

Starting the instrument from shutdown

1 Switch on the printer and the PC.

2 Switch on the instrument at the rear.

The lamps on the front panel light during initiation. When the initiation is ready, **Power** is lit, the **Temperature** lamp is lit or flashes, **Sensor chip** is not lit or flashes and **Run** is not lit

3 Start *Biacore X100 Control Software* from the Windows start menu.

4 In the login-dialog, enter your user name and password and click **OK**.



5 The software establishes connection with the instrument, which takes about 30 seconds.

6 Ensure that there is fresh buffer or water in the bottle on the left-hand tray and that both buffer tubes are inserted into the bottle.

7 Ensure that the waste bottle on the right-hand tray is empty and the waste tube is inserted in the bottle.

8 The system event log is displayed in the software and the startup procedure is recommended. Click **Run now** and follow the instructions on the screen. A previously used chip can be docked for the startup procedure

Once the startup procedure is finished, the instrument is automatically left in standby mode.

Preparing and loading buffer

1 Buffer preparation

Select buffer type depending on your specific experiments. For many protein interactions you may use HBS-EP+ buffer supplied by GE Healthcare. Use degassed deionized water when preparing buffers to prevent problems with air bubbles during

the run. If the instrument is equipped with the degasser provided with the Biacore X100 Plus Package, you do not need to degas the water. Always use buffer, fresh for the day and filtered through a 0.22 µm filter to remove particles. A volume of 200 ml is suitable for use during 24 hours.

2 Loading buffer

Prepare buffer in a bottle and place it on the left-hand tray and insert the two buffer tubes. The flow system is filled with buffer automatically when a chip is docked.

3 Changing buffer

If you need to change buffer when the instrument is in standby, stop the standby (**Tools:Stop Standby**) and change the buffer bottle. When both buffer tubes are inserted in the new buffer, select **Tools:Prime** to fill the system with the new buffer.

Inserting, removing or changing the sensor chip

Sensor chip and detection

The sensor chip is a gold-coated glass slide mounted on a supporting frame and enclosed in a protective cassette.

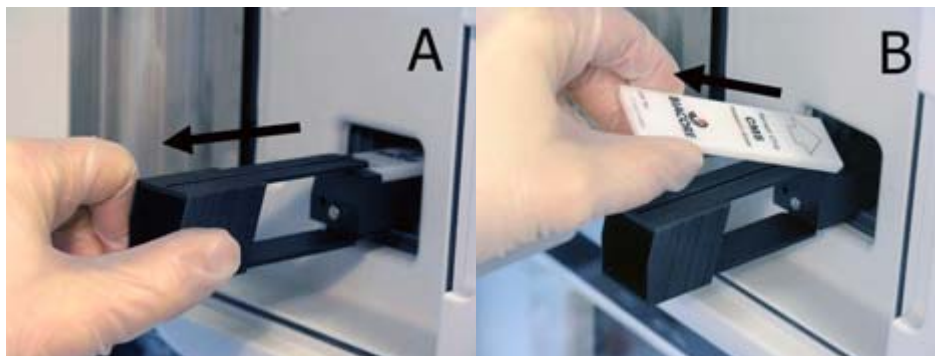
Sensor chip types

Biacore X100 should only be used with sensor chips from GE Healthcare. Series S sensor chips cannot be used with Biacore X100.

There are several different types of sensor chips appropriate for different molecules with different attachment chemistries.

Inserting a sensor chip

- 1 Open the upper front door of the instrument.
- 2 Click the undock chip icon, or select **Undock Chip** from the **Tools** menu. When undocking is completed, the **Dock Chip** dialog is displayed and the sensor chip lamp on the front flashes.
- 3 Pull out the chip slide, see Figure A.
- 4 If required, remove the old chip, see Figure B.
- 5 Insert the chip with the text on the upper side, see Figure C.



