right if necessary.</p> <p>3. Select  Export in the Action column.</p> <p>The file is exported in a proprietary format intended for import to another Biacore Insight database.</p>

Operation	Instruction
Export multiple objects	<ol style="list-style-type: none"> 1. Select a folder or perform a search to define the objects to be exported. All objects shown on the current page will be included in the export. 2. Choose Show more in the right-hand panel of the workspace. 3. Click Export objects, and select whether you want all objects in the same folder, or if you want the folder structure to be preserved.  <p>The files are exported in a proprietary format intended for import to another Biacore Insight database.</p> <p>Tip: <i>Use the search function to export objects of different types (runs, run methods, evaluations, evaluation methods) in a single operation.</i></p>
Import object(s)	<ol style="list-style-type: none"> 1. Navigate to the destination folder in the database. 2. Select  Import from the icons at the bottom of the panel. 3. Select file(s) to import. Supported file types are files exported from a Biacore Insight database and result files (.blr) from certain other Biacore systems (currently Biacore S200, Biacore T200, and some versions of Biacore T100). <p>Note: <i>Imported .blr files can only be opened in the Biacore Insight Evaluation Software.</i></p>


Managing folders

To manage folders, right-click on the folder in the left-hand panel or use the icons at the bottom of the panel.

Operation	Icon	Comments
Add folder		Enter the name for the folder.
Rename folder		Enter the new name for the folder.
Remove folder		You cannot remove a folder that contains subfolders or other objects.
Import		Use this function to import files to the Biacore Insight database.
Refresh		Refresh the display.

Searching for database objects

Follow the steps below to search for objects in the database.

Step	Action
1	Enter a search term in the Search field. Enclose phrases containing spaces in single or double quotation marks to search for the entire phrase (e.g., searching for new method will find new and method , but searching for "new method" will find only the complete phrase new method). Search terms are not case-sensitive.
2	Choose the appropriate search criteria, then click  Search . The available criteria may vary according to the type of object selected. You can restrict the search according to multiple criteria as described in the table below.

Category	Parameter	Description
Search for ¹	Runs	Finds runs with the search term in the name.
	Run methods	Finds immobilization and analysis methods with the search term in the name.

Category	Parameter	Description
	Evaluations	Finds evaluations with the search term in the name. Note: <i>Evaluations and evaluation methods cannot be opened in Biacore 8K Control Software.</i>
	Evaluation methods	Finds evaluation methods with the search term in the name.
Search within name and	Description	Finds objects where the object name or the description contains the search term.
	Ligand	Finds objects where the object name or the name of the immobilized molecule contains the search term. Note: <i>For runs that use captured ligand, searching for Ligand will find the capturing molecule.</i>
	Solution	Finds objects where the object name or the name of the Solution parameter in any command (including Capture commands) contains the search term.
Filter on	Date	Finds objects modified in the specified date range.
	Users	Finds objects modified by the specified user(s).
	Instrument id	Finds results and evaluations containing data from the selected instrument(s). Select the instrument id from the list that appears when this option is checked. The list includes all instruments that have been used for runs in the current data-base. Note: <i>Evaluations may contain data from more than one instrument.</i>

Category	Parameter	Description
	<i>Selected folder and subfolders only</i>	Searches only the selected folder and subfolders. If this option is not selected, the search is performed on the whole database.

¹ At least one category must be selected.

2 System description

About this chapter

This chapter describes the Biacore 8K/8K+ system.

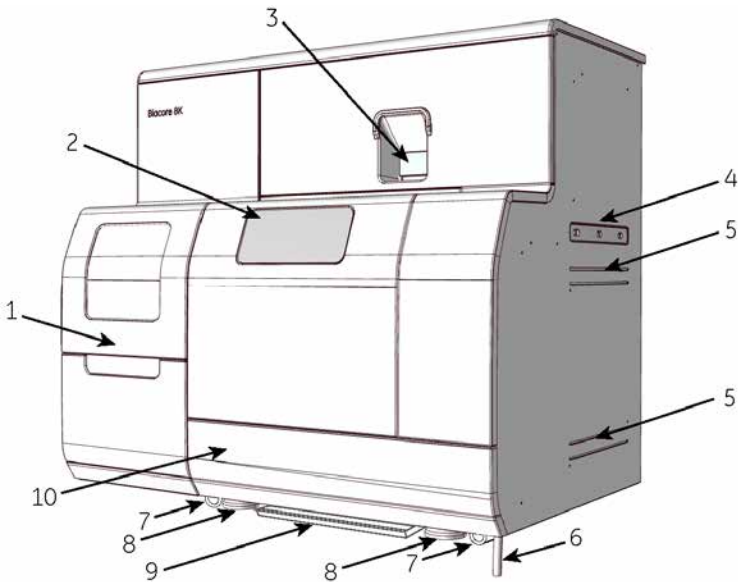
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2.1 Instrument components

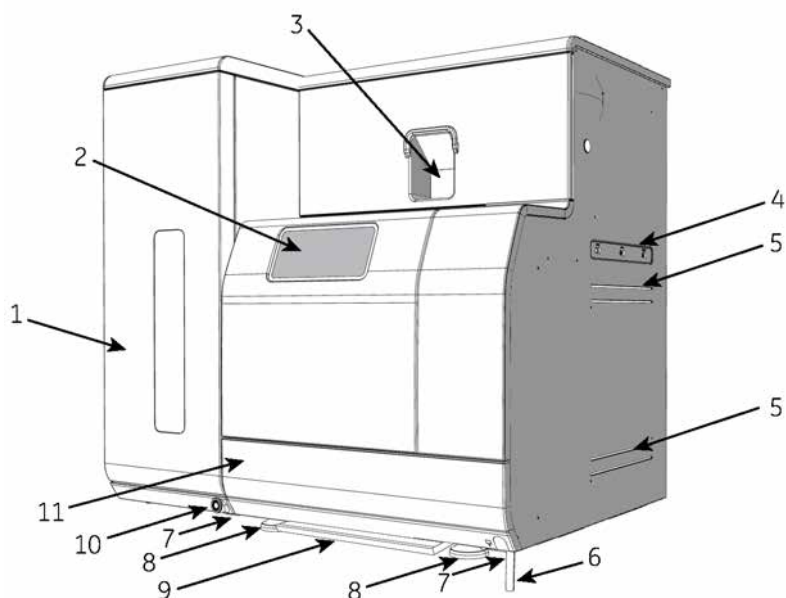
Overview

The main parts of the Biacore 8K instrument are identified in the illustration below.



Part	Function
1	Sample hotel door with window
2	Sample compartment with window
3	Sensor chip port
4	Tubing inlet panel
5	Rails for accessory holders
6	Waste tube
7	Fittings for lifting rods
8	Adjustable feet
9	Drip tray (under instrument)
10	Peristaltic pumps (behind hinged cover)

The main parts of the Biacore 8K* instrument are identified in the illustration below.

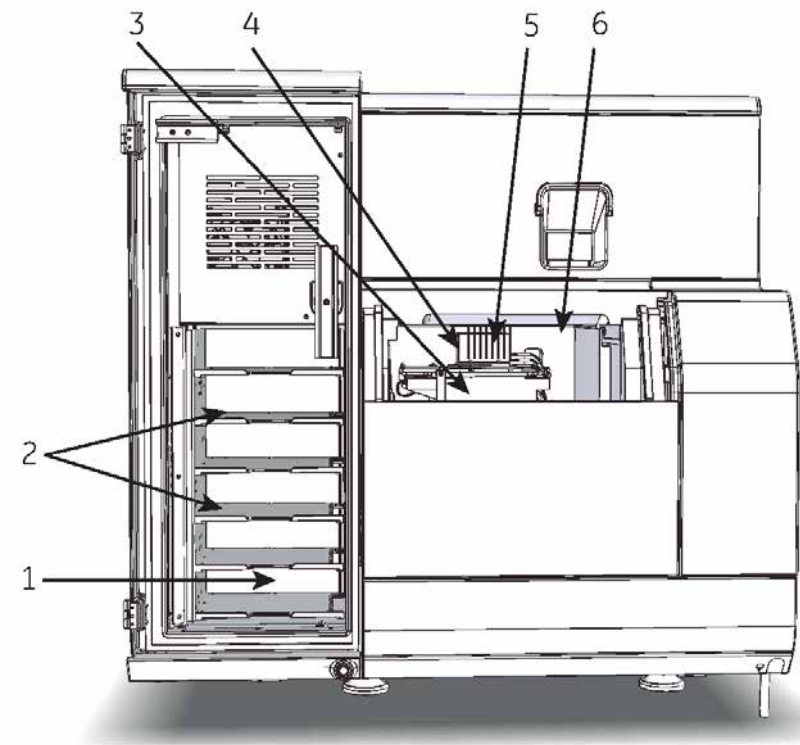


Part	Function
1	Sample hotel door with window
2	Sample compartment with window
3	Sensor chip port
4	Tubing inlet panel
5	Rails for accessory holders
6	Waste tube
7	Fittings for lifting rods (not visible in illustration)
8	Adjustable feet
9	Drip tray (under instrument)
10	Hotel door release button
11	Peristaltic pumps (behind hinged cover)

Sample hotel and sample compartment

The sample hotel is the area where trays carrying samples and reagents can be inserted and removed by the user. There are two tray positions in the Biacore 8K hotel, referred to as **upper** (upper) and **lower** (lower). The Biacore 8K+ hotel has 6 tray positions, numbered 1 to 6 from top to bottom.

The sample compartment holds one tray at a time. Trays are moved from the hotel to the sample compartment by an automatic sample loading mechanism as required. The sample compartment is the same in the Biacore 8K and Biacore 8K+ instruments. The illustration below shows a cutaway view of the Biacore 8K+ sample hotel and sample compartment.



Part	Function
1	Sample hotel
2	Sample tray positions (2 in Biacore 8K, 6 in Biacore 8K+)
3	Sample loading carriage
4	Liquid supply block

Part	Function
5	Eight parallel injection needles
6	Sample compartment

The sample hotel and sample compartment are maintained at the same nominal temperature, set in the Control Software (see [Section 2.3 Temperature control, on page 37](#)).

Sample hotel door

The sample hotel door may be opened at any time except when a sample tray is being transferred between the hotel and the sample compartment. Open and close the sample hotel door as described below.

Action	Biacore 8K instrument	Biacore 8K ⁺ instrument
Open sample hotel door	Release the sample hotel door by selecting the Open function in the Instrument control workspace in the Control Software, then lift the door upwards. Do not attempt to open the door without first releasing it in the software.	The sample hotel door is held in place by a magnetic lock. Release the lock by pressing the release button or selecting the Open function in the Instrument control workspace in the Control Software.
Close sample hotel door	Close the door, then push gently on the door until it clicks into place.	Close the door gently until the magnetic lock engages.



NOTICE

Do not leave the sample hotel door open unnecessarily, since this may affect temperature regulation of the sample compartment and sample hotel.

Hotel door release button (Biacore 8K⁺ only)

Illumination on the sample hotel door release button indicates the status as follows:

Illumination	Status
Steady	The button is active. Pressing the button will open the hotel door.

Illumination	Status
Off	The button is inactive (because of tray transfer between the hotel and the sample compartment). Pressing the button has no effect.
Flashing	The hotel door is open.

Sample illumination

Illumination in the sample hotel and sample compartment is provided by blue LEDs. Illumination may be switched on or off from the Control Software. Switch the illumination off if your samples are light-sensitive.

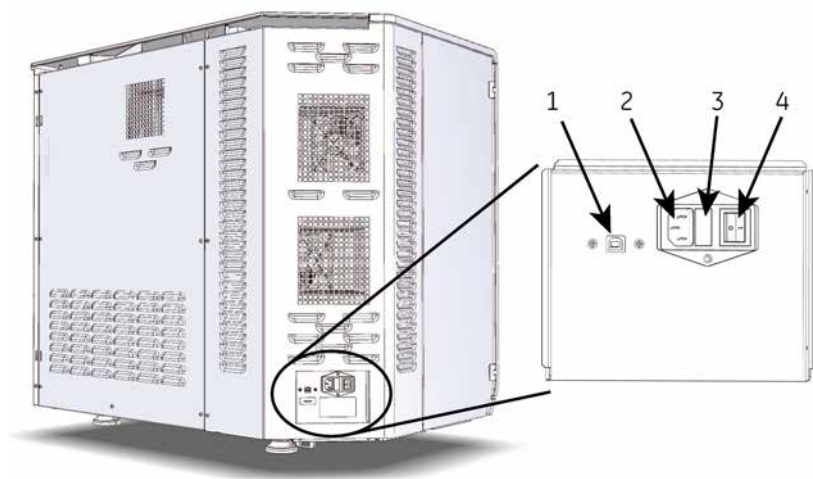
Sensor chip port

The sensor chip port is controlled from the software and cannot be opened by hand. See [Insert a sensor chip, on page 42](#) for further details.

Electrical connections

The electrical connection panel is located at the lower rear of the instrument on the left-hand side.

The illustration below shows the Biacore 8K* instrument. Electrical connections are in the equivalent position on the Biacore 8K instrument.



Part	Function
1	USB connector (for connection to controlling computer)
2	Mains power connector
3	Mains fuses
4	Mains power switch

2.2 Flow system

Liquid supply

Running buffer, distilled water and one optional large-volume reagent are supplied from bottles placed on the bench at the right of the instrument. Up to four different buffers can be used. Smaller bottles and tubes (up to 1000 mL) may be placed in accessory holders attached to the holder rails.

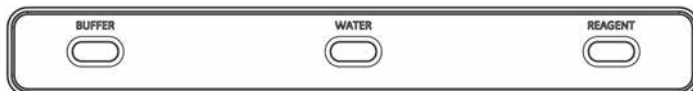
Liquid supply bottles

Glass bottles with caps (capacity 2 L and 5 L) for buffer, water and reagent are provided with the instrument. Any laboratory bottles with screw caps may be used. Bottle caps must be perforated for inlet tubing, and should be vented to prevent accumulation of over- or underpressure as the volume of liquid changes. Suitable caps are provided with the instrument.

Liquid filtering requirement

All buffers and large-volume reagents should be filtered through a 0.22 µm filter to avoid introducing unwanted particles into the flow system. Particles can both lead to disturbances in the SPR response and cause blockage or other malfunction of the microfluidic system.

Tubing inlet panel



Liquids are pumped in to the flow system through inlet tubes on the right-hand side of the instrument. The tube ports are labeled **BUFFER**, **WATER**, and **REAGENT**, used for running buffer, distilled water, and large-volume reagent respectively. The system has 4 buffer inlet tubes, labeled **BUFFER A** to **BUFFER D**, providing support for up to 4 different running buffers. Buffer inlets are selectable from the Control Software.

The **BUFFER A**, **WATER**, and **REAGENT** tubes should be supplied with liquid at all times during instrument operation, including standby. If a large-volume reagent is not used, insert the **REAGENT** tube into the distilled water bottle.

Buffer selector

The flow system includes a buffer selector that allows running buffer to be switched automatically between steps in the activity queue (see [Section 5.1 Activity queue, on page 61](#)). Up to four buffers are supported. Buffer cannot be switched within a step in the activity queue.

The buffer selector can be configured for 1 to 4 inlets from the Control Software (see [System setup tools, on page 67](#)).

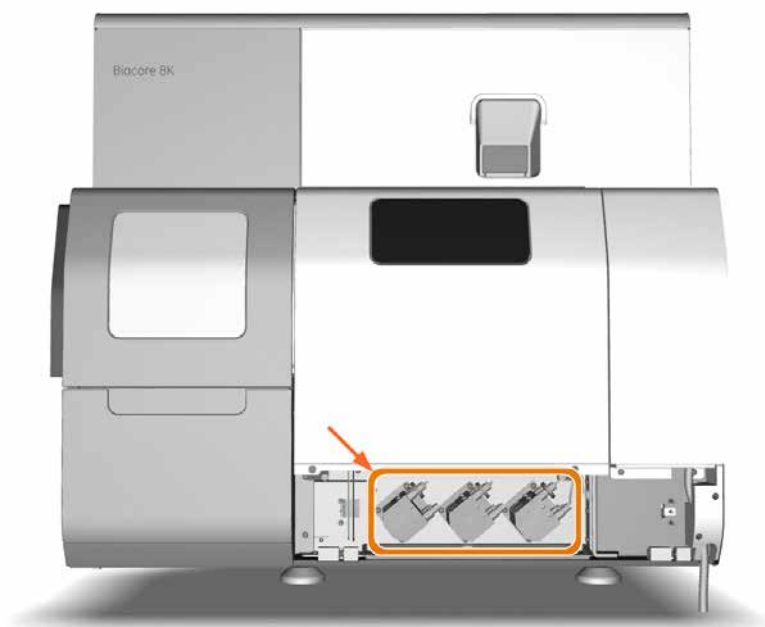
Continuous flow pumps

Continuous flow of liquid (running buffer or sample) over the sensor chip surface is managed by 16 high precision syringe pumps, housed inside the instrument. The syringe pumps are not accessible to the user.

Peristaltic pumps



Three peristaltic pumps provide a supply of running buffer and water for washing the needles during a run and provide regeneration or wash solution as needed.

The peristaltic pumps are placed at the lower front of the instrument, behind a hinged panel (shown with the panel removed in the illustration below). Open the panel by pulling outwards on the top edge, to access the pumps and release the pump clamps when the instrument is shut down. The illustration below shows the Biacore 8K instrument: peristaltic pumps in the Biacore 8K* instrument are the same.



