NGC™ Chromatography Systems and ChromLab™ Software

User Guide

Version 3.3

BIO-RAD

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Bio-Rad Technical Support Department

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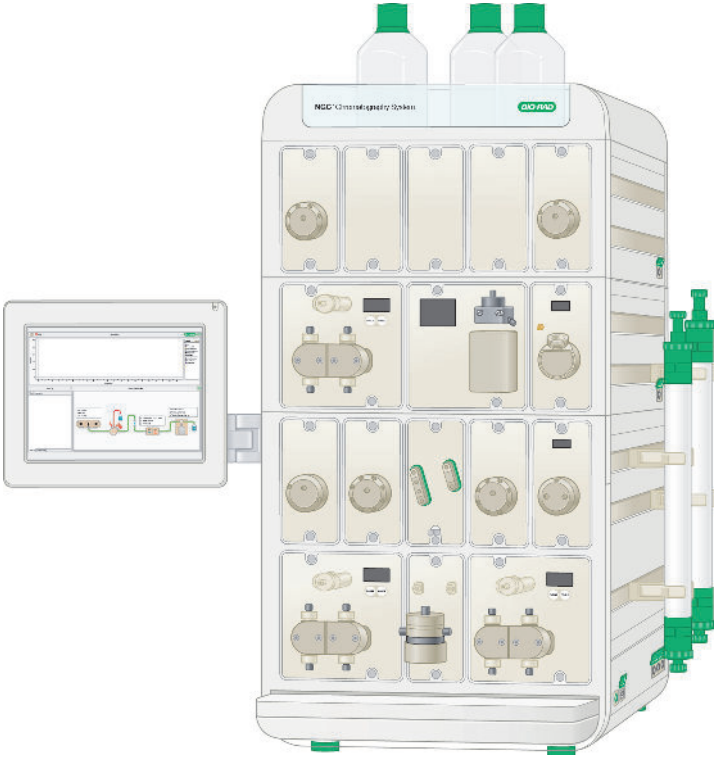
1 Introduction

NGC™ chromatography systems provide a general purpose purification platform for purifying all forms of biomolecules using a combination of chromatography techniques. The systems are also useful for developing and optimizing purification protocols. The systems can provide highly purified proteins, peptides, nucleic acids, monoclonal antibodies, and other small molecules.



ChromLab™ software enables you to set up and control an NGC instrument, run protein separations and other operations manually, program methods to automate purification runs, evaluate the results, and generate and print experiment reports. This user guide explains how to perform all these tasks.

Main NGC Features

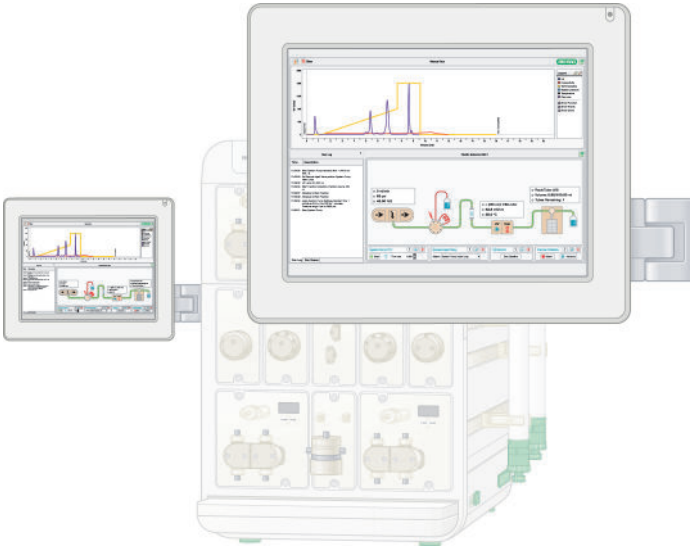
NGC chromatography systems enable you to do the following:

- Easily create purification and maintenance protocols from predefined method templates and protocol phases
- Automate multicolumn purification processes using preprogrammed templates and multiple column switching valves
- Automate multiple sample injections using either the sample inlet valve and the sample pump or the C-96 autosampler
- Expand sample monitoring using the signal import module (SIM) to export digital signals to and import digital signals from external detectors
- Collect large-volume fractions using multiple outlet valves while also collecting small-volume fractions using the BioFrac™ fraction collector
- Automatically prepare buffers using preprogrammed buffer blending protocols
- Analyze purification results through 1-click peak integration, determine protein concentration and calculate column performance
- Automate purification protocol optimization using the scouting wizard
- Easily locate fractions containing peaks of interest and view the protein concentration within each fraction
- Extend the preconfigured systems with additional valves for buffers, samples, and columns

- Organize the location of the modules to optimize separation performance based on method scale and complexity, and to minimize the system swept volume
- Minimize errors when connecting tubing using the Point-to-Plumb™ feature in ChromLab software

NGC Chromatography Systems

All NGC chromatography systems include ChromLab software and the NGC touch screen.