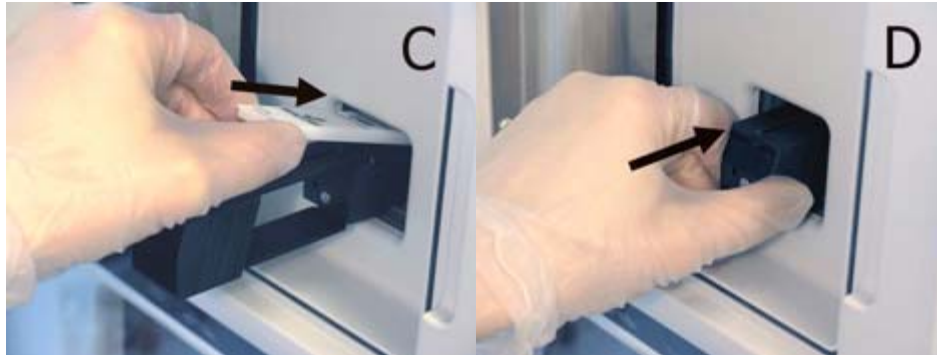


Figure . Removing and inserting a sensor chip, step A-D.

6 Push the chip slide all the way in, see Figure D.

7 Close the front door and check that the buffer tubes are placed in a bottle of buffer.

8 Fill in the information in the **Dock Chip** dialog and click **Dock Chip**. The chip is docked and the standby flow of buffer over the chip starts automatically.

Preparing and loading samples and reagents

Samples and reagents

Different reagents are used depending on the type of run to be performed. The requirements for your assay are displayed in the rack position list in the Control Software when the assay is set up. The list can be printed. If dilution of samples is needed, dilute in degassed running buffer.

The volumes specified in the rack position list are minimum volumes with due consideration for dead volumes.

1 Prepare the samples and reagents in 1.5 ml conical vials according to the volumes and concentrations in the rack position list and cap them to avoid evaporation during the run. Make sure there are no visible air bubbles in the tubes.

2 Fill the 4 ml vial with distilled water. Always use fresh water filtered through a 0.22 μm filter to remove particles. Do not cap the 4 ml vial! The water is used for cleaning the needle during the run and during standby.

Loading the rack

1 Click the **Load Samples** button in the Rack Positions dialog. You can also load the rack using the **Load Samples** icon in the toolbar if you do not have an application wizard open.

2 Wait until the **Rack locked** indicator below the rack compartment is switched off.

3 Lift out the rack and place the sample and reagent vials and the water vial according to the rack position list.

Note: Load samples in the rack outside the instrument.

4 Insert the rack in the sample compartment and make sure it sits properly on the rack base.

5 Click **OK** in the **Load Samples** dialog.

Creating a workflow

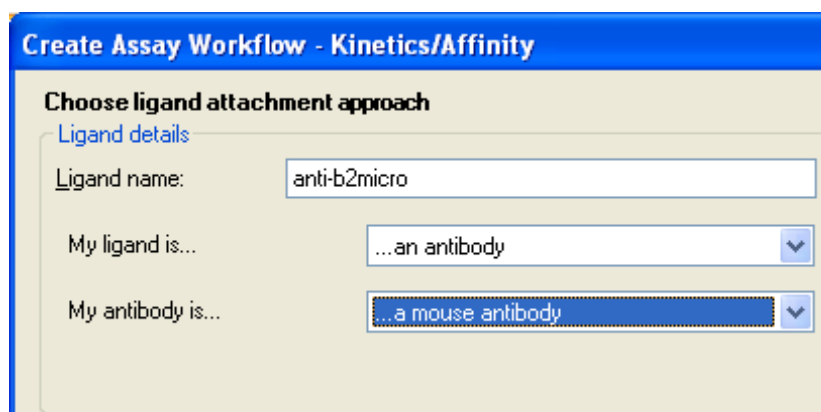
1 Click the relevant button to create a workflow for *Kinetics/Affinity* or for

Binding Analysis.



In the examples below a *Kinetics/Affinity* workflow is shown. The dialog sequence for a *Binding Analysis* workflow is similar.

2 In the *Create Assay Workflow* dialog that opens, enter ligand name and select the



type of ligand.

Depending on your input, you will get recommendations on what chip to use, suitable attachment approaches and what procedures to include in your workflow.