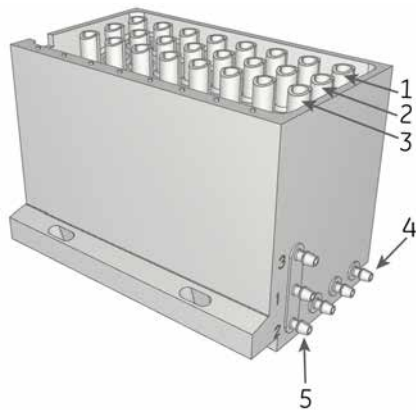
Each pump holds two pump tubes, which should be separately unclamped if the instrument is shut down and will remain unused for more than 2 weeks, and reclamped before the instrument is restarted. Follow the instructions below to open and close the pump clamps.

Note: Always open or close the clamps in both the outer (gray) and inner (blue) pumps.

Operation	Instruction
Open the pump clamps	<p>Squeeze the ridged area of the clamp on both sides of the pump and pull the clamp away from the pump body.</p>  <p>Do this for both clamps on each pump.</p>
Close the pump clamps	<p>Push the clamp towards the pump body until it clicks into place. Make sure the clamp stays aligned with the pump body on both sides as the clamp is closed.</p>  <p>Do this for both clamps on each pump,</p>

Liquid supply block

Buffer, water and one customer-selectable reagent are supplied to the injection needles through the **liquid supply block** in the sample compartment. Buffer and water are used for automatic needle wash, and the reagent may be regeneration or wash solution.



Part	Function
1	Water supply
2	Buffer supply
3	Reagent supply
4	Inlet ports
5	Outlet ports

Integrated microfluidic cartridge
(Integrated microfluidic cartridge,
IFC)

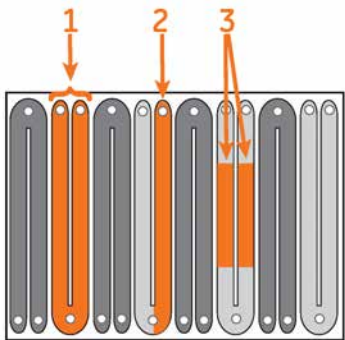
The IFC consists of a series of micro-channels and membrane valves encased in a plastic housing, and serves to control delivery of liquid from the liquid supply block to the sensor chip surface. Grooves on the IFC surface that comes into contact with the sensor chip form the flow cells when the sensor chip is docked in the instrument.

Channels and flow cells

The flow system has 8 separate channels over the sensor surface, each with two flow cells (**Fc1** and **Fc2**). Normally, ligand is attached to the surface in Fc2 and Fc1 is used as a reference.

The two flow cells in each channel can be addressed separately or in series. When the flow cells are addressed serially, liquid flows first over Fc1 and then over Fc2. Buffer and sample flow is controlled in parallel in all 8 channels, meaning that for any given cycle, the flow rate, sample contact times, and flow cell addressing are the same in all channels.

A schematic representation of the arrangement of channels, flow cells and detection spots on the sensor surface is shown in the illustration below.



Part	Function
1	Channel (one of 8 indicated). Channels are shown in different gray shades for clarity.
2	Flow cell (one of 16 indicated).
3	Detection spots (two of 16 indicated).

Waste drainage

The waste tube exits from the instrument at the bottom right corner. Waste drains from the flow system through the waste tube to a waste bottle placed under the instrument. A 15 L plastic waste bottle with cap is provided.

An additional drainage tube collects condensation and any spillage or leakage from the sample compartment, and drains to a drip tray underneath the instrument. Condensed water that drains from the sample compartment will normally evaporate, although condensation volumes may be significant if the instrument is used in a humid atmosphere, particularly with low temperatures in the sample compartment.

In the event of leakage from the flow system in the sample compartment, the drip tray should be cleaned and if necessary disinfected in accordance with applicable chemical handling regulations.

Note: *The volume of liquid draining to the drip tray should normally be small. Volumes may increase during operation at low sample compartment temperatures in a humid atmosphere. If significant volumes are observed under other circumstances, check the sample compartment for leaks or blockage in the waste drainage from the liquid supply block.*

2.3 Temperature control

Flow cell temperature

The temperature at the sensor chip is referred to as the **flow cell temperature**. SPR response is highly sensitive to temperature, and precise control of the flow cell temperature is essential for reliable performance.

The flow cell temperature is controlled within the range 4°C to 40°C (maximum 20°C below ambient). The temperature is set in the Control Software, and runs will not start (unless explicitly allowed to do so) if the actual temperature is not equilibrated to the set value.

Note: *The Control Software does not display the flow cell temperature at full precision. Consequently, the display may show the same value for the actual and set temperature before the temperature is reported as stable.*

Sample compartment temperature

The temperature in the sample compartment may be set within the range 4°C to 40°C. The compartment can be cooled to a maximum of 18°C below ambient. The sample compartment temperature is controlled with lower precision than the flow cell temperature, and does not affect the ability to start a run.

If the sample compartment temperature differs from the flow cell temperature, injected solutions equilibrate to the flow cell temperature during transfer from the microplate to the flow cells. However, for maximum performance (particularly at high flow rates), the sample compartment temperature should be set to the same value as the flow cell temperature.

Sample hotel temperature

The temperature in the sample hotel is not actively controlled in the Biacore 8K system. The sample hotel will maintain approximately the same temperature as the sample compartment provided that the sample hotel door is not opened more than necessary.

In the Biacore 8K+ system, the sample hotel is actively maintained at the same temperature as the sample compartment. For optimal temperature regulation, keep the sample hotel door closed except when handling sample trays in the hotel.

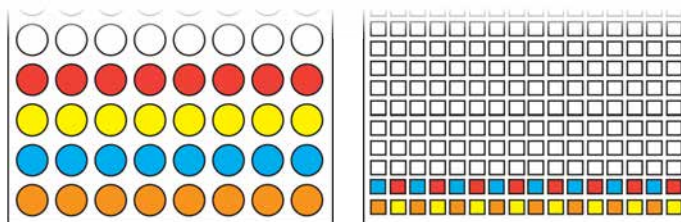
2.4 Sample handling

Microplates

Samples and low volume reagents are placed in 96- or 384-well standard or deep-well microplates. Microplate specifications and recommendations may be found on the **Related Documents** tab at [cytiva.com/Biacore8K-system](https://www.cytiva.com/Biacore8K-system).

Note: Do not use polystyrene microplates with samples that contain DMSO.

The 8 sample needles are spaced at a fixed distance from each other so that samples for one injection are taken from one complete row on a 96-well microplate and from every other position in a row on a 384-well plate. This is illustrated for 4 injections below, where different colors represent different injections.



The unattended processing capacity is 4 microplates in the Biacore 8K system and 12 microplates in the Biacore 8K+ system.

Microplate covers

Microplates should be covered directly after preparation to prevent evaporation. The cover is penetrated by the injection needles when solution is taken from the wells. Adhesive foil and septa for covering microplates are available from Cytiva.

- Use foil for microplates where solution is taken only once from each well.
- Use septa for microplates where solution is taken more than once from any well.



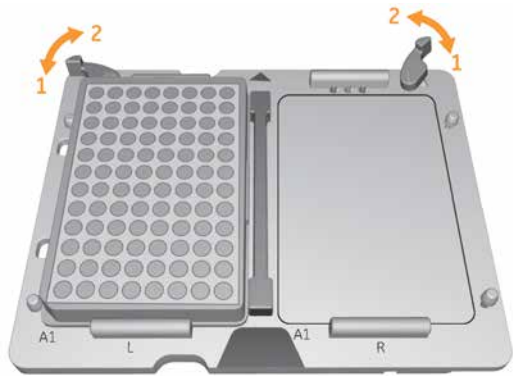
NOTICE

Place the foil or septa carefully on the microplates so that the well openings are free of adhesive. Adhesive that sticks to the injection needles will seriously impair system performance.

Sample trays

Microplates are mounted on a sample tray before they are loaded into the sample hotel. Each sample tray can hold two microplates. The microplate positions are identified as **L** (left) and **R** (right).

Place the microplate on the tray as shown, with well position A1 at the front left as marked on the tray. Close the locking lever (position 1) to lock the microplate in position. Open the lever (position 2) to release the microplate. The illustration below shows a tray with one microplate locked in position: the empty position is unlocked.

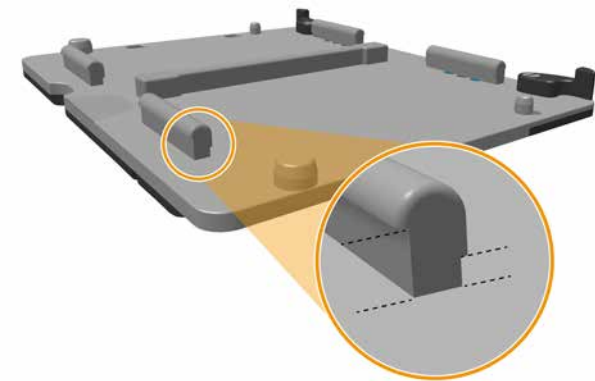


Position	State
1	Locked
2	Unlocked


Note: Corner shapes on microplates are not standardized, and the angled corner is not always at position **A1** as shown in this illustration. Orient the microplate by well position and not by corner shape.

Adjusting to microplate

To accommodate different brands of microplates, the stoppers on the sample tray have two edges with different heights to be able to securely fasten both low and high microplate flanges. See illustration below.



Follow the steps below to switch between low edge and high edge.

Step	Action
1	On the back of the sample tray, use a suitable screwdriver to loosen the screws that hold the four stoppers.
	
2	Turn the stoppers 180 degrees.
	
3	Tighten the screws to fasten the stoppers.

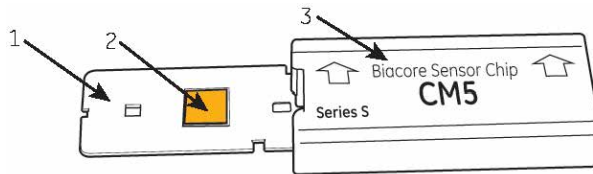
Sample injection

Samples are aspirated from the sample microplates and injected over the surface by the 8 parallel needles in the sample compartment. Switching between sample and running buffer during analysis is controlled by micro-valves in the IFC (see [Integrated microfluidic cartridge \(Integrated microfluidic cartridge, IFC\), on page 29](#)).

2.5 Signal detection and processing

Sensor chip

The sensor chip is a gold-coated glass slide mounted on a supporting frame, enclosed in a protective cassette. Do not remove the sensor chip from the cassette. The illustration below shows the sensor chip separated from the cassette for illustration purposes.



Part	Function
1	Frame
2	Gold-coated glass slide
3	Cassette

Surface plasmon resonance (Surface plasmon resonance, SPR)

Interactions are monitored through **surface plasmon resonance (SPR)** in the gold film on the sensor chip surface. SPR occurs under conditions of total internal reflection of incident light on the glass side of the gold film, and leads to a reduction in the intensity of reflected light at a specific combination of wavelength and angle of reflection (the **SPR angle**). The wavelength is fixed in Biacore systems. The SPR angle is sensitive to the local refractive index of solution very close (within about 150 nm) to the sensor surface on the opposite side of the gold film, so that changes in the SPR angle can be used to monitor the changes in concentration at the sensor surface as interaction proceeds. The light used to generate the signal does not pass through the sample.

SPR response data

The SPR response is monitored continuously in real time by a 2-dimensional detector array that measures the SPR angle for each detection spot. The response is expressed in **resonance units (resonance units, RU)**. As a rough approximation for proteins on Sensor Chip CM5, 1 RU corresponds to a change in surface concentration of 1 pg/mm². This correlation may differ for different molecules and on different sensor chip surfaces.

2 System description

2.5 Signal detection and processing

The raw SPR response is processed and buffered by a microprocessor in the instrument itself, before being transferred to the external computer for display and storage. This configuration means that real-time monitoring continues even when the processing capacity of the external computer is temporarily interrupted. The time resolution of the measurement may be set to 1 or 10 Hz.

3 Basic operation and strategies

About this chapter

This chapter describes the basic operation of the Biacore 8K/8K+ instrument together with essential considerations for the design and execution of experiments using the system.

In this chapter

Section	See page
3.1 Starting the system	38
3.2 Preparing for a run	41
3.3 Preparing and loading samples	44
3.4 Performing the run	46
3.5 Basic strategies	49

3.1 Starting the system

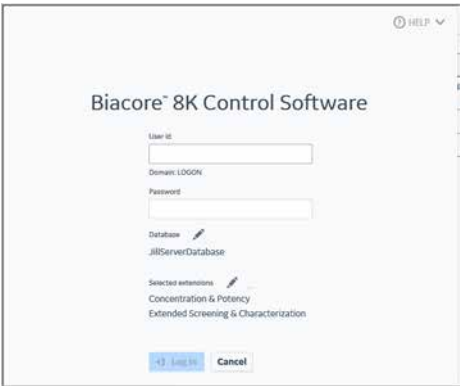
If the system is switched off

Follow the steps below to start the system and the Control Software:

Step	Action
1	Open the lower front panel and close both tube clamps on each of the three peristaltic pumps by pressing the clamps towards the pump body until they click into place (see Peristaltic pumps, on page 27). Make sure the clamps stay flush with the pump body on both sides as the clamp is closed.
2	Switch on the power to the instrument (see Electrical connections, on page 25).
3	Start the computer.
4	Start the Biacore 8K Control Software.

Result:

The login dialog is displayed.





Step	Action
------	--------

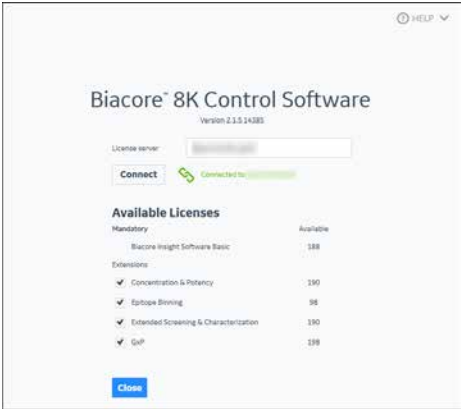
5	Make sure that the correct license server, database, and software extensions are selected.
---	--

Tip:

*If the software is running on another computer, open **Help > About** to compare license server, database, and software extensions.*

A warning symbol  next to the **Selected extensions** list indicates that the connection to the license server has not been specified or has been lost.


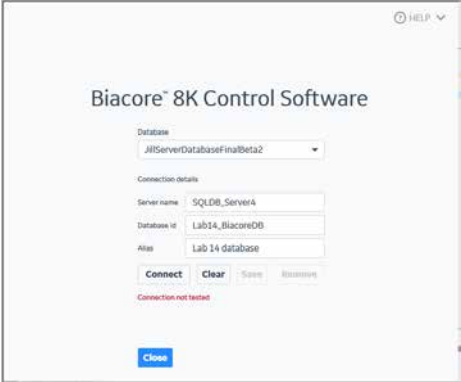
Click the pen icon  to specify the server details and to select extensions.



Enter the license server name and click **Connect**. Contact your system administrator if you need assistance.

Select the software extensions you wish to use. The number of available licenses for each extension is shown in the dialog.

Click **Close** when you have entered the details.

Step	Action
6	<p>Click the pen icon  next to the Database to change the selected database. Contact your database administrator if you need assistance.</p> 
	<p>Note:</p> <p><i>If you create a connection to a database that has not previously been accessed from this computer, click Connect then Save to establish and save the connection before closing this dialog.</i></p>
7	<p>Enter your account credentials. Your Windows user name and password are valid as credentials for Biacore 8K and Biacore Insight software.</p> <p>Note:</p> <p><i>Account credentials do not have to be the same for login to Windows and the Biacore 8K and Biacore Insight software, provided that valid credentials are used in both cases.</i></p> <p>Note:</p> <p><i>Biacore 8K/8K+ and Biacore Insight software do not support Windows Fast User Switching.</i></p>
8	<p>Click Login.</p>
9	<p>Wait until the instrument self-test is completed and connection to the computer is established as indicated by the instrument number at the top of the screen.</p>

If the system is in standby mode

If the system is in standby mode (the instrument status panel at the bottom of the screen shows **Running standby flow**), no further action is needed. Standby mode will be stopped automatically when a new instrument activity is started.

3.2 Preparing for a run

Set the temperature

Set the flow cell temperature and sample compartment temperature well in advance of starting the run, to allow time for the system to equilibrate. Equilibration time for a temperature change of 5°C is about 40 minutes.

Methods will not start if the flow cell temperature has not reached the set value. (Methods will however start if the sample compartment temperature has not reached the set value.)

Follow the steps below to set the temperature. Repeat the steps for each of the flow cell temperature and sample compartment temperature if you need to set both.

Step	Action
1	From the Instrument Control workspace, add Set flow cell temperature or Set sample compartment temperature to the activity queue.
2	Enter the required temperature in the activity workspace.
3	Select Set temperature . Temperature equilibration will start immediately if there are no prior activities in the queue.
	Note: <i>The Set ... temperature activities are executed and removed from the queue quickly. However, temperature equilibration normally takes a longer time.</i>

Set up the liquid supply

Follow the instructions below to provide running buffer, distilled water, optional large-volume reagent and waste bottle for the flow system. Required volumes of running buffer and reagent for a run are shown in the **Instrument control** workspace. Volumes shown are minimum requirements, calculated from estimated consumption plus a dead volume in the bottle.

Note: *Volumes shown do not include requirements for queued activities that will start automatically or for standby flow following the run. Add the appropriate volumes of buffer, reagent and water to those shown in the workspace (see [Standby mode, on page 47](#) for volumes required during standby).*

Step	Action
1	Fill a bottle with distilled water and place it on the right-hand side of the instrument. Insert the tube marked WATER .