NGC chromatography systems are available in several combinations. Each system is equipped with either two 10 ml system pumps (the 10 series) or two 100 ml system pumps (the 100 series).

The NGC Quest™ chromatography system includes the following:

- Two system pumps
- Mixer
- Sample injection valve
- Conductivity monitor with either a single-wavelength UV detector or a multi-wavelength UV/Vis detector (available on the Plus systems)

The NGC Scout™ chromatography system includes the following:

- All modules on the Quest system
- pH detector valve
- Buffer blending valve

The NGC Discover™ chromatography system includes the following:

- All modules on the Scout system
- Column switching valve
- Two buffer inlet valves
- Sample pump

The NGC Discover Pro chromatography system includes the following:

- All modules on the Discover system
- Fourth expansion tier
- Sample inlet valve
- Outlet valve

Finding Out More

After you install NGC documentation from the NGC Chromatography Systems Documentation DVD, you can access installed NGC guides and tutorials on the Help menu in any ChromLab view.

More information about the NGC chromatography systems and ChromLab software is available from the following sources.

- The NGC Chromatography Systems and ChromLab Software Installation Guide is available on your NGC Chromatography Systems Documentation DVD as a .pdf file. This guide explains how to set up your environment, set up and install the NGC instrument in the lab, install ChromLab software, and connect ChromLab to the NGC system.
- The NGC Chromatography Systems and ChromLab Software Instrument Guide is available on your NGC Chromatography Systems Documentation DVD as a .pdf file. This illustrated guide details the modules that make up the NGC instrument and includes troubleshooting and maintenance information.
- For ChromLab Help, click the question mark in the upper right corner in dialog boxes to access relevant information. Screen-level help is also available on the Help menu.
- NGC video tutorials are available on the NGC Chromatography Systems Documentation DVD as .mp4 files.

  Tip: You can click the Bio-Rad logo in the upper left corner of any ChromLab window to launch the Bio-Rad website.

2 The Workspace

ChromLab™ software provides an intuitive interface for developing chromatography methods, operating an NGC™ instrument, and analyzing data from chromatography runs.

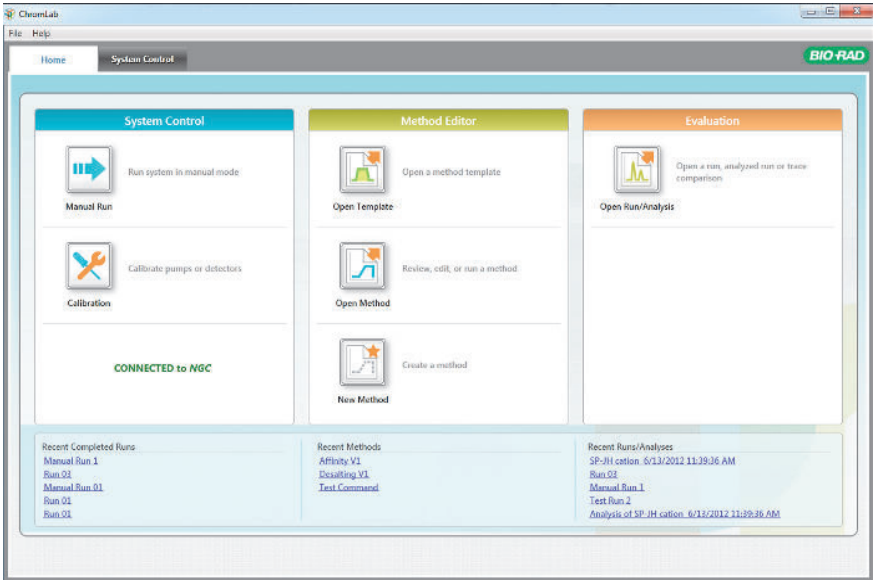
ChromLab software presents four primary workspaces.