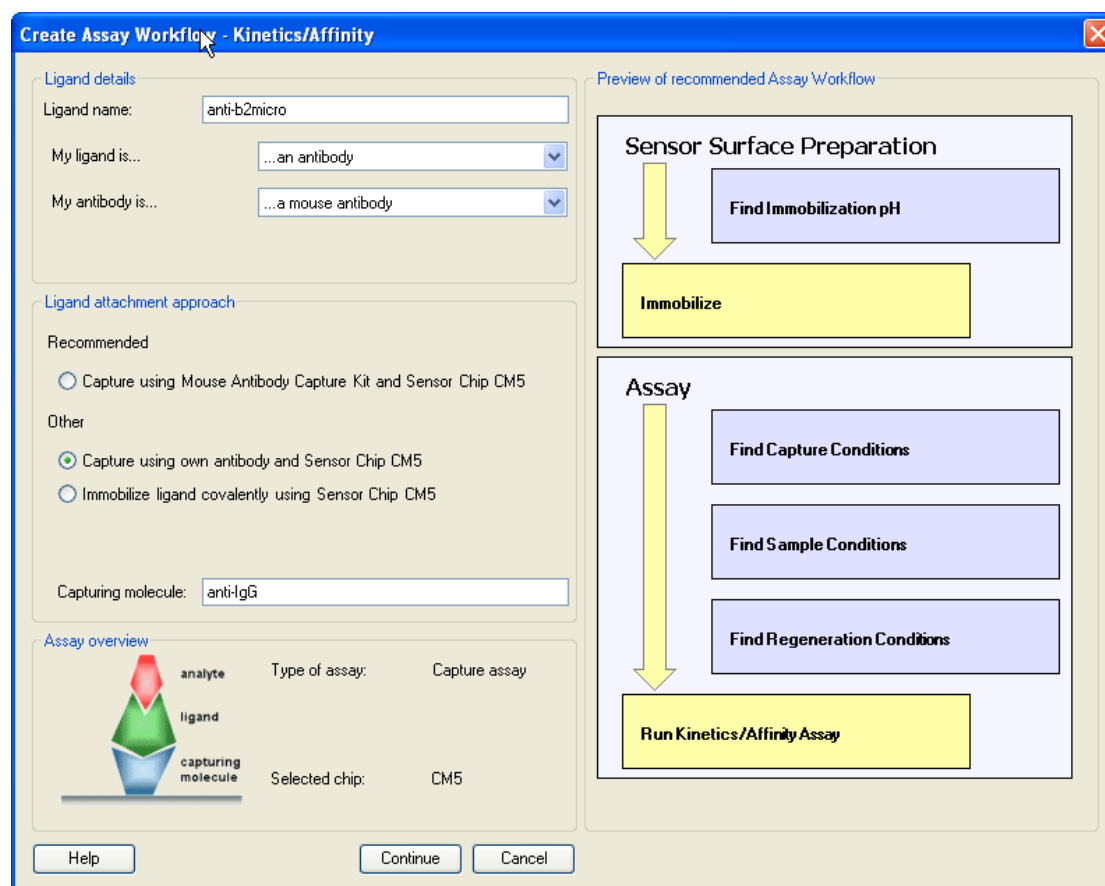


Figure . Example of the creation of a *Kinetics/Affinity* workflow with a preview of the recommended assay workflow to the right in the dialog.

3 Select the ligand attachment approach, and if required enter a capturing molecule. A preview of the recommended assay workflow for the current selection is displayed to the right.