3 Basic operation and strategies

3.2 Preparing for a run

Step	Action
2	<p>For instruments without buffer selector, fill a bottle with running buffer and place it on the right-hand side of the instrument. Insert the tube marked BUFFER.</p> <p>For instruments with buffer selector, fill up to 4 bottles with running buffer(s) as required and place them on the right-hand side of the instrument. Insert the buffer inlet tubes according to the buffer requirements for the run. Configuration of the buffer selector is described in System setup tools, on page 67.</p> <p>Note: <i>The BUFFER A tube should always be placed in buffer, even when another buffer is used as running buffer. Other unused buffer tubes should be capped or placed in water or buffer.</i></p> <p>Configuration of the buffer selector is described in the <i>Biacore 8K/8K+ User Manual</i>.</p>
3	<p>If required, prepare a bottle with large-volume reagent and place it on the right-hand side of the instrument. Insert the tube marked REAGENT.</p> <p>If a large-volume reagent is not used, insert the REAGENT tube into the water bottle.</p>
4	<p>Make sure that all liquid supply tubes are securely placed at the bottom of the liquid.</p>
5	<p>Place the waste container on the trolley shelf under the instrument. Insert the waste tube.</p>

Insert a sensor chip

If the instrument is in standby mode, a sensor chip will be docked in the instrument. Normally, you will need to replace this sensor chip with one appropriate for your run. Follow the steps below to change the sensor chip. Follow the same steps but omit step 2 if the instrument has been restarted from shutdown.

Step	Action
1	Add Change chip to the activity queue.
2	If a sensor chip is already docked in the instrument, click Undock chip and remove the chip from the sensor chip port.
3	Select New chip and enter the details of the sensor chip for the run. Alternatively, select Existing chip and choose a chip from the list.
4	Insert the sensor chip and close the chip door.

Step	Action
5	Click Dock chip .
	<p>Note:</p> <p>If you click Dock chip before closing the chip door, a notification will be issued. Close the door and select Retry in the notification.</p>
Note:	For runs requiring highest performance, allow the flow system to equilibrate in standby mode at least overnight after changing the sensor chip or changing solutions. Extend the equilibration time to at least 24 h if the detergent concentration in the running buffer is changed.

Select or create a method


Follow the instructions below to set up the method for the run. See [Chapter 7 Methods workspace, on page 72](#) for more details.

Step	Action
1	Go to the Methods workspace in the Control Software.
2	Open a predefined or existing method from the database. Make any modifications that may be required to the method definition.
3	Go to the Variables and positioning tab. Enter or modify the variable details as required including sample information, microplate types and position assignments.
4	(Optional) Go to the Cycle overview tab and check that the run is set up correctly. If you need to make any adjustments, return to the Method Definition tab and adjust the necessary settings.
5	(Optional) Print the Plate layout information as an aid in preparing microplates.

3.3 Preparing and loading samples

Prepare samples and reagents

Follow the steps below to prepare samples and reagents in a microplate.

Step	Action
1	<p>Dispense the samples and reagents into the microplate wells according to the Plate layout information in the method. Make sure that there are no air bubbles trapped at the bottom of the microplate wells. It is particularly easy to trap air bubbles in 384-well microplates. Use of a microplate centrifuge to remove air bubbles is recommended.</p> <p>Note:</p> <p><i>Buffer will be injected over any channels that are not used. You will need to add buffer to the appropriate wells on the microplate(s).</i></p> <div><p>NOTICE</p><p>Do not exceed the volume specified in the plate type.</p></div>
2	<p>Cover the microplates as recommended (see Microplate covers, on page 32) to prevent evaporation from the samples during analysis.</p> <p>Note:</p> <p><i>Microplate foils and septa may cover the well position identifiers on the microplates. You may want to mark the A1 corner of the microplate after attaching the foil or septum. Take care not to mark the foil or septum directly over a well position.</i></p>

Mount the microplate on a sample tray

Follow the steps below to mount the microplate on the sample tray.

Step	Action
1	<p>Verify that the sample tray suits the microplate flange height. If needed, adjust the sample tray (see Adjusting to microplate, on page 33).</p>
2	<p>Open the locking lever on the sample tray and place the microplate on the tray with position A1 at the front left, as marked on the tray (see Sample trays, on page 32).</p>

Step	Action
3	Close the locking lever and make sure that the microplate is properly seated on the tray (see Sample trays, on page 32).

3.4 Performing the run

Start the run

Follow the instructions below to start the run in the software.

Step	Action
1	Click Send to queue in the method workspace. <i>Result:</i> The method is added to the activity queue.
2	Select the running buffer for the method. Note: <i>All buffer tubes that are active in the buffer selector configuration (see Buffer selector, on page 26) should be placed in buffer or water, even those that are not used as running buffer.</i>
3	Assign the tray(s) to sample hotel positions.
4	If the sample hotel door is closed, select Open from the instrument status pane in the Control Software.
5	Open the hotel door fully (see Sample hotel door, on page 23).
6	Place the sample tray(s) on the correct shelf in the sample hotel as assigned in the software.
7	Close the hotel door. Make sure that the status is shown as Closed in the software.
8	Click Ready to start ¹ .
9	Navigate to the required folder and provide a name for the run. Click Save . <i>Result:</i> The run will start as soon as all previously queued activities are complete. Actual run start may be delayed if the flow cell temperature is not stable (see Flow cell temperature, on page 31).

You can load the sample tray(s) into the sample hotel at any time except when a tray is being moved between the hotel and the sample compartment. If a sample tray is not present in the hotel when required by the method, a notification will be issued and the run will not continue until the tray is provided.

**NOTICE**

The system checks that a sample tray is present in the hotel, but does not detect the presence or type of the microplate(s) on the tray. It is the user's responsibility to ensure that microplates corresponding to the software settings are mounted on the tray. Using incorrect microplates may damage the injection needles.

Monitor the run

Sensorgrams generated as the run progresses are displayed in the **Instrument control** workspace (see [Section 5.4 Display during a run, on page 70](#)).

At the end of the run, the next activity in the queue will start automatically unless user input is required. Otherwise, the system will automatically be placed in standby mode.

Clean-up after the run

The following activities should be performed as required after a run:

- Remove any microplates from the sample hotel.
- Empty the reagent bottle and replace with water or buffer.
- Make sure there is sufficient liquid for the intended standby period.
- Empty the waste bottle.
- Clean the drip tray if necessary.

Standby mode

Always leave the system in standby mode unless the instrument is to be shut down. Standby mode will use the current buffer tube.

Tip: *The current buffer tube is highlighted in blue in the status bar in the Control Software.*

Standby mode maintains a continuous low flow of liquid through the flow system. Recommended liquids for standby operation over longer periods are buffer for the **BUFFER** supply tubing and water for **WATER** and **REAGENT** tubing. Liquid from the **BUFFER** inlet, but not from **WATER** or **REAGENT**, passes over the sensor surface during standby.

A sensor chip is required in the instrument during standby operation.

The maximum unattended standby period is 7 days. Select **Restart** in the instrument status pane to extend the standby period by resetting the timer to 7 days. Make sure there is sufficient liquid supplied to each inlet for the intended standby period. Approximate liquid consumption for each inlet tubing is listed below:

Tubing	Consumption (mL/24 h)	Passes over sensor surface
BUFFER	250	Yes
WATER ¹	150	No

3 Basic operation and strategies

3.4 Performing the run

Tubing	Consumption (mL/24 h)	Passes over sensor surface
REAGENT ¹	25	No

¹ Place both the **REAGENT** and **WATER** tubing in the water bottle.

3.5 Basic strategies

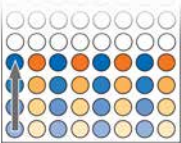
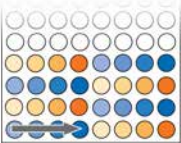
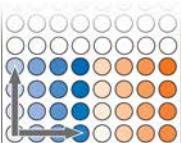
Introduction

The 8-channel capacity in the Biacore 8K/8K+ system offers considerable flexibility for experimental design. The following general consideration may affect the design of experiments and should be borne in mind:

- Buffer flow and injections are controlled in all 8 channels in parallel. It is not possible, for example, to perform an injection in some channels but not others, or to use different flow rates in different channels.
- The same running buffer is used throughout a method. A different running buffer may be selected at the start of a new method.
- The same running buffer is used in all channels, although buffer conditions for sample injections can be varied by using the **A-B-A** command (see [A-B-A command, on page 93](#)).
- Solutions for each injection are taken from all 8 positions in a row in a 96-well plate, or every second position in a row in a 384-well plate. All positions used must contain solution. If some positions are left empty, air will be injected over the surface in the corresponding channel.
- Each channel has two flow cells, normally used as active (Fc2) and reference (Fc1) surfaces. Injections may be addressed to one or both flow cells. Flow cell addressing applies to all channels. If only one flow cell is addressed, buffer will flow through the other flow cell.
- Subtraction of reference from active sensorgrams (reference subtraction within the same cycle) is performed using data from two flow cells in the same channel. Reference subtraction between channels is not supported.
- Subtraction of sensorgrams from different cycles (usually blank subtraction, performed in the Biacore Insight Evaluation Software) may only be performed within channels.

Serial and parallel modes

Applications that involve groups of the same interactants (usually with varying analyte concentration) can be set up in *serial* or *parallel* mode. A combined mode (*2D mode*) is supported for kinetic analysis.

Mode	Description
Serial mode	<p>The concentration series is distributed within a column in the microplate. One analysis cycle injects a single concentration for up to 8 analytes. The number of concentrations in the series is limited only by the capacity of the microplate.</p> 
Parallel mode	<p>The concentration series is distributed within a row in the microplate. One analysis cycle injects a series of up to 8 concentrations for one analyte or 4 concentrations for 2 analytes.</p> 
2D mode	<p>This mode combines serial and parallel features, with concentration series in both rows and columns in the microplate. This allows efficient coverage of a wider range of analyte concentrations. All concentrations of the same analyte are treated as a single series.</p> 

Ligand immobilization

Ligand may be immobilized on the flow cell surfaces in a variety of arrangements, and combined with the use of 8 channels for each injection according to the demands of the experiment. For example, the run may be set up with the same ligand but different analytes in all channels to increase sample throughput, or with different ligands but the same analytes in different channels to enable comparative studies on different ligands in one run. Intermediate arrangements are of course also possible.

When the same ligand is immobilized in different channels in a single cycle, the resulting levels of immobilized ligand may vary slightly even though the procedure is identical (performed in parallel) between channels.

- Minor variations are generally not significant for evaluation of kinetic determinations. Affinity measurements in parallel mode are not recommended.
- Parallel concentration measurements are supported by **Channel normalization** as part of the assay setup (see [Step purposes, on page 87](#)).
- For screening experiments that are evaluated in terms of response levels, variations in ligand level are often compensated by adjustment of sample responses when the data is evaluated in the Biacore Insight Evaluation Software. Beware however of basing conclusions on comparison of non-adjusted sample responses in different channels, particularly if immobilized ligand levels are low.

Achieving low levels of immobilized ligand

Experience with amine coupling of some proteins on Sensor Chip CM5 has shown that consistent low immobilization levels between channels are best achieved by maintaining the ligand concentration and ligand contact time in combination with one or both of the following approaches:

- Reduce the activation time of the surface with EDC/NHS. Activation times as short as 30 seconds have proved useful.
- Reduce the proportion of EDC mixed with NHS. Using 20% EDC and 80% NHS can reduce the immobilization level by about 50%.

A predefined surface preparation method for amine coupling that implements both of these approaches is provided with the software. Adjust the activation time and/or proportion of EDC and NHS to suit your requirements.

More details may be found in publications on the website (see [User documentation on the web, on page 9](#)).

Assay development

Assay development work frequently involves comparison of ligand and analyte behavior under different conditions. The Biacore 8K/8K⁺ system may be used to increase efficiency of assay development either by reducing the number of sensor chips required or by completing assay development in a shorter time.

Assay development principles are described in the *Biacore Application Guides* (available from Cytiva). Some examples of steps supported by predefined analysis and/or evaluation methods in the Biacore 8K/8K⁺ system are listed below:

- pH scouting for immobilization conditions
- Binding test using a single-cycle kinetics format
- Interaction characteristics
- Buffer scouting using the **A-B-A** injection
- Regeneration scouting

3 Basic operation and strategies

3.5 Basic strategies

Detailed design of assay development work is outside the scope of this handbook. Supporting material for experimental design may be found on the web (see [User documentation on the web, on page 9](#)).

Screening applications

To exploit the capacity of the Biacore 8K/8K* system fully in screening applications, ligand should be immobilized or captured in one flow cell (Fc2) in all channels. An arrangement with the same ligand immobilized in the same way provides maximum sample throughput, with 8 different samples analyzed in each cycle. Alternatively, different ligands or different immobilization chemistries can be used in the different channels, providing comparative binding information for a reduced set of samples. Results from different channels and cycles can be grouped in the Evaluation Software to reflect the intention of the experiment.

Immobilized ligand is inevitably constant between cycles. However, if ligand is captured, the ligand can be varied between cycles. With the **A-B-A** injection (see [A-B-A command, on page 93](#)), it is possible to vary the solution conditions between cycles and channels, for example, for analysing interactions in the presence of a competitor or cofactor.

Concentration analysis

The **Concentration & Potency** extension adds support for determination of analyte concentrations using a calibration curve obtained by analysis of known standard samples. Predefined analysis methods are provided for a range of different experimental setups, including parallel and serial microplate layouts, with and without enhancement injections. Concentration measurements are evaluated using dedicated functionality in the Evaluation Software, described in the *Biacore Insight Evaluation Software Manual*.

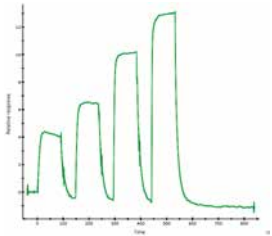
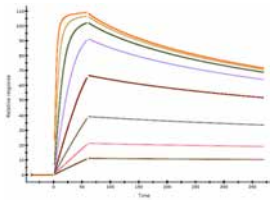
Parallel line analysis and EC₅₀ determination

Potency of a pharmaceutical candidate or product can be expressed in terms of EC₅₀ (half maximal effective concentration) or in comparison to a reference compound by PLA (parallel line analysis, also called parallel line assays).

Support for both PLA and EC₅₀ determination is included in the **Concentration & Potency** extension.

Kinetics and affinity

Kinetic and affinity determinations both rely on analysis of the interaction over a range of analyte concentrations, and can be approached in several ways, depending on the requirements of the application. Some underlying principles and recommendations are listed in the table below.

Approach	Considerations
Single cycle approach	<p>Increasing analyte concentrations are injected sequentially in the same cycle, with no regeneration between injections.</p> <p>Sample concentrations must be arranged serially in the microplate. Up to 8 determinations, one in each channel, can be performed in one cycle (excluding blanks).</p> 
Multi-cycle ¹ approach	<p>Each analyte concentration is injected in a separate cycle or channel. Regeneration is required between cycles.</p> <p>Samples may be arranged serially or in parallel in the microplate.</p> <ul style="list-style-type: none">• In serial mode, up to 8 determinations, one in each channel, can be performed in one series (excluding blanks).• In parallel mode, one determination with up to 8 concentrations or two determinations with up to 4 concentrations each can be performed in one cycle (excluding blanks). 
Blank cycles	<p>During evaluation in Biacore Insight Evaluation Software, sensorgrams are corrected by subtraction of blank cycles (analysis cycles with zero analyte concentration). Blank subtraction applies only within channels. For single cycle determinations, blank cycles occupy the same number of positions in the microplate as the analyte cycles.</p>

Approach	Considerations
Duplicate cycles	<p>For multi-cycle determinations in serial mode, at least one non-zero analyte concentration may be run in duplicate, with duplicates well separated in the cycle sequence, to provide a check on consistency of surface capacity during analysis.</p> <p>Duplicate analyte concentrations are not relevant to single-cycle determinations or to multi-cycle in parallel mode.</p>

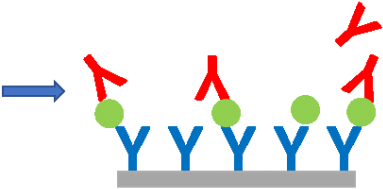
¹ The term "multi-cycle" derives from earlier Biacore systems where parallel mode is not supported. It is not strictly correct in the Biacore 8K/8K+ system, since kinetics and affinity can be determined in a single cycle in parallel mode, and evaluated in the same way as a multi-cycle experiment. The Evaluation Software handles both single- and multi-cycle runs equally.

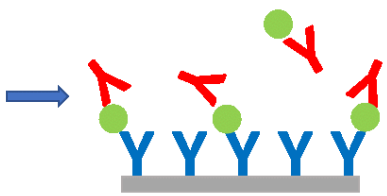
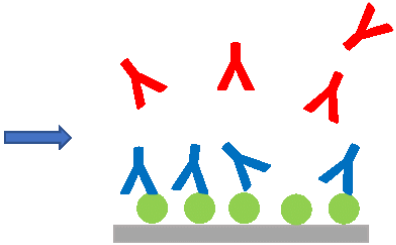
Single- and multi-cycle approaches give fully comparable results.

Epitope binning

Antibodies specific to the same target antigen are tested in a pairwise combinatorial manner to assess whether or not they block one another's binding to an epitope of the antigen. Antibodies that compete for the same epitope and share a common blocking profile are binned together. Predefined analysis methods are provided for the most commonly used assay formats sandwich, premix, and tandem. Epitope binning runs are evaluated using dedicated functionality in the Evaluation Software, described in the *Biacore Insight Evaluation Software Manual*.