- The Home window
- The System Control window
- The Method Editor window
- The Evaluation window

Each workspace is shown and described in this chapter. The NGC instrument touch screen is also described.

The Home Window

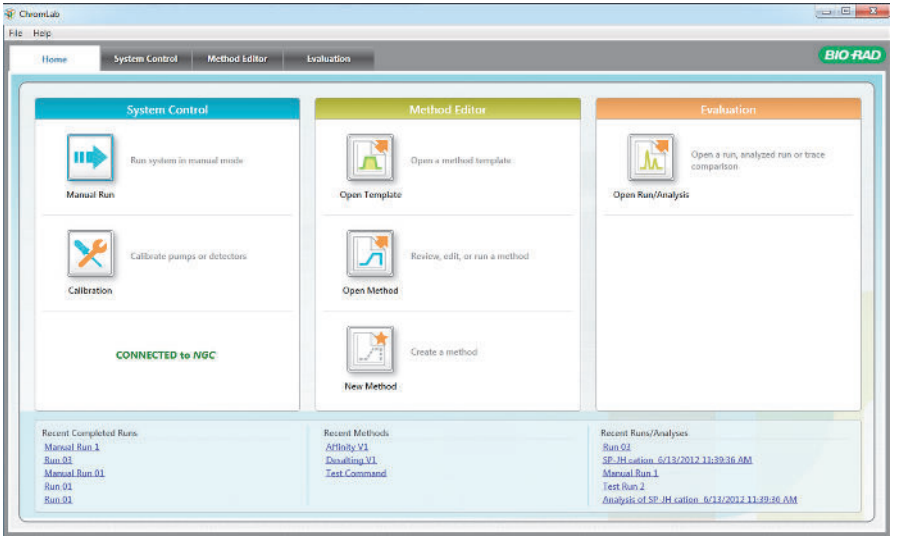
ChromLab software opens with the Home window, which displays three panes and the System Control tab.



The three panes provide quick access to the system control, method editor, and evaluation workspaces. The status of connection to the NGC instrument is also displayed. Links to recently completed runs, recently created methods, and recently accessed or analyzed runs and analyses appear listed at the bottom of the window.

Tip: Clicking the Bio-Rad logo in the upper right corner of any ChromLab window launches the Bio-Rad website.

Method Editor and Evaluation tabs become visible in the Home window when you select tasks in the Method Editor and Evaluation panes.



File Menu Commands

Connect to System — opens a dialog box that enables you to choose an NGC chromatography system to connect to. ChromLab detects the NGC systems available on the same subnetwork or those systems that are directly connected to the computer. Their system name, network name, and IP address appear in the table. To connect to a system, you can:

- Select a name in the list of detected systems and click Connect.

  Note: If your system does not appear in the list, click Detect. ChromLab searches the network for available NGC systems and refreshes the list.

- Select the appropriate radio button, enter the system's name or IP address, and click Connect.

  Tip: To obtain the system's name and IP address select System Information on the instrument touch screen dropdown menu.

Disconnect System — starting ChromLab connects you to the NGC system. This command enables you to disconnect ChromLab software from the system so you can work offline.

Manual Run — opens the System Control window in manual mode so you can perform a manual run or set up your system manually.

Calibrate — opens the Calibration dialog box, which displays instructions and options for selecting a module and calibrating it. See Calibrations on page 66 for details.

Open Template — opens the Template dialog box in which you can select a method template from template folders organized by technique.

Open Method — opens the Method dialog box in which you can select from a list of your saved methods.

New Method — opens the Method Editor window in which you can create a method using standard method phases and steps.

Import — displays links from which you can import the following:

- **NGC File** — opens a dialog box in which you can import a method, a method with runs, or a run exported from ChromLab software running on another NGC system. See Importing an NGC Method or Run on page 263 for more information.
- **Unicorn Data** — opens a dialog box in which you can import a Unicorn data file into the NGC database. See Importing Unicorn Data Files on page 265 for more information.
- **DuoFlow Data** — opens a dialog box in which you can import a BioLogic DuoFlow™ data file into the NGC database. See Importing BioLogic DuoFlow Data Files on page 267 for more information.

Export — displays links from which you can export the following:

- **Methods/Method Runs** — opens a dialog box in which you can export both single or multiple methods and single or multiple methods with associated runs.
- **Runs** — opens a dialog box in which you can export single or multiple runs.