Developing and running assays

Creating assay workflows

4 Click Continue and save the workflow in one of your folders.

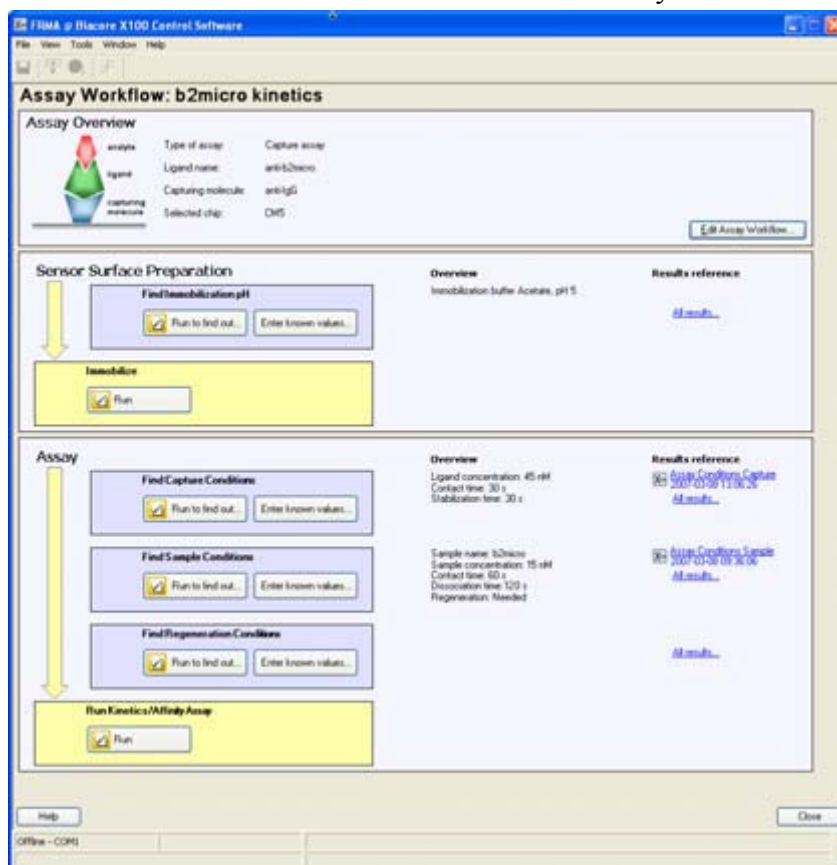


Figure . Example of a saved Kinetics/Affinity workflow with required steps in yellow boxes and optional assay development steps in blue boxes.

Runs are started from the buttons in the boxes

5 Continue by running the desired steps from top to bottom (required steps are yellow, optional steps are blue). You can find detailed support for each step in the Support Navigator.

Editing a workflow

Before any runs have been started in a workflow, you can edit ligand name, ligand type and ligand attachment approach by clicking the **Edit Assay Workflow** button in the workflow. If any runs have been performed in the workflow, you can view the workflow definition but you cannot edit the settings.

Automatic display of result data

The results from the assay run (the last yellow box in a workflow) are displayed automatically in the Evaluation Software. All other results are automatically displayed

in the Control Software when the run is finished.

Other ways to run assays on Biacore X100

Apart from workflow assays you can also run assays using the wizards outside a workflow or using manual runs. See the following sections for more details.

Using wizards

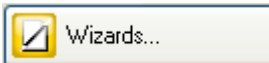
Wizards provide direct entry to specific types of run. Wizards support the same types of experiment as workflows but without the integrated assay overview and automatic transfer of settings to subsequent steps. If the Plus Package is installed, wizards are also available for concentration analysis and customdesigned assays. Settings for a wizard run can be saved in a wizard template.

Wizard	Corresponding workflow step
Immobilization pH Scouting	Find immobilization pH
Immobilization	Immobilization
Assay Conditions Capture	Find capture conditions
Assay Conditions Sample	Find sample conditions
Regeneration Scouting	Find regeneration conditions
Concentration Analysis Using calibration Calibration-free	–
Kinetics/Affinity	Assay step, kinetics/affinity workflow
Binding Analysis	Assay step, binding analysis workflow
Custom Assay Wizard	–