Support for epitope binning is included in the **Epitope binning** extension.

Assay format	Description
Sandwich	<p>The first antibody is immobilized or captured to the surface. Antigen is then injected over the first antibody, followed by injection of the second antibody.</p> 

Assay format	Description
Premix	<p>The first antibody is immobilized or captured to the surface. The premixed solution with antigen and second antibody is then injected over the first antibody.</p>  A diagram illustrating the Premix assay format. A blue arrow points to a surface where five blue Y-shaped antibodies are immobilized. Above the surface, a mixture of green circular antigens and red Y-shaped antibodies is shown. Some red antibodies are already bound to the green antigens, while others are free in the solution.
Tandem	<p>The antigen is immobilized to the surface. The first antibody is then injected over the antigen, followed by the second antibody.</p>  A diagram illustrating the Tandem assay format. A blue arrow points to a surface where five green circular antigens are immobilized. Four blue Y-shaped antibodies are bound to these antigens. Above the surface, three red Y-shaped antibodies are shown, representing the second antibody being added to the assay.

Support for regulated environments

The **GxP** extension provides support for work in regulated environments in accordance with the requirements of §21 part 11 of the Federal Code of Regulations. Functionality added by the **GxP** extension is described in the separate *Handbook 29312548*.

4 Software overview

About this chapter

This chapter describes the general organization of the Biacore 8K Control Software. The software requires a connection to the instrument in order to control instrument-related operations such as starting and monitoring a run. However, the software can also be used on a computer that is not connected to the instrument, such as an office computer, to create methods and examine runs stored in a network database.

Workspaces

The interface is organized into four main workspaces:

Workspace	Description	For details, see
Instrument control	Provides tools for instrument management.	Chapter 5 Instrument control workspace, on page 59.
Activity history	Displays a log of instrument activity.	Chapter 6 Activity history workspace, on page 71.
Methods	Used for creating and editing methods.	Chapter 7 Methods workspace, on page 72.
Runs	Displays results of runs.	Chapter 8 Runs workspace, on page 110.

Software extensions

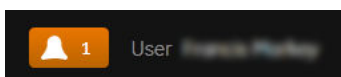
The software for the Biacore 8K/8K+ system is available as a basic package with optional software extensions. Currently available extensions are listed in the table below.

Extension	Description
Concentration & Potency	Adds predefined method templates and functionality for determination of analyte concentration based on calibration curves.
Data Integration	Provides support for data export in JSON and XML format.
Epitope Binning	Adds predefined method templates and functionality optimized for epitope characterization.

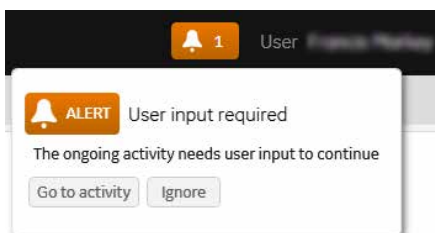
Extension	Description
Extended Screening & Characterization	Adds predefined method templates and functionality optimized for screening and characterization of low molecular weight analytes.
GxP	Provides support for work in regulated environments, including restricted access for routine users and audit trails. Functions in the GxP extension are described in the separate <i>Biacore Insight GxP Handbook</i> .

Notifications

Alerts and malfunctions are indicated by notifications in the top banner of the screen. The number of notifications is shown in the notification symbol. Notifications are also issued as reminders when scheduled instrument maintenance is due.



Click on the notification symbol to open details of the notification. Most notifications require user action for instrument operation to continue. In some cases, software buttons for user action are shown with the notification text.

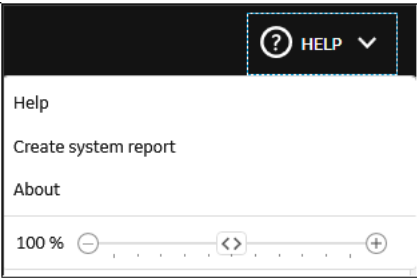



Time stamps

Time stamps are created as local time on the computer where the stamped operation is performed, and displayed with an offset to local time in a different time zone (for example, a run performed at 15:00 EST will be shown as 12:00 PST).

Help

The following functions are provided in the **Help** menu:



Function	Description
Help	Provides top-level help on the current workspace. Follow the links in the help system to find the information you need.
Create system report	Use this function to generate a report containing information on the software installation and environment, and on the status of the connected instrument (if any). The report is intended to assist Cytiva service representatives in troubleshooting system errors.
About	Provides information about the current software version, the connected database, and the selected extensions.
<div> Zoom slider</div>	<p>Use the slider to zoom the display in or out.</p> <p>Note: <i>Zooming in to the display may obscure some elements of the interface.</i></p>

5 Instrument control workspace

About this chapter

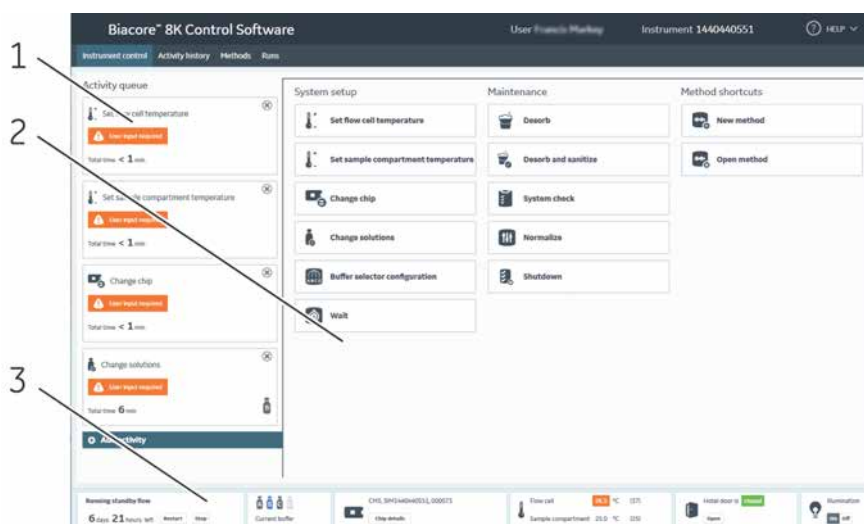
This chapter describes the functions and use of the **Instrument control** workspace.

In this chapter

Section	See page
5.1 Activity queue	61
5.2 Instrument status	64
5.3 Instrument control tools	67
5.4 Display during a run	70

Introduction

Operation of the Biacore 8K/8K+ instrument is managed from the **Instrument control** workspace. The workspace is divided into three main areas. The area borders are fixed.

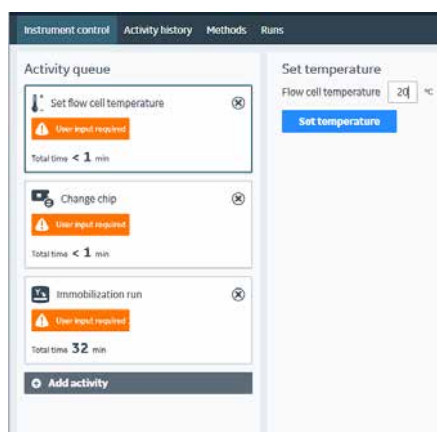


Part	Function
1	<p>Activity queue.</p> <p>Programmed activities (methods and tools) are placed in the queue to be executed on the instrument. See Section 5.1 Activity queue, on page 61 for details.</p>
2	<p>Main workspace.</p> <p>Shows the available tools when no activity is selected in the queue.</p> <p>Shows the details and any user input requirements for activities selected in the queue.</p>
3	<p>Instrument status.</p> <p>Provides information on the current status of the instrument to which the software is connected, and holds some direct action controls for instrument operation such as opening the sample hotel door and turning the sample compartment illumination on or off.</p>

5.1 Activity queue

Introduction

Operations on the Biacore 8K/8K+ instrument are initiated through the **Activity queue**, displayed in the left-hand panel of the **Instrument control** workspace.



An activity that is added to the queue will start automatically when required user input has been provided and all previously queued activities are completed. Conditions that prevent a run from starting are highlighted in orange.

Activities are executed in order from top to bottom. When an activity is completed, the item is automatically removed from the queue.

Adding activities to the queue

To place a tool from the **Instrument Control** workspace in the queue, simply click the required tool button. If the workspace displays the activity details for an activity already in the queue, select **Add activity** in the **Activity queue** panel to return to the tool selection display.

Methods are added to the queue from the **Methods** workspace (see [Section 7.1 Managing methods, on page 73](#)).

Activities are added to the bottom of the queue, and can be moved by dragging with the mouse. An activity cannot be moved to a position before the one that is currently being executed.

User input

All activities require some kind of user input before they will start. This may be simple confirmation that the activity is ready to start, or more detailed input in the form of parameter values, settings, and tray positions. User input is provided in the main workspace that is displayed when the activity is selected in the queue (and when the activity is first added to the queue).

User input for queued activities can be provided in advance while activities earlier in the queue are being executed. If all input is provided in advance, the activity will start automatically as soon as the preceding activity has been completed.

Selecting buffer inlet

The buffer inlet can be selected when a method or **Change solutions** activity is added to the activity queue. The available inputs are determined by the **Buffer selector configuration** (see [System setup tools, on page 67](#)). The buffer inlet cannot be changed during an ongoing activity.

An example of the user input workspace for **Change solutions** with the buffer selector configured for 3 inlets is shown below.



The current buffer inlet (buffer **B** in the example above) is selected by default. There is no default selection if the buffer selector configuration is changed to exclude the current inlet.

Note: *The name of running buffer for a method is set in the method, and is independent of the buffer inlet used.*



Estimated time for completion of activities

Each activity entry in the queue show the estimated time required for completion of the activity. If the selected buffer inlet for an activity differs from the previous setting, the estimated time will include the time required to change the buffer in the system.

Managing the activity queue

Procedures for managing activities in the queue are listed in the table below.

Operation	Procedure
Move an activity	Drag the activity item to the new position with the mouse. You cannot move an activity to a position before the activity currently being executed.

Operation	Procedure
Remove an activity	Select  Remove in the activity item, then confirm that you want to remove the activity. Any user input provided for the activity will be lost.
Abort an ongoing activity	Select  Stop in the activity item, then choose whether to abort with or without washing the system. New confirmation will be required for all following items in the queue when an activity is aborted.

5.2 Instrument status

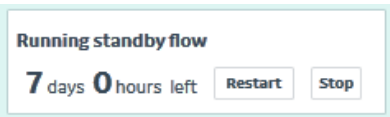
Overview

The instrument status and other information is shown in the panel at the bottom of the **Instrument control** workspace.



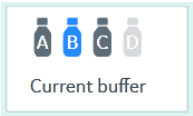
Current instrument activity

This item displays the current instrument activity. When no activity is being executed, buttons for managing standby flow are displayed:






Function	Description
Start standby flow	Displayed when the instrument is idle. Click to start standby flow. Make sure liquid is supplied to all inputs before starting standby flow.
Restart	Displayed while standby flow is running. Click to reset the standby flow timer to 7 days.
Stop	Displayed while standby flow is running. Click to stop standby flow. Note: <i>Use this function only if you want to stop standby flow for maintenance purposes. Standby flow stops automatically whenever another activity is started.</i>

Buffer inlets



This item shows the current buffer selector configuration and selected buffer inlet. Buffer symbols are colored as follows:

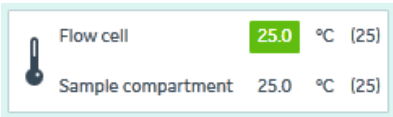
Color	Meaning
 Blue	Currently selected.
 Black	Available but not currently selected.
 Gray	Not included in the current buffer selector configuration.

Sensor chip



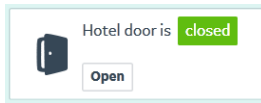
This section displays properties of the currently docked sensor chip. Select **Chip details** for more extensive information, including ligand identity and immobilized level in each flow cell and channel.

Temperature



This item displays the current flow cell and sample compartment temperature (with the set values in parentheses). The flow cell temperature is shown in red if the set temperature has not been reached.

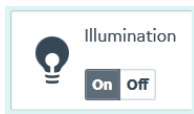
Sample hotel door



This section displays the status of the sample hotel door. Select **Open** to release the hotel door lock (see [Sample hotel door, on page 23](#)).

The door cannot be opened while sample trays are being transferred between the sample compartment and the hotel.

Sample illumination

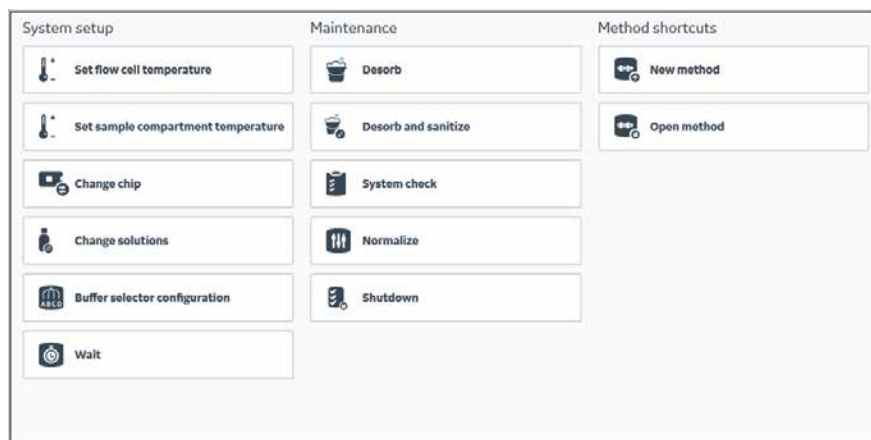


This section displays the status of the sample compartment and hotel illumination. Use the buttons to turn the illumination on and off.

5.3 Instrument control tools

Introduction

The **Instrument control** main workspace panel provides access to tools for performing instrument operations and shortcuts to creating a new method or opening an existing method. Click on a tool to add the tool to the activity queue. Click on a method shortcut to go to the **Methods** workspace.



System setup tools

Tool	Description
<i>Set flow cell temperature</i>	Sets the temperature at the flow cell.
<i>Set sample compartment temperature</i>	Sets the temperature in the sample compartment. The sample compartment temperature is set independently of the flow cell temperature: however, for best performance, the sample compartment temperature should be the same as or close to the flow cell temperature.
<i>Change chip</i>	Undocks and docks the sensor chip in the instrument. Note: <i>For runs requiring highest performance, allow the flow system to equilibrate in standby mode at least overnight after changing the sensor chip.</i>

Tool	Description
Change solutions	<p>Fills the flow system with solutions. Use this tool when buffer or reagent is changed, when the system is started from shutdown, and after docking a chip. Run Change solutions even when changing to a new batch of nominally the same solution, and to change the selected buffer inlet within the scope of the buffer selector settings.</p> <p>Note:</p> <p><i>For runs requiring highest performance, allow the flow system to equilibrate in standby mode at least overnight after changing solutions. Extend the equilibration time to at least 24 h if the detergent concentration in the running buffer is changed.</i></p>
Buffer selector configuration	<p>Configures the buffer selector for 1 to 4 buffer inlets and selects the current inlet:</p> <ul style="list-style-type: none"> • One inlet (A) • Two inlets (AB) • Three inlets (ABC) • Four inlets (ABCD) <p>The tool flushes and empties buffer tubes as appropriate in a sequence of steps that depends on the initial and target configurations. Each step requires user input to start. Full instructions are given in the tool.</p> <p>Tubes that are configured as not used should be fitted with protective caps to prevent dust from entering the tubes. Tubing caps are provided with the system.</p> <p>This tool is only available for instruments fitted with a buffer selector (see Buffer selector, on page 26).</p>
Wait	<p>Inserts a user-defined delay period into the activity queue. Buffer flow continues during the Wait period.</p>

Maintenance tools

Tool	Description
Desorb	Cleans the flow system. Run this tool at regular intervals (recommended at least once a week).

Tool	Description
Desorb and sanitize	Cleans and disinfects the flow system to prevent growth of microorganisms. Run this tool at regular intervals (recommended at least once a month).
System check	Checks system performance. Run this tool when a malfunction is suspected or when instructed by Cytiva service.
Normalize	Normalizes the detection system to compensate for small differences in the light distribution in the detection system. For best performance, run this tool monthly and after change of analysis temperature.
Shutdown	Empties the flow system and shuts down the instrument. Run this tool when the instrument will be left unused for more than 7 days.

See the *Biacore 8K/8K+ Operating Instructions* for details of maintenance operations.

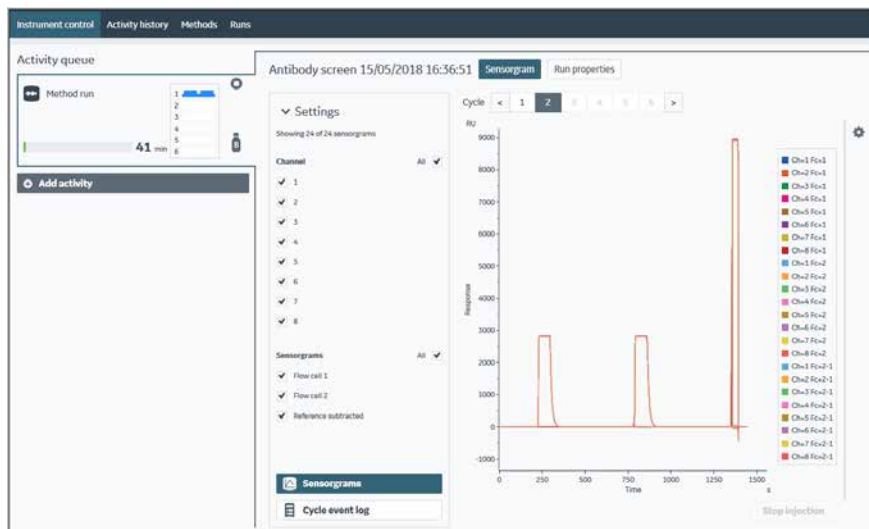
Method shortcuts

Tool	Description
New method	Opens the Methods workspace with the New option selected.
Open method	Opens the Methods workspace with the Open option selected.