See Exporting Data as an NGC File on page 269 for more information.

Open Run/Analysis — opens a dialog box in which you can select a run or analysis to view or analyze.

Preferences — opens dialog boxes in which you can do the following:

- Select pressure units for all system and software pressure values. This is a global setting.
- Set up an SMTP server to receive email messages about system notifications from the ChromLab computer.

Exit — closes ChromLab.

Help Menu Commands

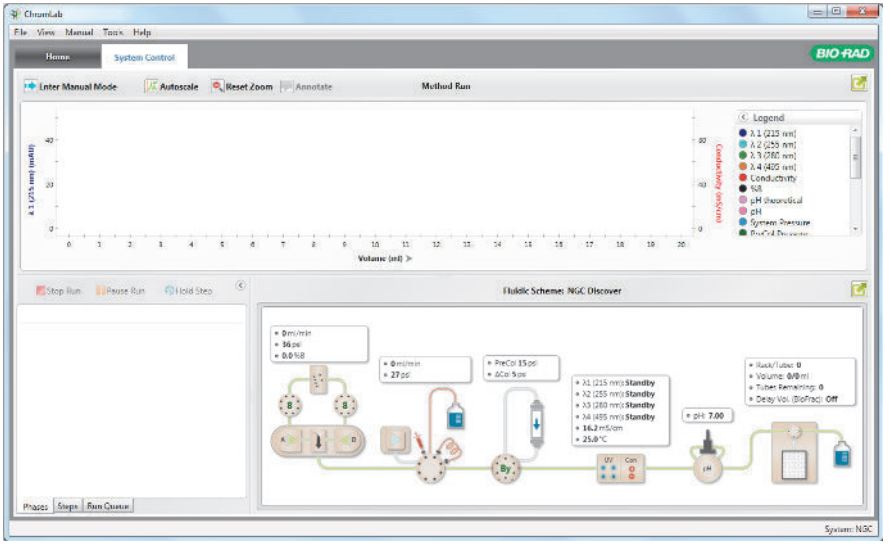
Help — displays screen-level help topics and links to installed manuals.

Export Diagnostic Logs — opens the Export Diagnostic Logs dialog box in which you can export all critical information that Bio-Rad Technical Support requires to diagnose issues. The log files and data are zipped and saved to a location that you choose. See Exporting Diagnostic Logs on page 275 for more information.

About — displays ChromLab copyright and version information.

The System Control Window

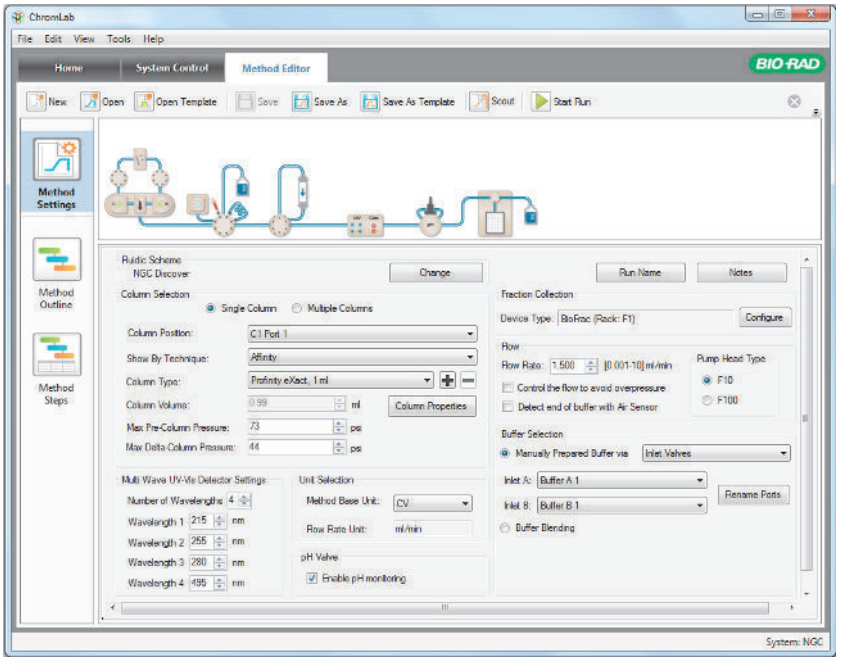
The System Control window enables you to run the instrument manually, monitor method runs while they are running, select fluidic schemes, calibrate pumps and detectors, and verify the accuracy of instrument plumbing. This window displays a chromatogram during a run. A fluidic scheme graphically depicts the flow path of all the modules on the system. A status panel appears above each module displaying its real-time status. In manual mode, clicking a module displays its controls and detailed settings. The Run Log documents each action that occurs during a run. The Run Queue lists all runs ready to be started.