Developing and running assays

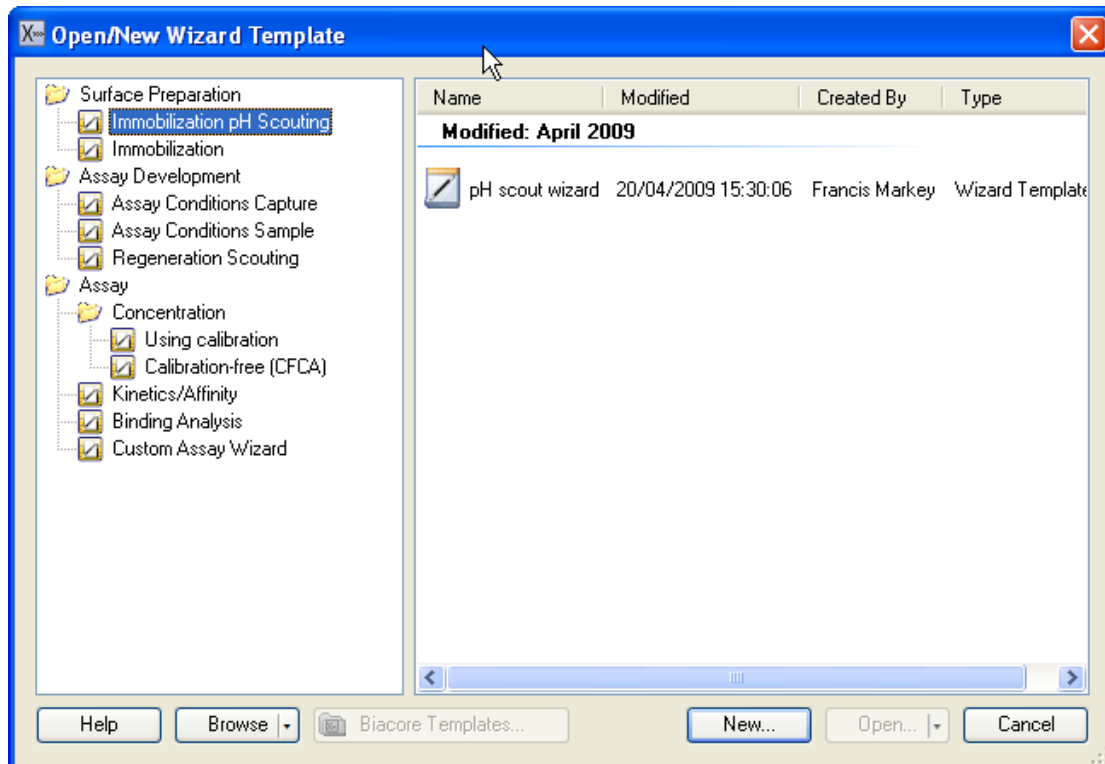
Other ways to run assays on Biacore X100

1 Click  >> to expand the choices.

2 To set up a new wizard template or start a wizard run, click .

3 Double-click on a wizard type in the left panel, or select the wizard type and click **New**, to create a new wizard template of that type. The Concentration Analysis wizards and the Custom Assay Wizard are only available if you have the optional Biacore X100 Plus Package installed.

To open an existing wizard template from the right panel in the dialog, double-click on the template or select the template and click **Open**. Only templates corresponding to the currently selected wizard type are shown. You may also click **Browse** to open templates stored as files outside the Biacore X100 database. For the Custom Assay Wizard, click **Biacore Templates** to access templates provided with the software installation.



4 Continue with the setup and the actual run. Detailed help for each step is provided in the Support Navigator to the right all through the process.

Automatic display of result data


Result data from surface preparation wizard runs and assay development wizard runs are automatically displayed in the Control Software when the run is finished. All other wizard results are automatically displayed in the Evaluation Software when the run is finished.

Manual runs

There is an option to run the instrument with manual commands to control the run interactively. All settings can be changed during a run. Commands are placed in a queue if the instrument is busy when a command is issued: queued commands that have not yet been started can be edited or deleted from the queue. Use this option for quick interactive experiments such as one-shot yes/no binding tests.

The results from a manual run are saved as a Results item, and can be evaluated in the Evaluation Software. The results cannot however be evaluated with the tools for concentration, kinetics/affinity and binding analysis. It is only possible to view sensorgrams and create plots.

1 Click  >> to expand the choices.

2 Click  to open the **Manual Run** dialog. The option is only available on the system controller. Detailed help is provided in the Support Navigator.

Specifications

Automation	Maximum 15 samples, 24h unattended operation
Molecular weight detection	Down to 100 Da in various sample environments
Required sample volume	Injection volume + 20-30 μ l (application dependent)
Sample/reagent capacity	15 sample/reagent vials, volume 1.5 ml 1 water vial, volume 4 ml
Analysis time per sample	Typically 5-15 min
Detection limit	Typically 0.1 nM for >10 000 Da analytes. Typically 1 nM for <10 000 Da analytes.
Typical range for kinetics: Association rate constant (k_a):	10^3 – 10^7 $M^{-1}s^{-1}$ (for typical protein-protein interactions)
Dissociation rate constant (k_d):	10^{-5} – $0.1s^{-1}$
Number of flow cells	2
Flow cell height	Approximately 0.05 mm
Flow cell volume	Approximately 0.06 μ l

Sample rack

Capacity	15 sample vials + 1 water vial
Sample/reagent vials	1.5 ml, conical bottom, with cap
Water vial	4 ml, flat bottom, no cap

Liquid containers provided

Running buffer	500 ml, screw cap
Waste	500 ml, screw cap