5.4 Display during a run

Description

During an ongoing run, the main instrument control workspace panel displays the progress of the sensorgrams from the run. The **Activity queue** panel is still accessible. If you select **New activity** to add a new activity to the queue, you can return to the run display by selecting the run activity in the queue.



Note: The illustration above shows sensorgrams from a simulated run.

The display is the same as that for the **Runs** workspace (see [Chapter 8 Runs workspace, on page 110](#)), with two exceptions:

- The activity queue panel is accessible alongside the run display. The activity queue item shows the sample hotel configuration and highlights the sample tray currently in use.
- A **Stop injection** button appears at the bottom right of the sensorgram panel for some injections. Click this button to stop the current injection. This function applies to the ligand injection in immobilization methods and to **Capture** and **General** injections (including dissociation time, if any) in analysis methods. The method will continue automatically after the stopped injection. Use this function to interactively stop an injection when the required response is reached.

Note: There will be a few seconds delay after selecting the **Stop injection** function before the injection is actually stopped.

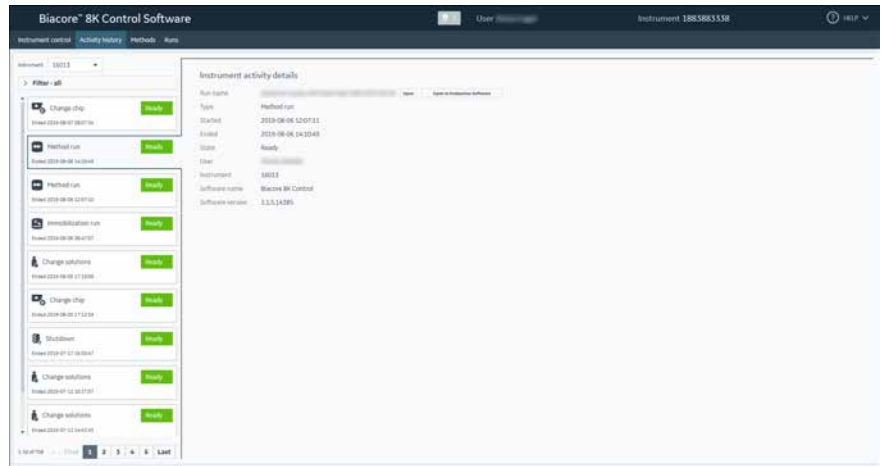
Note: The **Stop injection** button is not shown if no injections in the cycle can be stopped.

6 Activity history workspace

About this chapter

This chapter describes the **Activity history** workspace.

Description



The **Activity history** workspace lists activities performed on a selected instrument. By default, the activity history is shown for the currently connected instrument. Other instruments that are or have been connected to the same database can be selected from the **Instrument** list.

Select an activity to display the activity details. Where appropriate, results generated by the activity can be opened from the details panel.

Activity filter

With the filter it is possible to choose which activities that are shown. It is possible to show analysis runs, immobilization runs, different instrument control tools, and to filter on date.

7 Methods workspace

About this chapter

Biacore 8K/8K+ software supports two kinds of methods. Both kinds are created and edited in the **Methods** workspace.

- Immobilization methods, for covalently attaching molecules to the sensor surface.
- Analysis methods, for performing interaction analysis experiments.

In this chapter

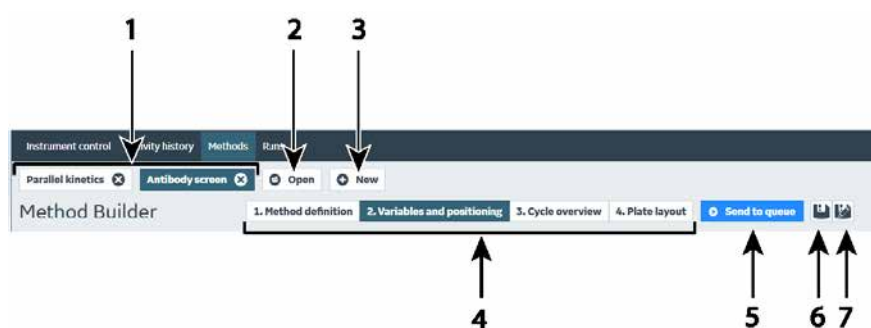
Section		See page
7.1	Managing methods	73
7.2	Immobilization methods	78
7.3	Analysis methods	82

7.1 Managing methods

Introduction

This section describes the **Methods** workspace and provides instructions for managing methods. The following sections describe immobilization and analysis methods in detail.

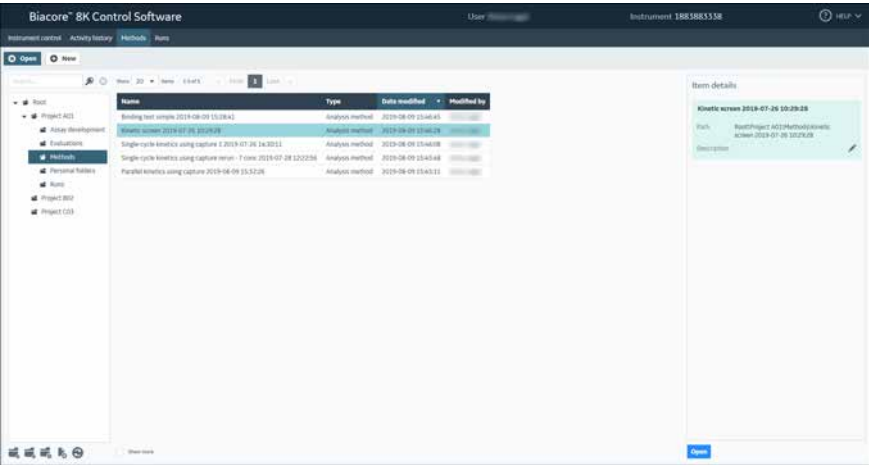
Methods workspace header



Part	Function
1	Tabs for open methods. Multiple methods may be open at the same time. The currently displayed method is highlighted in blue. <ul style="list-style-type: none"> Select a tab to make the corresponding method current. Click Close on a tab to close that method.
2	Open button. Select to open an existing method from the database.
3	New button. Select to create a new method.
4	Method management steps. The steps are different for immobilization and analysis methods.
5	Add the method to the activity queue.
6	Save the method to the database.
7	Save a copy of the method to the database with a new name.

Opening a method from the database

Follow the steps below to open a method in the **Methods** workspace. A shortcut to this workspace is also provided in the **Instrument Control** workspace.



Step	Action
1	Select Open in the Methods workspace.
2	Navigate to the required folder or use the Search function (see Searching for database objects, on page 16).
3	Choose the method. Summary details of the chosen method are shown in the panel at the right.
4	Select Open or double-click the method. The method will be opened in a new tab.

Opening a method from a run

The method definition used for a run is saved with the results of the run, and can be opened from the run item even if the original method item has been changed or deleted. You can edit the method if required and save it with a new name.

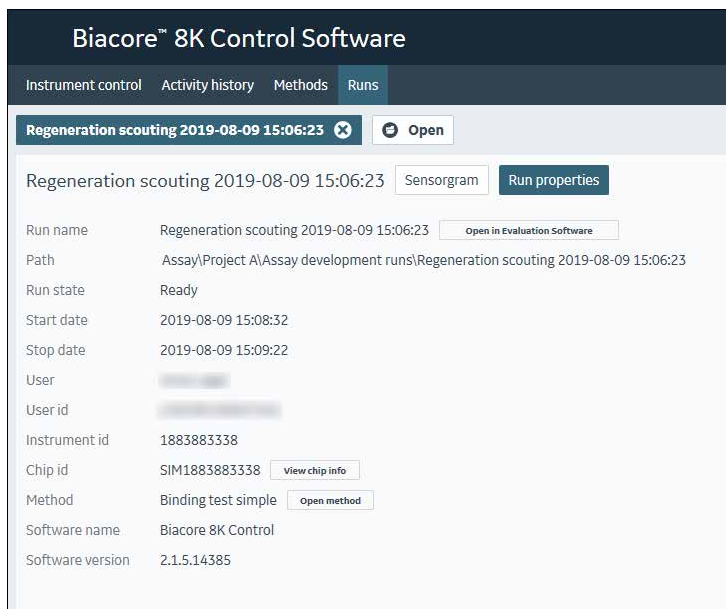
Note: *The method definition saved in the run item cannot be changed.*

Follow the steps below to open the method saved in the run item.

Step	Action
1	Open the run (see Section 8.1 Opening a run, on page 111).

Step	Action
------	--------

- | | |
|---|-------------------------------------|
| 2 | Open the Run properties tab. |
|---|-------------------------------------|



- | | |
|---|-----------------------------|
| 3 | Choose Open method . |
|---|-----------------------------|

Result:

The method is opened in a new method workspace, exactly as it was used to perform the run.

Creating a new method

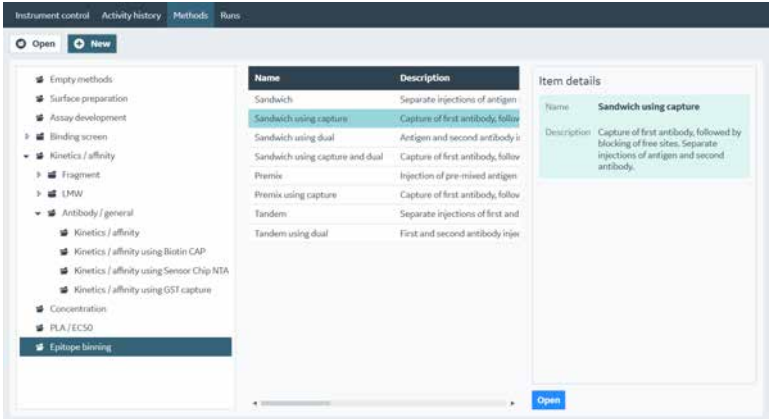
New methods are created using predefined methods as templates. Predefined methods are provided for common application requirements.

Note: You can also create a new method by editing an existing method and saving it with a new name.

Follow the steps below to create a new method from the **Methods** workspace. A shortcut to this workspace is also provided in the **Instrument Control** workspace.

Step Action

- 1 Select **New** in the **Methods** workspace.
- 2 Select an appropriate predefined method as a template for your method.




- 3 Select **Open** or double-click on the predefined method. The method template will be opened in a new tab.
- 4 Edit, save and/or run the method as required.


Note:
Predefined methods cannot be overwritten.

Editing method definitions

To edit a method, enter new steps, commands and parameter values as required.
See [Section 7.2 Immobilization methods, on page 78](#) or [Section 7.3 Analysis methods, on page 82](#) for detailed instructions.

Saving methods

Select  **Save method** to save the method with the current name. The previous version of the method will be overwritten.

Select  **Save as new method** to save the method with a new name.

Note: *Predefined method templates cannot be overwritten. If you create a new method based on a template and select **Save method** you will automatically be redirected to **Save as new method**.*

Note: *A copy of the method is automatically saved with the results when the method is run. Methods that have been run but not explicitly saved can be recovered from the results (see [Opening a method from a run, on page 74](#)).*

Method verification

Methods are verified automatically in two respects:

- Syntax verification is performed on the fly, to check that steps and commands are correctly specified.
- Variable verification is performed when the **Cycle overview** is accessed or the method is added to the activity queue, to check that values are provided for all required variables.

Note: *Verification determines only that the method is correctly specified and can be executed. The software cannot check that the method fulfills the intended application purpose.*

Errors are indicated by a red frame around the missing or incorrect information. The example below shows a missing solution in the command **Regeneration 1**.

Property	Variable	Value
Solution	<input type="checkbox"/>	
Contact time	<input type="checkbox"/>	30 s
Flow rate	<input type="checkbox"/>	30 µl/min

Flow path: ☒ both flow cells, ☐ flow cell 1, ☐ flow cell 2

Errors in closed steps and commands are indicated by a warning symbol in the respective tab. The example below shows that there are one or more errors in the step **Startup 1**.

Note: *The warning symbol is hidden if the step or command is selected. Instead the error indication is visible.*

Running methods

To run a method, select **Send to queue** from the method workspace and provide a name and location for the results. The method will be placed in the activity queue, and will start when the required information has been provided and all preceding activities have been completed.

The **Send to queue** button is not available from the **Method definition** step.

7.2 Immobilization methods

Introduction

Immobilization methods are used to attach capturing molecules or ligands to the sensor surface. Standard attachment methods on each sensor chip type are supported directly. Custom methods can also be created, either to modify details of standard methods or to create methods for new attachment approaches.

Immobilization may be performed in all or selected channels in one step (using the same settings for each channel). An immobilization method may contain multiple steps with different procedures in different steps. Each step results in one cycle when the method is run.

More details of coupling chemistry and attachment methods may be found in the *Biacore Sensor Surface Handbook*.

Note: *Reversible capture of ligands on the sensor surface by binding to an immobilized capturing molecule is not performed with an immobilization method. This step is part of the analysis cycle in an analysis method.*

Setting up standard immobilization methods

Only the ligand injection conditions can be changed in standard immobilization methods. To change other injection settings, use a custom method (see [Setting up custom immobilization methods, on page 81](#)).

Follow the steps below to set up a standard immobilization method.

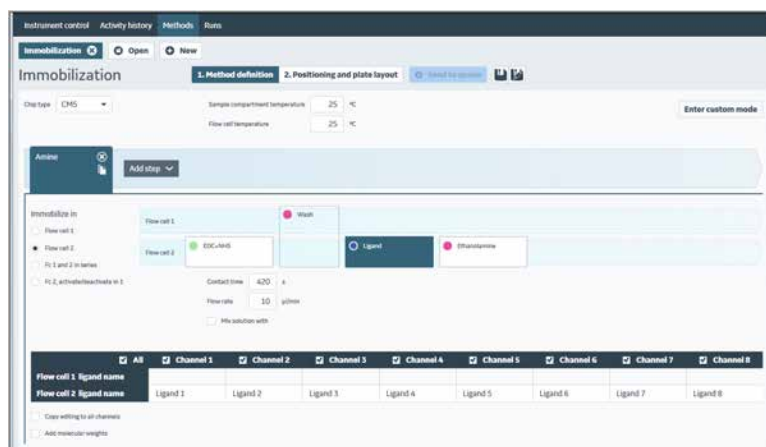
Note: *The immobilization method templates provided with the system are set up for amine coupling. To create a method for a different immobilization chemistry, remove the **Amine** step and add a step for the appropriate chemistry.*

Step	Action
1	Select New in the Methods workspace and choose a suitable predefined immobilization method template from the Surface preparation folder. Click Open .

Step	Action
------	--------

Result:

The method opens in a new method tab.

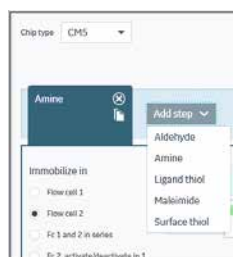


- 2 Select the **Chip type** that you wish to use. The default is **CM5**. Select **Custom** if you are using a chip type that is not listed.

If you select a chip type that is not compatible with steps in the method, you will be prompted to discard the steps.

- 3 Remove any steps that you do not want from the predefined method and add steps that you require.

To add a step, select **Add step** and choose the immobilization chemistry. Only chemistries applicable to the selected **Chip type** are listed.



Note:

Steps added to a method based on the predefined **Immobilization low levels** method will not include the adaptation to low immobilization levels (see [Achieving low levels of immobilized ligand, on page 51](#)).

Step	Action
4	<p>Choose the flow cells where the ligand is to be immobilized. This choice will apply to all channels addressed in the step.</p> <p>The option Fc2, activate/deactivate in 1 injects all solutions except ligand over both flow cells. Buffer replaces the ligand injection in Fc1. This creates a reference surface that has been activated and deactivated to give charge properties that more closely resemble the ligand surface.</p> <p>Note:</p> <p><i>When both flow cells are selected, solutions will pass over Fc1 followed by Fc2 in a single injection. Immobilization levels may be slightly lower in Fc2.</i></p>
5	<p>Change the contact time and flow rate for the ligand injection if required.</p> <p>Contact time and flow rate are fixed for all injections except the ligand in standard methods. Select an injection to display these parameters.</p> <p>If you want to modify fixed parameters, set up a custom immobilization method (see Setting up custom immobilization methods, on page 81).</p>
6	<p>Select Mix with to mix the ligand with another solution immediately before injection. Specify the name of the solution to be mixed and the percentage in the final mixture (for example, specifying "90% buffer" will mix 1 part of ligand with 9 parts of buffer). This can be used, for example, to dilute the ligand immediately before immobilization when the ligand shows reduced stability in immobilization buffer.</p> <p>Note:</p> <p><i>Mixing is performed in the microplate and requires an empty row of positions in the plate. The empty positions must be on the same tray (but not necessarily the same microplate) as the solutions to be mixed.</i></p> <p><i>Mixing positions are also required for preparing the EDC/NHS mixture used in most immobilization methods.</i></p> <p><i>Mixing is only supported in 96-well microplates with well volume 250 or 650 μL.</i></p>
7	<p>Select the channels where the immobilization is to be performed. Buffer will flow through channels that are not used.</p>
8	<p>Provide ligand name(s) for the selected channels and flow cells. If the option Copy editing to all channels is checked, names entered in one channel will automatically be applied to all channels that are selected for the immobilization step.</p>
9	<p>Select Add molecular weights and enter ligand molecular weights if required. This information is used for calculation of expected binding capacity (R_{max}) values in some evaluation procedures.</p>



Step	Action
10	When the method definition is complete, go to the Positioning and plate layout step to manage sample and reagent positioning in the microplates and to print positioning information as a guide to microplate preparation (see Section 7.3.7 Sample positioning, on page 103).