System Control functionality is detailed in Chapter 3, System Control.

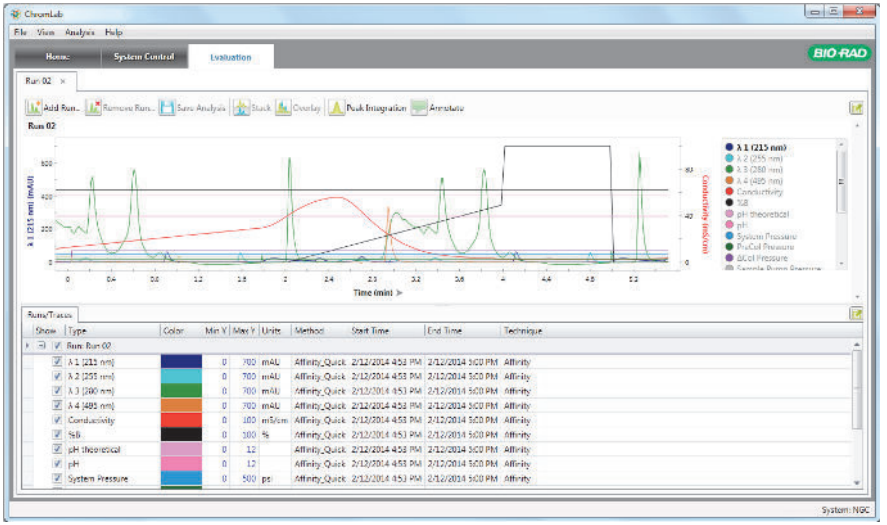
The Method Editor Window

The Method Editor window enables you to open, create, review, edit, and run a method. You can also open and edit a method template to create a new template. Method Editor functionality is detailed in Chapter 5, Method Editor. See also Chapter 6, Creating a Method.



The Evaluation Window

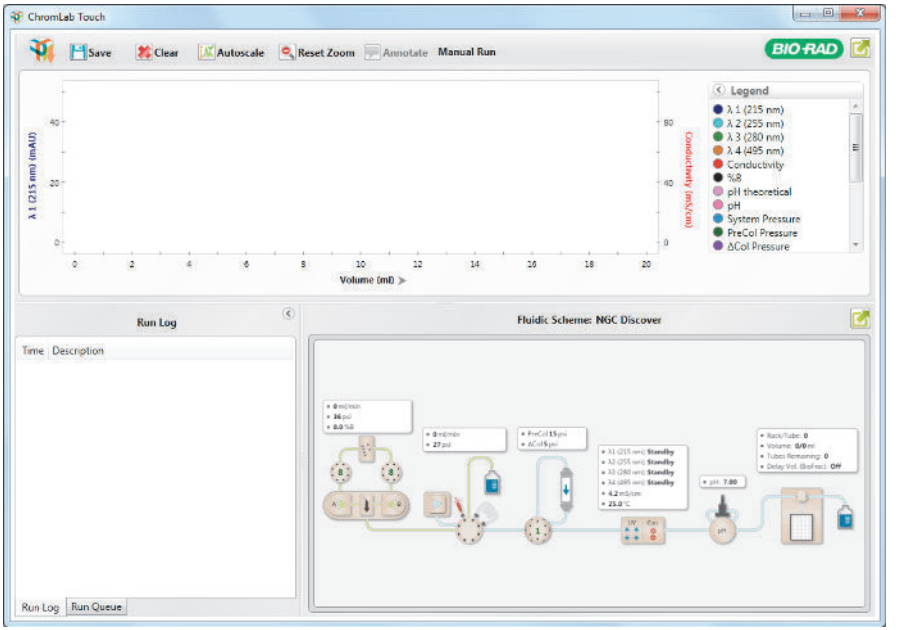
The Evaluation window enables you to view and compare run data, perform peak integration, and save run data as analyses. Evaluation functionality is detailed in Chapter 7, Evaluating Results.



Instrument Control Touch Screen

In addition to ChromLab software running on a computer, the instrument is equipped with a touch screen that accesses system control functionality. You can use this touch screen to run, control, and monitor a run independent of ChromLab. See System Control on page 31 for more information.

Tip: When the NGC system has been inactive for two hours the LED display screens on the instrument turn off, the touch screen dims, and a dialog box appears on the touch screen informing you that the system is in standby mode. You can take the system out of standby mode by touching OK in the dialog box, starting the system pumps by initiating a manual or method run, or clicking on a module in the fluidic scheme that has an LED display.



Touch Screen Menu Commands

Calibrate — opens the Calibration dialog box, which displays instructions and settings for selecting a module and calibrating it. See Calibrating a Module on page 66 for details.

Point-to-Plumb — starts the Point-to-Plumb™ feature and simultaneously turns off instrument LED lights so you can visually verify or change instrument plumbing. Displays the current fluidic scheme. See Verifying Plumbing with the Point-to-Plumb Feature on page 69 for details.

Change Fluidic Scheme — opens the Fluidic Scheme Selector dialog box in which you can edit the fluidic scheme or choose another one. See Fluidic Scheme Configurations on page 53 for details.

Map Fluidic Scheme — opens the Fluidic Scheme Mapping dialog box in which you can map devices on your instrument to their position in the fluidic scheme. See Fluidic Scheme Mapping on page 62 for details.

System Settings — opens the System Settings dialog box in which you can customize system settings. See System Settings on page 71 for more information about customizing your system.

System Information — opens the System Information dialog box, which lists the serial number and other general information about the NGC device as well as information about the system components, processes, and UV and UV/Vis detectors.

Service — for Bio-Rad technical service staff use only. Do not select this command.

Help — displays detailed information about touch screen menu commands.

About — displays ChromLab version and copyright information.

Shut Down — shuts down the NGC system, including the connected computer.

Touch Screen Toolbar Commands

Save — saves in a data file the steps executed during a manual run.

Clear — deletes manual run data from the touch screen display.

Autoscale — automatically scales the chromatogram's primary y-axis to the tallest peak height during the run. Autoscaling is enabled by default.

Reset Zoom — resets the view to show the full chromatogram.

Annotate — adds a note to the chromatogram at points on the x-axis during a run or after the run completes.

3 System Control

The ChromLab™ System Control window is the main interface to the NGC™ chromatography system instrument. This interface also appears on the instrument touch screen. System Control settings enable you to perform a manual run, monitor and control a method run, verify the device plumbing with the Point-to-Plumb™ feature, control and calibrate the system, and map two or more valves of the same type on your instrument to their position in the fluidic scheme.

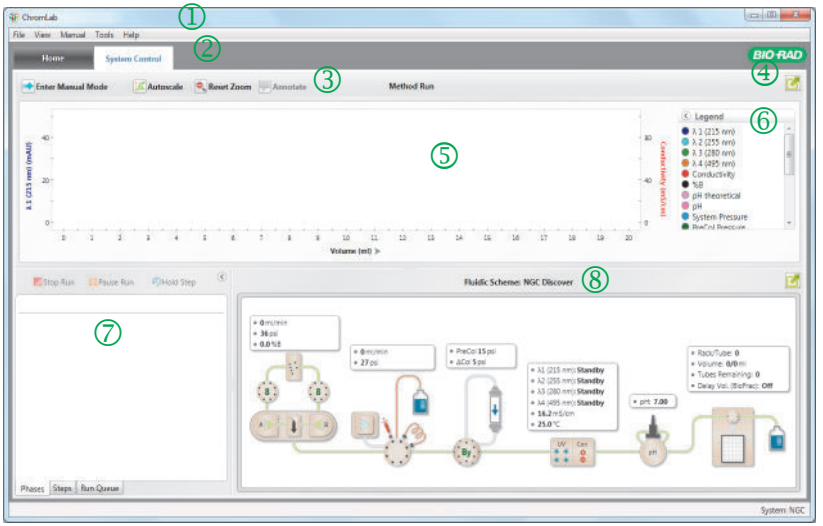
In the Home window, you can access the System Control window by selecting the System Control tab.

System Control Window

The System Control window displays a chromatogram viewer and a graphical fluidic scheme. The chromatogram is a time-, volume-, or column volume-based view of the run data acquired from the instrument. The fluidic scheme is a real-time view of the instrument status and flow.