Setting up custom immobilization methods

To customize an immobilization method, select **Enter custom mode** in the **Method definition** step. Custom mode adds the following features to the method definition:

- Parameters can be changed for all commands.
- Commands can be added, copied, moved and deleted in the command sequence.

Follow the instructions below to use these customization features.

Action	Instruction
Change command parameters	<ol style="list-style-type: none"> 1. Select the command in the command sequence. 2. Change the parameter settings as required.
Add a new command	Select Add command and choose the command type. Available types are Injection and Wash . The new command will be added at the end of the command sequence.
Copy a command	Click  Copy on the command. A copy of the command will be added to the command sequence. You can change the command name for all commands except Wash and Ligand .
Move a command	Drag the command to the new position in the command sequence.
Delete a command	Click  Remove on the command.

7.3 Analysis methods

Introduction

Analysis methods are used to run experiments using the Biacore 8K/8K+ system. Predefined methods for a range of applications are provided with the system, and are recommended starting points for development of custom methods (see [Creating a new method, on page 75](#)). For many purposes, the predefined methods are ready to run after sample information has been provided. This section describes the principles of creating a method from start. Apply the principles as appropriate when editing predefined or existing methods.

In this section

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7.3.1 Method overview

Method structure

Analysis methods are built from the components summarized in the table below. Steps and commands are represented by tabs, with the currently selected tab expanded to show the tab details.

Component	Description
Steps	Steps define the overall structure of the method and represent one or more repeated cycles. Steps have a specified purpose which determines how the cycle will be handled during evaluation of the results.
Commands	A command represents an individual operation, usually injection of solution over the sensor surface. The commands are sequentially ordered and determines the series of operations performed in a step.
Variables	Command parameters can be set as constant or variable. Constant parameters are applied every time the command is executed. Variables may be given different values for different cycles.

In the illustration below, the **Analysis** step is selected and expanded at the top, the **Regeneration 1** command is selected and expanded in the middle, and its command parameters are displayed at the bottom.

Property	Variable	Value
Solution	<input type="checkbox"/>	Regeneration solution
Contact time	<input type="checkbox"/>	30 s
Flow rate	<input type="checkbox"/>	30 µl/min

Flow path: ☒ both flow cells, ☐ flow cell 1, ☐ flow cell 2

☐ Predip, ☐ High viscosity solution

Method definition workflow

The steps below give a brief summary of the workflow for setting up analysis methods. For most purposes, you will start from a predefined method template that includes steps and commands appropriate for the method purpose. You may however want to modify the predefined steps and commands or to add new ones to the method.

Note: *The flow cell temperature in predefined method templates is generally set to 25°C. This is also the default flow cell temperature for new steps. If you want to use a different flow cell temperature, make sure that the setting is changed for all steps.*

Step	Action
1	Define the steps in the method. For each step, set up the command sequence that will determine operations in the step.
2	Exclude any channels that you do not want to use. Note: <i>Buffer will be injected over unused channels. You will need to add buffer to the appropriate wells in the microplate(s). Unused channels will be automatically excluded from the evaluation in Biacore Insight Evaluation Software.</i>
3	Assign cycles to each step and assign values to variable parameters in the cycles. This may be done either by adding cycles and entering variable values manually, or by importing variable information from the Windows clipboard or an external file. Cycles that have no variable parameters must be added manually to the respective steps.
4	Adjust the positions of solutions in the microplates if required. Positions are assigned automatically for solutions that are fixed in the method definition and for variable solutions that are entered manually. Imported sample data may include positioning information (for example, when the information is obtained from a laboratory robot used to prepare the microplates).
5	Examine the Cycle overview to ensure that samples are run in the desired order (in particular, that repeated cycles are appropriately placed). The cycle overview also displays the estimated duration of the run.
6	Save the method if required.
7	Use the Plate layout information to prepare the microplates if required, then send the run to the activity queue.

7.3.2 General settings

General method settings are specified at the top of the **Method definition** workspace.



Setting	Description
Data collection rate	The number of data points registered per second from each flow cell during a run. Choose between 1 and 10 Hz. 1 Hz is sufficient for most general applications. Use 10 Hz when resolution of fast binding events is important.
Sample compartment temperature	Temperature in the sample compartment. Choose between set to fixed with a specified value and vary with flow cell temperature . Note: <i>The flow cell temperature is set separately for each step in the method.</i>
Concentration unit	Choose the unit that will apply for concentration values entered in the method.
Running buffer	Enter a name for the running buffer if required. This name will be shown in the results and run documentation.


7.3.3 Method steps

Introduction

Steps define the overall structure of the method and represent one or more repeated cycles. Steps have specified purposes which determine how the step will be handled during evaluation of the results. The assigned purpose does not affect how the step will be run.

Adding a new step

Follow the instructions below to add a new step to the method.

Step	Action
1	Select Add step and choose the step purpose (see Step purposes, on page 87). You can change the step purpose if required after the step has been added.
2	<p>Provide a name for the step.</p> <p>Tip:</p> <p><i>New steps are named by default with the step purpose and a serial number (for example, Analysis 2). Changing to a more informative name can help in understanding the method structure.</i></p>
3	<p>Set the flow cell temperature for the step if necessary.</p> <div><p>NOTICE</p><p>The default flow cell temperature for a new step is always 25°C. If you are creating a method for a different temperature, make sure that the setting is correctly specified in all steps.</p></div>
4	<p>Select Repeat within if the step is to be repeated within the context of another step (referred to as the parent step). Choose the parent step in the Repeat within field, and choose between repeating at a set frequency or distributing a set number of repeated steps evenly between the cycles in the parent step. Choose also whether to perform the step additionally at the beginning and end of the parent step.</p> <p>Use this option for cycles such as solvent correction or control samples that are repeated at regular intervals throughout the run.</p> <p>Examine the Cycle overview to see how the repeated step will be distributed through the run (see Section 7.3.8 Cycle overview, on page 107).</p>

Step	Action
------	--------

Note:

If you want to run the repeated step only at the beginning and/or end of the series, check the appropriate option and set either the number of distributed occurrences to 0 or the frequency of occurrence to a number higher than the number of cycles in the series.

- | | |
|---|---|
| 5 | Define the command sequence for the new step (see Section 7.3.4 Command sequence, on page 90). |
|---|---|

Step purposes



One of the following purposes is assigned to each step in a method:

Purpose	Description
Conditioning	Used to condition the sensor surface at the start of an assay. Conditioning at the beginning of a method is required, for example, for some pre-immobilized sensor chips such as Sensor Chip SA.
Startup	Used to equilibrate the flow system and sensor surface at the start of an assay, before the first sample is analyzed. The first few cycles on a newly immobilized or docked chip often show drifting response as the system stabilizes, and startup steps prevent such drift from affecting the first analysis cycles. Startup cycles should preferably use the same injection conditions as analysis steps.
Calibration	Used for calibration cycles in concentration analysis applications. Calibration curves are created from samples run in Calibration steps. Calibration steps are only available if the Concentration & potency extension is active.

Purpose	Description
Channel normalization	<p>Used to provide response values for normalizing the response between channels in parallel mode concentration assays by injecting a single sample concentration over all channels.</p> <p>The Channel normalization step should be placed directly before the Calibration step. Settings should be identical to the Calibration step, except that the sample solution is constant.</p> <p>Channel normalization steps are only available if the Concentration & potency extension is active. During evaluation, channel normalization is only applied to concentration assays in parallel mode.</p> <p>Tip:</p> <p><i>To add a Channel normalization step to a method, make a copy of the Calibration step and change the step purpose to Channel normalization. This will ensure that the settings are correct.</i></p>
Analysis	Used for sample analysis.
Solvent correction	Used specifically for solvent correction cycles. The step contains one Solvent correction command (see Solvent correction command, on page 95), and should typically be repeated at intervals throughout the assay.
Rmax control	<p>Used specifically for determining the analyte binding capacity of the surface by injecting a high concentration of a known binder. This information is used in evaluation of weak binding, most commonly in work with fragments and small molecules (see the <i>Biacore Insight Evaluation Software Manual</i> for details).</p> <p>Rmax control steps are only available if the Extended screening and characterization extension is active.</p>
General	Used for steps that do not fit any of the other purposes.

Managing steps

Follow the instructions in the table below to copy, move and delete steps in the method.

Action	Instruction
Copy a step	Select  Copy in the step. A copy of the step including its command sequence is added to the step sequence. Any steps repeated within the step being copied are also copied.
Move a step	Drag the step to a new position in the step sequence. A repeated step cannot be moved separately from its parent step.
Remove a step	Select  Remove . If a parent step is removed, the child steps are also removed.

7.3.4 Command sequence

Introduction

The command sequence in a method step defines the operations that will be performed in each cycle in the step. Manage the command sequence in the same way as the step sequence (see [Managing steps, on page 88](#)).

The various injection commands differ in the way the injection is performed and in how the cycles are handled in evaluation of the results. More detailed descriptions are given in the sections that follow.

Command names

Commands are automatically named with a serial number. If there are more than one command of the same type they are always sequentially numbered, that is, **Wash1** is executed before **Wash2**. An automatic renumbering is done if the order is changed.

The name of a command cannot be altered, but a comment can be added. The comment is displayed below the command name.

Fixed and variable values

Many command settings may be either fixed or variable. Values for fixed settings are entered directly in the command definition and apply to all channels every time the command is executed. Values for variable settings are entered in the **Variables and positioning** step, and determine the number of cycles that will be run in the step. Variables may refer to sample names or command parameters such as flow rate or contact time.

User-defined variables can be added to some commands.

As an example, the analyte injection command in a sample analysis step may have fixed values for **Contact time** and **Dissociation time**, and variable values for **Solution** (identifying the sample that will be injected) and **Concentration** (used in evaluating the results). A user-defined variable may be added, for example, to hold the batch number of the sample.

General command settings

General command settings are described in the table below. Settings that are specific to one or a few commands are described in the detailed command descriptions that follow this section.

Setting	Description
Solution	Name of the solution to be injected.
Contact time	Contact time for the injection.
Dissociation time	Time after the end of the injection to allow analyte dissociation before the next command is executed.

Setting	Description
Flow rate	Flow rate, maintained for the duration of the command.
Flow path	Flow path for the injection. Choose Flow cell 1 , Flow cell 2 or Both flow cells . The choice is illustrated graphically by the placing of the tab in the command sequence.
Predip	Check this option to dip the needles in a separate row of wells containing the injection solution before aspirating the solution to be injected. This will rinse the needle tips briefly to minimize carry-over effects from the previously injected solution. A separate row of predip positions is created in the microplate for each command that uses the option.
Mix	<p>Mix the sample with another solution before injecting. Specify the name of the solution to be mixed and the percentage in the final mixture (for example, specifying Mix with 20% buffer will mix 8 parts of sample with 2 parts of buffer).</p> <p>Note:</p> <p><i>Mixing is performed in the microplate and requires an empty row of positions in the plate. The empty positions must be on the same tray (but not necessarily the same microplate) as the solutions to be mixed.</i></p> <p>Mixing is only supported in 96-well microplates with well volume 250 or 650 µL.</p>
Concentration	<p>(Optional) Enter the sample concentration or check this option to include concentration as a variable for the injected solution.</p> <p>Note:</p> <p><i>Do not include concentrations as part of the sample name. This will affect grouping by sample in the Evaluation Software.</i></p>
Molecular weight	(Optional) Enter the sample molecular weight or check this option to include molecular weight as a variable for the injected solution.
Dilution	<p>(Optional) Check this option to include sample dilution as a variable for the injected solution.</p> <p>The Dilution value is used for calculating the original concentration from concentration measurements on diluted samples in evaluation of concentration measurements. The value is taken as 1 if the field is left blank</p> <p>Dilution is only available for Analyte and General commands, and only when the Concentration & potency extension is active.</p>

Setting	Description
Add variable	Select this option to add a user-defined variable. Provide a name for the variable and specify the format (Text or Numeric). Numbers entered in a text variable will be treated as text for evaluation purposes.

7.3.5 Command descriptions

Introduction

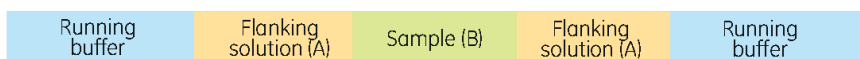
This section describes the commands that can be used in analysis methods. See [General command settings, on page 90](#) for settings that are common to several commands.

Commands are listed here in alphabetical order.

A-B-A command

This command injects analyte ("**B**") flanked by a different solution ("**A**") which may be buffer (other than running buffer) or another sample. Use this option for example for buffer scouting without changing running buffer or for investigating interactions in the presence of different cofactors. The flow path for the analyte can be set to flow cell 1, flow cell 2, or both flow cells, while the flanking solution always is injected over both flow cells. Contact times for flanking solution before and after the sample are set separately. The same flow rate is used throughout the injection.

Dissociation times are not supported for A-B-A injections. The **Post-analyte contact time** for flanking solution is equivalent to a dissociation time for the analyte.



Analyte command

This command is intended for injection of analyte.

Choose the type of injection from the following options.

Type	Description
High performance	Optimizes the injection for best performance. This option increases sample consumption.
Low sample consumption	Optimizes the injection for low sample consumption. This injection type is adequate for most applications.
Fast injection	Optimizes the injection for minimum overhead time. This injection type is primarily intended for Clean Screen runs in fragment screening (see the <i>Biacore Insight Evaluation Software Manual</i> for a description of Clean Screen). The Fast injection type is only available if the Extended screening and characterization extension is active.

Capture command

This command is intended for injection of ligand over a capturing molecule at the beginning of a cycle. The injected solution, contact time and flow rate can be set as variables.

The **Capture** command injects solution by default over flow cell 2, leaving flow cell 1 as a reference surface without ligand.

Carry-over control command

This command injects a 20 s pulse of buffer over both flow cells at a flow rate of 30 $\mu\text{L}/\text{min}$, in order to determine whether there is carry-over of analyte or other material from the preceding command. The command is suitably placed at the end of the cycle. The command has no user-definable settings.

Dual command

This command injects first analyte A and then analyte B, without buffer in between. The contact times for the two injections are set separately. The two analytes are injected over both flow cells, and the same flow rate and flow path is used for the entire command.

Enhancement command

This command is intended for injection of a secondary enhancement reagent following the sample injection. Enhancement reagents are most commonly used to confirm the identity of the bound analyte.

Note: *In other techniques, such as immunoassays, enhancement may be used to amplify the sample response. Due to the high sensitivity of the system, this is seldom relevant in Biacore 8K/8K+ applications.*

General command

This command is a general-purpose injection that supports the same settings as the **Analyte** command (see [Analyte command, on page 93](#)). **General** injections are not automatically recognized as analyte injections for evaluation purposes, but can be selected for evaluation in the Biacore Insight Evaluation Software.

Regeneration command

This command is intended for injection of a regeneration solution following the sample injection.

Select **High viscosity solution** if the regeneration solution has a relative viscosity higher than about 3 (corresponding to about 35% glycerol or 40% ethylene glycol at 20°C). This will adapt the solution aspiration and injection procedure to handle the higher viscosity solution.

Single-cycle kinetics command

This command injects a series of sample concentrations in the same cycle, intended specifically for single-cycle kinetics analysis (see [Kinetics and affinity, on page 52](#)). The samples are injected in direct sequence, separated only by the time required to prepare the next injection. A dissociation time is included after the last sample injection.

Solvent correction command

This command injects a series of solvent correction solutions through each channel in a single cycle, with a contact time of 20 s for each solution at a flow rate of 30 $\mu\text{L}/\text{min}$. Use this command in experiments where samples contain dimethyl sulfoxide (DMSO) to maintain analyte solubility.

One solvent correction command is automatically included in a method step with purpose **Solvent correction**. Solvent correction commands cannot be added to the command sequence in any other way. Repeat the step at regular intervals (recommended at the beginning and end of the run and every 30 to 50 cycles depending on the demands of the experiment).

Solvent correction solutions consist of running buffer containing varying concentrations of DMSO, typically covering a range of -0.5% to +1.0% from the nominal DMSO concentration in the samples (for example, if the samples are prepared in 2% DMSO, the solvent correction solutions should cover a range of 1.5% to 3.0% DMSO). The recommended number of solvent correction solutions is 4: up to 8 solutions can be used.

Note: *Each solvent correction solution will occupy 8 positions in the microplate, one for each channel. Solutions for repeated instances of the command are placed in separate positions by default but may be pooled if desired.*

The principles and application of solvent correction are described in the *Biacore Insight Evaluation Software Manual*.

Wait command

This command pauses the method for the specified length of time. Data collection continues during the wait period.

Wash command

This command washes the flow system with the specified solution. The solution does not pass over the sensor surface.

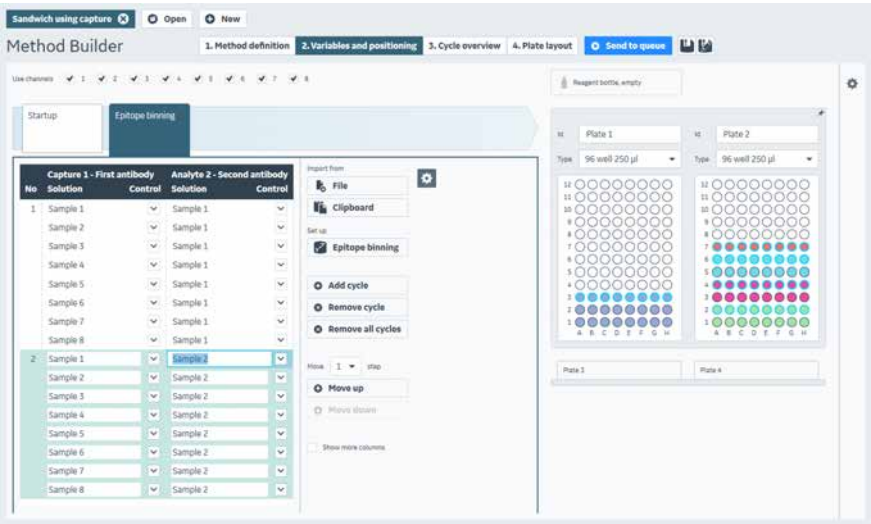
7.3.6 Entering variables and managing cycles

Introduction

Cycles are managed in the **Variables and positioning** step of method creation.

Cycles are added to steps in the method either manually using the **+ Add cycle** button or by importing variables. Importing variables adds as many cycles as required to hold the imported sample information.

Samples and reagents are also assigned to microplate positions in this step (see [Section 7.3.7 Sample positioning, on page 103](#) for details).



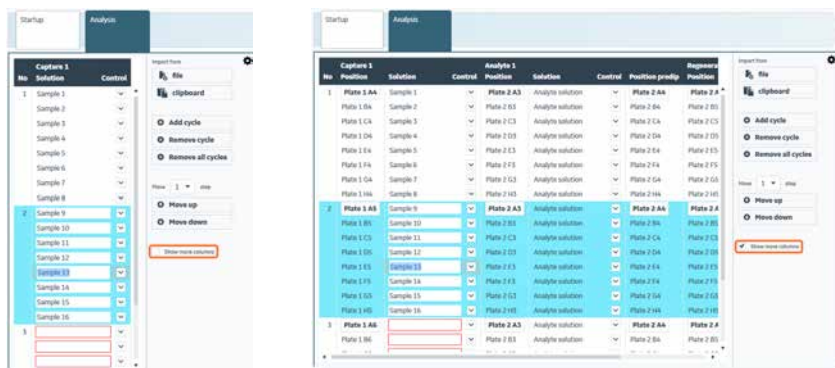
Channel usage

If you do not want to use all 8 channels for analysis, uncheck the appropriate channels under **Use channels**. Unused channels will be shaded in gray in the microplate illustrations and injected solutions for all injections in all steps will be set to **Buffer**.

Variable table


The variable table lists variables for the commands in the currently selected step. There are 8 rows in the table for each cycle, representing channels 1 to 8 in order from top to bottom. Click anywhere within a cycle group to highlight both the cycle and the row. For variables that apply to all channels in one cycle (for example, flow rate or contact time), values are entered on the first row of each cycle only.

Check **Show more columns** to display additional columns, including microplate positioning and information for commands in steps that do not include variables.








Default

Show more columns

Click  **Expand/Collapse** in the pane at the right of the variables table to expand or collapse the pane. Collapsing the pane reduces the controls to icons, to provide more area for the variable table. Some controls are hidden when the pane is collapsed.

Managing cycles

Manage cycles from the control pane as described in the following table. Cycles are managed independently for the different steps in a method.

Action	Instruction
Add a cycle	<p>Click  Add cycle. The cycle will be added at the end of the cycle list.</p> <p>Note:</p> <p><i>You do not need to add cycles manually if variable information is imported.</i></p>
Move a cycle	<p>Cycles are executed in order from top to bottom of the list.</p> <p>Select a cycle and click  Move up or  Move down to move a cycle up or down.</p> <p>The selected cycle will be moved by the number of steps specified in Move ... step.</p>
Remove a cycle	Click  Remove cycle .
Remove all cycles	<p>Click  Remove all cycles.</p> <p>Note:</p> <p><i>A method that includes a step with no cycles cannot be run.</i></p>

Entering variable values

To enter variable values manually, simply type the values in the table. Values must be provided for all active channels in each cycle. The value is fixed as **Buffer** for inactive channels.



Values may be copied and pasted within or between tables using standard Windows copy-paste operations.

Importing variable values

Variable values may be imported from external text files or from the Windows clipboard. Data may be separated by tabs, commas or semi-colons. Data from Excel files may be imported via the Windows clipboard. Only one method step can be addressed in one import operation.

Columns in the source file are either mapped to variables in the target method step or set to **Ignore**. New user-defined variables can be created by the import operation if required. If positioning information is imported, solutions will be positioned according to the specification in the source file. If samples are imported without positioning information, rows in the source file will be assigned in sequence to rows in the variable table.

Follow the steps below to import data to the variable table. An example of variable import is shown in [Variable import example, on page 100](#).

Step	Action		
1	<p>Set up the source material in a table with one sample per row and sample details in separate columns. Include microplate id and well position if you want to import samples to specific microplate positions.</p> <p>Use a header row to identify the columns. The content of the header row is for identification purposes only and will not be imported.</p> <p>The number of columns is not important provided that all detail that will be imported is represented. Additional columns can be ignored in the import operation.</p> <p>Note:</p> <p><i>Do not construct the source table as a direct representation of the microplate.</i></p>		
2	<p>Select whether to import from  file or  clipboard.</p> <p>For importing from a file, select the source file.</p> <p>For importing from the clipboard, the data must have been copied to the clipboard before the import operation is started.</p>		
3	<p>Select the appropriate settings for the import operation:</p> <table><tr><td>Decimal separator</td><td>Choose Dot or Comma as appropriate.</td></tr></table>	Decimal separator	Choose Dot or Comma as appropriate.
Decimal separator	Choose Dot or Comma as appropriate.		

Step	Action
------	--------

Default import to	Specify the default command to which the data will be imported. The target command can be changed individually for each imported parameter when data is mapped to variables.
Includes column headers	Check this option if the imported data includes column headers. Column headers help to identify columns but are not imported.
Remove existing cycles	Check this option if you want to clear all existing cycles from the target step. If the option is not checked, imported data will be added to the existing data as new cycles.

- 4 Map the columns in the imported data to variables in the method step. For each column, select the target command and the variable for import. Choose **Ignore** in the **Variable** header if you do not want to import a particular parameter. Choose **New user defined** to create a new user defined variable to hold imported values that are not mapped to existing variables. If positioning information is imported, samples will be placed in the specified positions. Existing cycles that are kept will be moved to the first available positions after the imported cycles.

Tip:

*Importing the text **Reagent bottle** to the variable **Position** will place the imported solution in the reagent bottle. Solution name and other variables must be identical for all instances of the imported solution.*

- 5 If **Plate id** is imported, assign plates to tray positions at the top right of the workspace.
- 6 In the **Included** column, remove the checkmark from any rows that you do not want to import.
- 7 Examine the parameters in the import preview carefully to ensure that the import operation is set up correctly.
- 8 Select **Import into method**.

Excluded channels are skipped when variable values are imported.

If positioning information is imported, solutions that are imported from different rows to the same position will be pooled, provided that all other parameters are identical and that the position has sufficient capacity.

7.3.6 Entering variables and managing cycles

The example below shows part of a Microsoft Excel sheet containing sample data and a corresponding mapping into variables in a method. Note that the source information for concentration is ignored (it is not included as a variable in the method) and that the **Wash solution** used in the command **Wash 1** is imported to the reagent bottle.

	A	B	C	D	E	F	G
	Plate ID	Well position	Sample	MW	Conc µM	Wash solution	
1							
2	1	A1	LMW1	475	2.5	Reagent bottle	
3	1	C1	LMW2	451	2.5	Reagent bottle	
4	1	E1	LMW3	436	2.5	Reagent bottle	
5	1	G1	LMW4	466	2.5	Reagent bottle	
6	1	I1	LMW5	477	2.5	Reagent bottle	
7	1	K1	LMW6	467	2.5	Reagent bottle	
8	1	M1	LMW7	498	2.5	Reagent bottle	
9	1	O1	LMW8	466	2.5	Reagent bottle	
10	1	A2	LMW9	454	2.5	Reagent bottle	
11	1	C2	LMW10	469	2.5	Reagent bottle	
12	1	E2	LMW11	487	2.5	Reagent bottle	
13	1	G2	LMW12	423	2.5	Reagent bottle	
14	1	I2	LMW13	487	2.5	Reagent bottle	
15	1	K2	LMW14	487	2.5	Reagent bottle	
16	1	M2	LMW15	428	2.5	Reagent bottle	
17	1	O2	LMW16	493	2.5	Reagent bottle	
18	1	A3	LMW17	525	2.5	Reagent bottle	
19	1	C3	LMW18	474	2.5	Reagent bottle	
20	1	E3	LMW19	487	2.5	Reagent bottle	
21	1	G3	LMW20	449	2.5	Reagent bottle	
22	1	I3	LMW21	518	2.5	Reagent bottle	
23	1	K3	LMW22	402	2.5	Reagent bottle	
24	1	M3	LMW23	477	2.5	Reagent bottle	
25	1	O3	LMW24	474	2.5	Reagent bottle	
26	1	A4	LMW25	495	2.5	Reagent bottle	
27	1	C4	LMW26	471	2.5	Reagent bottle	
28	1	E4	LMW27	456	2.5	Reagent bottle	
29	1	G4	LMW28	486	2.5	Reagent bottle	
30	1	I4	LMW29	497	2.5	Reagent bottle	
31	1	K4	LMW30	487	2.5	Reagent bottle	
32	1	M4	LMW31	518	2.5	Reagent bottle	
33	1	O4	LMW32	486	2.5	Reagent bottle	
34	1	A5	LMW33	474	2.5	Reagent bottle	
35	1	C5	LMW34	490	2.5	Reagent bottle	

Import into step Samples

Decimal separator: Dot
Default import to: Analyte 1
☒ Includes column headers
☒ Remove existing cycles in step

Plate selections

Plate id	Type	Located
1	384 well 120 µl	Tray 1, left

Included	Plate ID	Well position	Sample	MW	Conc pH	Wash solution
	Analyte 1	Analyte 1	Analyte 1	Analyte 1	Analyte 1	Wash 1
	Plate id	Position	Solution	Molecular weight (Da)	Ignore	Position
<input checked="" type="checkbox"/>	1	A1	LHW1	475	2.5	Reagent bottle
<input checked="" type="checkbox"/>	1	C1	LHW2	451	2.5	Reagent bottle
<input checked="" type="checkbox"/>	1	E1	LHW3	436	2.5	Reagent bottle
<input checked="" type="checkbox"/>	1	G1	LHW4	468	2.5	Reagent bottle
<input checked="" type="checkbox"/>	1	I1	LHW5	477	2.5	Reagent bottle
<input checked="" type="checkbox"/>	1	K1	LHW6	467	2.5	Reagent bottle
<input checked="" type="checkbox"/>	1	M1	LHW7	498	2.5	Reagent bottle
<input checked="" type="checkbox"/>	1	O1	LHW8	468	2.5	Reagent bottle
<input checked="" type="checkbox"/>	1	A2	LHW9	434	2.5	Reagent bottle
<input checked="" type="checkbox"/>	1	C2	LHW10	469	2.5	Reagent bottle
<input checked="" type="checkbox"/>	1	E2	LHW11	487	2.5	Reagent bottle
<input checked="" type="checkbox"/>	1	G2	LHW12	423	2.5	Reagent bottle
<input checked="" type="checkbox"/>	1	I2	LHW13	487	2.5	Reagent bottle
<input checked="" type="checkbox"/>	1	K2	LHW14	487	2.5	Reagent bottle
<input checked="" type="checkbox"/>	1	M2	LHW15	428	2.5	Reagent bottle

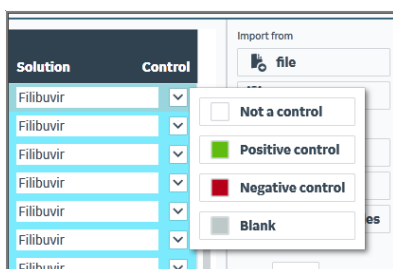
Import into method Cancel

Note: *Importing values creates the number of cycles required to hold the imported values. You do not need to create empty cycles before importing values.*

Note: *Control sample status cannot be imported, but will be assigned if imported sample names have already been defined as controls.*

Setting control sample properties

Control sample properties are defined in the variable table, and may be set for the injected solution in most commands. Expand the variable table to show more columns if you want to set control properties for constant solutions.



The control sample property affects how the sample is handled in evaluation.


Setting a property for a given sample in the variable table for one command will set the property for all occurrences of the identical solution name in all steps and commands in the method. If you want to use the same substance as a control and a sample in different contexts of the method, distinguish the usages by using different sample names.

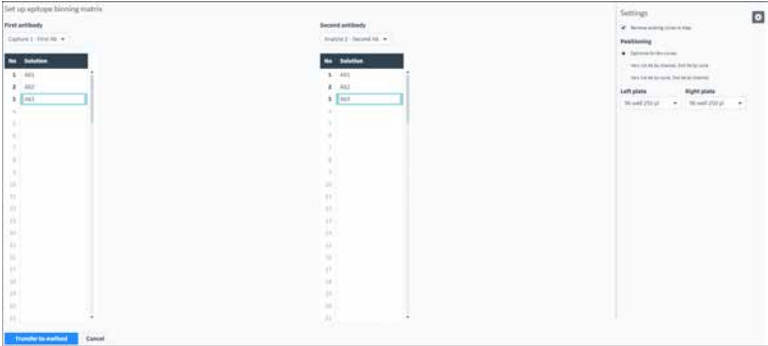
Control sample properties can also be set in the **Variables** tool in the Evaluation Software (see the *Biacore Insight Evaluation Software Manual* for details).

Setting up epitope binning

With the **Epitope Binning** extension enabled, the software has support for generation of a sample matrix that holds all possible combinations of first and second antibody.

Follow the steps below to set up the matrix.

Step	Action
1	Click Epitope binning .
	 Epitope binning
2	Verify that correct commands are selected for the first and second antibody. If needed, select correct commands from their drop-down menus.

Step	Action
3	<p>Enter the names of the solutions.</p>  <p>Note: Enter every solution only once per column, the software makes all combinations.</p> <p>Tip: Solution names can be copied and pasted.</p>
4	<p>Choose option for positioning.</p> <p>Note: The choice of plate type will affect all plates in the entire method.</p>
5	<p>Click Transfer to method.</p> <p>Result:</p> <p>Note: The sample table is populated with cycles covering all combinations.</p>

7.3.7 Sample positioning

Introduction

Samples and other solutions are automatically assigned to positions in microplates in accordance with the positioning settings in the method. Positioning settings are overridden if sample information including positions is imported from an external source. Positions are handled in groups of 8 corresponding to the 8 flow channels, occupying all wells in a row on a 96-well microplate and every second well in a row on a 384-well microplate (see [Microplates, on page 32](#)).

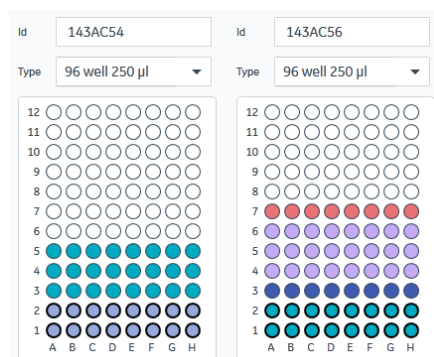
Occupied positions in the microplate are colored according to the solution and command.

Positions are managed in the **Variables and positioning** step. Equivalent functions are available for immobilization methods in the **Positioning and plate layout** step.

Positioning information can be printed from the **Plate layout** step as a guide to microplate preparation.


Position identification

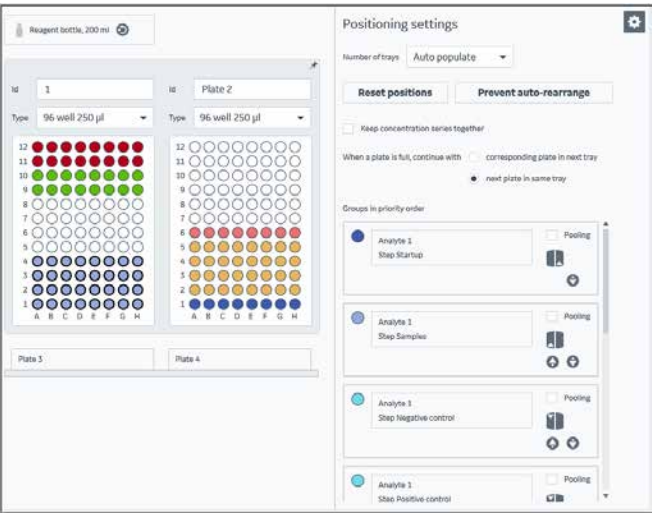
Microplate positions are identified by plate id and well coordinates (e.g., **Plate 1 G3**). Microplates are numbered sequentially by default. Choose the microplate **Type** and change the **Id** if desired in the plate representation on the right of the workspace.



Note: You may use a barcode reader to enter the microplate **Id** if you are using coded microplates. The microplate **Type** is not however linked to the microplate barcode. Make sure that the correct **Type** is selected.

Positioning settings

Click  **Positioning settings** to access and change settings that determine the automatic positioning of samples. Positions that have been assigned automatically are rearranged directly if the settings are changed, unless the function is switched off (see below).





The following settings apply to all solutions:

Setting	Description
Number of trays	Select either Auto populate or a fixed number of sample trays. Auto populate will use sample trays as required for the run.
Reset positions	Choose this function after manually repositioning solutions to restore all solutions in the microplates to the positions determined by the current positioning settings. Solutions imported with positioning information are not affected.
Prevent auto-rearrange	Choose this function to prevent automatic rearrangement of positions where possible when manual changes are made. Automatic rearrangement may be necessary if solutions are manually repositioned to a different tray or to already occupied wells.
Keep concentration series together...	Check this option to position samples within a concentration series in the same microplate as far as possible.

Setting	Description
When a plate is full...	Choose whether to continue with the corresponding plate (left or right) on the next tray or to continue with the next plate on the same tray.

Solutions may be grouped together so that the settings apply to all solutions in the group. To split a group, drag one of the commands from a group to the empty placeholder at the bottom of the group list. To combine solutions in a group, drag the solutions together. The following settings apply separately to groups:

Setting	Description
Pooling	Check this option to allow multiple injections to be taken from the same microplate well. Note: <i>Pooling is only relevant for solutions that are used repeatedly.</i>
	Select whether solution placement will start at the top/bottom and left/right of the tray. Note: <i>Channels are always mapped from left to right within the microplate.</i>
	Use these icons to move positioning groups in the group list. Solutions are positioned in the microplate in order from top to bottom of the list. Changing the order in the list will affect the positioning.

Moving solutions manually

Default microplate positions are assigned by the software or by imported positioning information.

To change the positions, drag a solution to a new position in the graphical microplate representation.

Alternatively, follow the steps below:

Step	Action
1	Select Show more columns to expand the sample table and show the Position column.

Step	Action
2	Click on the first position in the cycle you want to reposition. Choose whether the target position is the reagent bottle or a specified tray. To reposition solutions in the reagent bottle, all solutions affected must have identical parameters.
3	For target positions on a tray, choose the position in the blank microplate illustration that appears.

All samples in the affected cycle (a complete row in a 96-well microplate or every second sample in a 384-well plate) will be moved together. Solutions cannot be moved in such a way that would require sample tray change within a cycle (for example, analyte solutions and regeneration solutions for the same cycles cannot be on different tray).

Note: For 96-well plates, the entire row is moved regardless of which position within a row you select as source or destination for the move. With 384-well plates, however, take care when selecting source and destination positions, since adjacent positions in a row represent different cycles.

Existing positions are rearranged according to **Positioning settings** when manual changes are made, unless **Prevent auto-rearrange** is active. Positions that have been imported or manually moved are marked with a heavy black border in the microplate illustration and are protected from auto-rearrangement.


Using the reagent bottle

To place a solution in the reagent bottle, drag the solution from the microplate to the bottle. All microplate positions with the same solution will be moved, regardless of the **Pooling** setting in **Positioning settings**. You cannot move solutions to the reagent bottle if the same microplate row contains different solutions in different channels.

Solutions can also be imported directly to the reagent bottle (see [Importing variable values, on page 98](#)).

The minimum volume in the reagent bottle is 200 mL.

Note: If less than 8 channels are used, solution from the reagent bottle will be injected over the unused channels even if the corresponding microplate positions are identified as **Buffer**.

Click  **Reset bottle solution...** on the reagent bottle position to restore solution from the reagent bottle to the default positions in the microplates.

7.3.8 Cycle overview

Introduction



The **Cycle overview** step provides an overview of the cycle order and the estimated run time for the run. Use this information to check that the run is set up as intended, in particular for steps that are repeated in the context of other steps.

Note: *The estimated time for the run shown in **Cycle overview** does not include preparation steps such as temperature change, and may differ from the time shown when the method is added to the activity queue.*

You cannot make any changes to the cycle order or parameter values in this step.

By default, the list shows only the first microplate position of every cycle.

Method Builder

1. Method definition 2. Variables and positioning 3. Cycle overview 4. Plate layout [Send to queue](#)  

Estimated run time **2 h 17 min**

Cycle	Step name	Capture 1 Position	Solution	Analyte 1 Position	Solution	
1	Startup	Plate 1 A1	Ligand	Plate 2 A1	Buffer	<input type="checkbox"/>
2	Startup	Plate 1 A1	Ligand	Plate 2 A1	Buffer	<input type="checkbox"/>
3	Startup	Plate 1 A1	Ligand	Plate 2 A1	Buffer	<input type="checkbox"/>
4	Controls	Plate 1 A1	Ligand	Plate 1 Aa	Control sample	<input checked="" type="checkbox"/>
5	Analysis	Plate 1 A1	Ligand	Plate 2 A3	Sample 1	<input type="checkbox"/>
6	Analysis	Plate 1 A1	Ligand	Plate 2 A4	Sample 9	<input type="checkbox"/>
7	Analysis	Plate 1 A1	Ligand	Plate 2 A5	Sample 17	<input type="checkbox"/>
8	Controls	Plate 1 A2	Ligand	Plate 1 A5	Control sample	<input checked="" type="checkbox"/>
9	Analysis	Plate 3 A1	Ligand	Plate 4 A1	Sample 25	<input type="checkbox"/>
10	Analysis	Plate 3 A1	Ligand	Plate 4 A2	Sample 33	<input type="checkbox"/>
11	Analysis	Plate 3 A1	Ligand	Plate 4 A3	Sample 41	<input type="checkbox"/>
12	Controls	Plate 1 A2	Ligand	Plate 1 A6	Control sample	<input checked="" type="checkbox"/>
13	Analysis	Plate 3 A1	Ligand	Plate 4 A4	Sample 49	<input type="checkbox"/>
14	Analysis	Plate 3 A1	Ligand	Plate 4 A5	Sample 57	<input type="checkbox"/>
15	Analysis	Plate 1 A2	Ligand	Plate 2 A6	Sample 65	<input type="checkbox"/>
16	Controls	Plate 1 A2	Ligand	Plate 1 A7	Control sample	<input checked="" type="checkbox"/>

Settings

Use the toolbar at the right of the workspace to customize the information that is displayed.

Icon

Description

Allows selection of which columns are displayed and in what order.

- Columns are grouped by command. Use the symbol at the left of the command name to expand or collapse the list of individual columns.
- Select a command or column and use the arrows to move the selection between **Available columns** and **Selected columns** and/or to reposition the selection in the display.

Expands the display to show information for all channels.

		Capture 1		Analyte 1	
Cycle	Step name	Position	Solution	Position	Solution
1	Startup	Plate 1 A1	Ligand	<input type="checkbox"/>	Plate 2 A1
		Plate 1 B1	Ligand	<input type="checkbox"/>	Plate 2 B1
		Plate 1 C1	Ligand	<input type="checkbox"/>	Plate 2 C1
		Plate 1 D1	Ligand	<input type="checkbox"/>	Plate 2 D1
		Plate 1 E1	Ligand	<input type="checkbox"/>	Plate 2 E1
		Plate 1 F1	Ligand	<input type="checkbox"/>	Plate 2 F1
		Plate 1 G1	Ligand	<input type="checkbox"/>	Plate 2 G1
		Plate 1 H1	Ligand	<input type="checkbox"/>	Plate 2 H1
2	Startup	Plate 1 A1	Ligand	<input type="checkbox"/>	Plate 2 A1
		Plate 1 B1	Ligand	<input type="checkbox"/>	Plate 2 B1
		Plate 1 C1	Ligand	<input type="checkbox"/>	Plate 2 C1
		Plate 1 D1	Ligand	<input type="checkbox"/>	Plate 2 D1

7.3.9 Plate layout

Description

Plate 1

Leftmost position	Volume μ l	A Channel 1	B Channel 2	C Channel 3	D Channel 4	E Channel 5	F Channel 6	G Channel 7	H Channel 8
A6	118	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
A5	118	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
A4	118	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
A3	118	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
A2	118	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
A1	118	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8

Plate 2

Leftmost position	Volume μ l	A Channel 1	B Channel 2	C Channel 3	D Channel 4	E Channel 5	F Channel 6	G Channel 7	H Channel 8
A6	126	Regeneration solution	Regeneration solution	Regeneration solution	Regeneration solution	Regeneration solution	Regeneration solution	Regeneration solution	Regeneration solution
A5	137	Regeneration solution	Regeneration solution	Regeneration solution	Regeneration solution	Regeneration solution	Regeneration solution	Regeneration solution	Regeneration solution
A4	114	Buffer	Buffer	Buffer	Buffer	Buffer	Buffer	Buffer	Buffer
A3	223	Buffer	Buffer	Buffer	Buffer	Buffer	Buffer	Buffer	Buffer

Plate layout provides a summary of the sample positioning in microplates and required solution volumes, as an aid when preparing samples. Select whether you want the workspace to display trays with microplates (select **Trays**), or a summary of the total volumes that need to be prepared for each solution (select **Volume summary**).

For trays, you can sort the table rows in ascending or descending order of position. The plate layout display cannot be edited in any other respect. Make any required adjustments to the plate layout in the **Variables and positioning** step (see [Section 7.3.6 Entering variables and managing cycles, on page 96](#)).

Note: The graphical representation of the microplates is not affected by the table sort order.

Volumes listed in the plate layout are recommended minimum values. Extra volumes for pipetting are not included in the volume summary.

Click  **Print** to print a copy of the current workspace.

8 Runs workspace

About this chapter

This chapter describes the **Runs** workspace, where the results of ongoing and stored runs are displayed.

In this chapter

Section		See page
8.1	Opening a run	111
8.2	Display information	112
8.3	Sensorgram view settings	115

8.1 Opening a run

Procedure

Ongoing runs are displayed automatically in the **Runs** workspace (see [Section 5.4 Display during a run, on page 70](#)).

Finished runs are opened from the database. Follow the procedure below to open a finished run.



Step	Action
1	Select Open if the list of runs is not displayed.
2	Navigate to the required folder or use the Search function (see Searching for database objects, on page 16).
3	Double-click on the required run or choose the run and select Open at the bottom right of the workspace.

8.2 Display information

The **Runs** workspace displays two or three kinds of information for the selected run. Use the buttons at the top of the workspace to choose the information to display.

- **Results**
- **Sensorgram**
- **Run properties**

Results display

The **Results** display is only shown for immobilization runs, and certain tools such as **System check** that generate numerical and/or text results.

Immobilization 14/02/2017 16:56:28 **Results** Sensorgram Run properties

Immobilization result
Chip: CMS

Date	Channel	Flow Cell	Chemistry	Method	Ligand	Response Bound (RU)	Response Final (RU)	Molecular Weight
14/02/2017 16:56:43	1	2	Amine	Immobilization	Ligand 1	1067.5	16.8	
14/02/2017 16:56:43	2	2	Amine	Immobilization	Ligand 2	1067.3	16.9	
14/02/2017 16:56:43	3	2	Amine	Immobilization	Ligand 3	1067.7	16.6	
14/02/2017 16:56:43	4	2	Amine	Immobilization	Ligand 4	1064.2	16.7	
14/02/2017 16:56:43	5	2	Amine	Immobilization	Ligand 5	1064.2	17.1	
14/02/2017 16:56:43	6	2	Amine	Immobilization	Ligand 6	1095.7	16.4	
14/02/2017 16:56:43	7	2	Amine	Immobilization	Ligand 7	1077.6	16.5	
14/02/2017 16:56:43	8	2	Amine	Immobilization	Ligand 8	1063.7	16.9	

For immobilization runs, two response levels are reported. **Response Bound** is the difference in response level directly before and after the ligand injection. **Response Final** is the difference in response level before the activation injection and after the deactivation injection, and is used as the immobilization level in **Chip** details.

Sensorgram display

The sensorgram display shows the sensorgram details for one cycle at a time. Choose the cycle to display from the bar at the top of the panel.

Tip: Select a cycle in the cycle bar and then use the left and right arrow keys on the keyboard to browse rapidly through the cycles.

The sensorgram display may contain up to three subpanels, for **Sensorgrams**, **Cycle event log**, and **Report point table**. Use the buttons at the bottom of the **Settings** panel to control which subpanels are displayed.

Subpanel	Description
Sensorgrams	Displays the sensorgram curves as selected in the Settings panel at the left of the workspace.
Cycle event log	Displays a detailed list of instrument control events in the current cycle. Events are marked and identified on the sensorgram display.

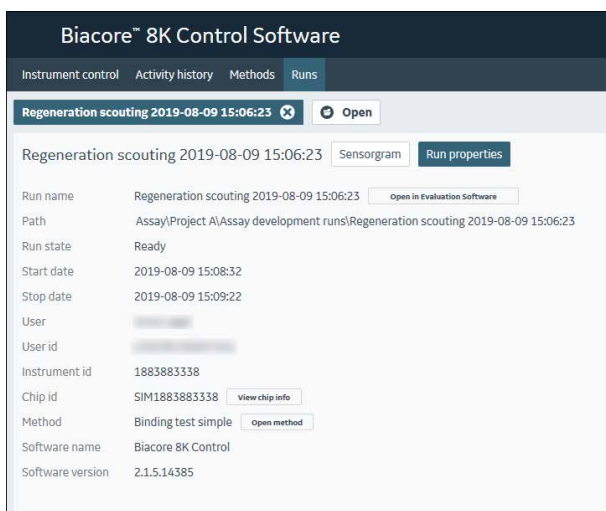
Subpanel	Description
Report point table	Displays the report point table for immobilization runs. This button is not available for analysis runs. Report points for analysis runs are created in the Evaluation Software.

To zoom in to a selected region of a sensorgram, drag around the area to be enlarged with the mouse. After zooming, double-click in the display to return to the previous zoom level.

Use the view settings to control the content of the **Sensorgrams** display. See [Section 8.3 Sensorgram view settings, on page 115](#) for details.

Run properties display

Run properties shows details of the run.



The following functions are available from **Run properties**:


Function	Description
Open in Evaluation Software	Opens the run in a new instance of the Evaluation Software. If this function is used for an ongoing run, data up to and including the most recently completed analysis cycle is opened in the Evaluation Software. Evaluation of data from an ongoing run cannot be saved or exported from the Evaluation Software.
View chip info	Displays sensor chip information in a separate window.

Function	Description
<i>Open method</i>	<p>Opens the method for the run in the Methods workspace.</p> <p>Note:</p> <p><i>This function opens the copy of the method stored in the result file, that was actually used when the run was executed. Any later changes that might have been made to the method in the database are not included.</i></p>

8.3 Sensorgram view settings

Introduction

The content and appearance of the sensorgram display is controlled in two ways:

- Use the **Settings** panel at the left of the workspace to control which sensorgrams are displayed.
- Use  **Chart settings** at the right of the workspace to control how the run data is displayed.

In addition, you can drag over an area of the sensorgram display to zoom in to that area.

Settings

Check the channels and sensorgrams you want to include in the display. All options are selected by default.

Chart display settings



Click  **Chart settings** at the right of the workspace to access the following options:

Chart display settings


☒ Sensorgram start at zero

☐ Zoom lock

☐ Show reference Line

Baseline

Show
☐ injections

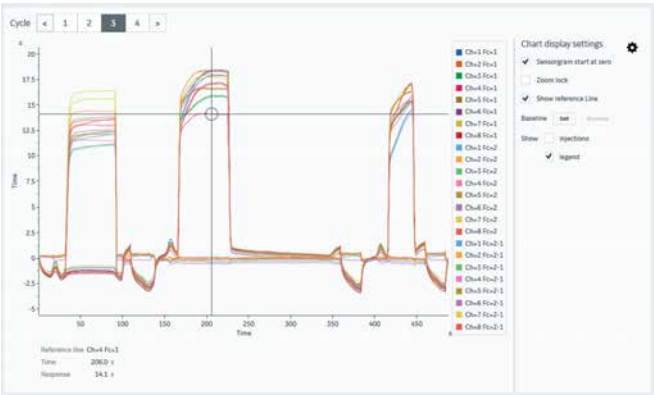
☒ legend

Option	Description
Sensorgram start at zero	Aligns all sensorgrams to start at zero response and time.
Zoom lock	Maintains the current zoom factor when cycle or sensorgram selection is changed. If this option is not checked, the display will zoom automatically according to the current display data when selections are changed.
Show reference line	Displays crosshairs indicating the coordinates of the currently selected point on a sensorgram. See Using the reference line, on page 116 for instructions on how to use the reference line.

Option	Description
Show injections	Highlights the duration of injections (including dissociation time) with a heavy black line.
Show legend	Displays a legend with sensorgram identification.

Using the reference line

When the reference line is activated, the position of the reference line is identified by sensorgram name, time and response values in the sensorgram panel.



Instructions for using the reference line are given in the table below.

Action	Instruction
Move the reference line	Drag the vertical reference line left or right. Alternatively, click on the horizontal reference line at a new time position.
Change the current sensorgram	Click on a different sensorgram. The time position of the reference line will not change.
Set a local baseline	Click Baseline → Set in Chart display settings . A local baseline will be set at the current position of the reference line. As the reference line is moved, the local baseline remains fixed and the value of Response from baseline is displayed. If the current sensorgram is changed, the local baseline is applied to the new current sensorgram.
Remove a local baseline	Click Baseline → Remove in Chart display settings .

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