For enhanced viewing, you can maximize the chromatogram or the fluidic scheme using the Expand buttons on the right side of the window. This is especially useful for touch screen viewing.

The fluidic scheme graphically depicts the flow between modules and how the system is configured and plumbed for an experiment. Each module's real-time status appears next to its image. For manual runs, a list of executed commands appears in the Run Log pane. In method mode, phases and steps of the method being run appear in their respective tabs, along with controls to stop or pause the run and hold the step. The Run Queue pane lists all the runs that are ready to be started. The Run Queue pane is accessible in both manual and method modes.



LEGEND

1	The menu bar provides quick access to File, View, Manual, Tools, and Help menu commands.
2	Tabs provide quick navigation among open windows (Home, System Control, Method Editor, and Evaluation).
3	The tab toolbar provides commands to save the current run, autoscale the UV trace, change the chromatogram view, annotate the chromatogram, and delete manual run data from the display.
4	Expand buttons expand the selected pane to fill the screen.
5	The chromatogram viewer displays data acquired from the instrument as traces based on time, volume, or column volume.
6	The chromatogram legend matches each trace to its trace type and color and displays the wavelength value in nanometers for UV traces. You can view or hide traces by clicking them.
7	In manual mode, the Run Log pane presents a time-stamped record of run steps and events. In method mode, run data appear in the Method Editor Phases and Step tabs. In both modes, the Run Queue tab lists runs that are waiting to start.
8	The Fluidic Scheme pane depicts graphically how modules are configured and plumbed for an experiment.

File Menu Commands

Connect to System — connects your computer to the NGC system if it is not already connected. In the dialog box that appears enter the DNS name of the NGC system and click Connect.

Disconnect System — when you start ChromLab, you are automatically logged in to the NGC system to which your computer is connected. This command enables you to disconnect ChromLab software from that system so you can work offline.

System Settings — opens the System Settings dialog box in which you can customize system settings. See System Settings on page 71 for more information about customizing your system.

System Information — opens the System Information dialog box, which lists the serial number and other general information about the NGC device as well as information about the system components, processes, and UV and UV/Vis detectors. See System Information on page 88 for more information.

Preferences — opens dialog boxes in which you can do the following:

- Select pressure units for all system and software pressure values. This is a global setting.
- Set up an SMTP server to send email messages about system notifications from the ChromLab computer. See Preferences on page 91 for more information about setting up an SMTP server.

Exit — closes ChromLab.

View Menu Commands

Show Chromatogram — displays a chromatogram of the current run data. Clearing this command hides the chromatogram from view.

Show Fluidics — displays the fluidic scheme. Clearing this command hides the fluidic scheme from view.

Manual Menu Commands

Enter/Exit Manual Mode — toggles ChromLab between manual and automatic modes.

Save Recorded Manual Run — in manual mode, saves in a data file the steps executed during a manual run.

Clear Recorded Data — deletes manual run data from the display.

Tools Menu Commands

Calibrate — opens the Calibration dialog box, which displays instructions and settings for selecting a module and calibrating it. See Calibrating a Module on page 66 for details.

Point-to-Plumb — starts the Point-to-Plumb feature and simultaneously turns off instrument LED lights so you can visually verify port locations during instrument plumbing. Displays the current fluidic scheme. Gray lines indicate the flow path. Clicking a line in the window turns on LED lights on the instrument corresponding to ports to be connected. See Verifying Plumbing with the Point-to-Plumb Feature on page 69 for details.

Change Fluidic Scheme — opens the Fluidic Scheme Selector dialog box in which you can edit the fluidic scheme or choose another one. See Fluidic Scheme Configurations on page 53 for details.

Map Fluidic Scheme — opens the Fluidic Scheme Mapping dialog box, which displays the location of two or more valves of the same type in the fluidic scheme, for instance two or more inlet valves or column-switching valves. You can use this dialog box to map the device on your instrument to its position in the fluidic scheme. See Fluidic Scheme Mapping on page 62 for details.

Flow Rate Converter— opens the Flow Rate Converter tool, which enables you to determine the flow rate to use for each column in the method based on the column size and the initial rate entered. A rate entered in ml/min is converted to cm/h and L/h; a rate entered in cm/hr is converted to ml/min and L/h. You can copy the result in the converter and paste it into your method.

Help Menu Commands

Help — displays screen-level help topics and links to installed manuals.

Export Diagnostic Logs — opens the Export Diagnostic Logs dialog box in which you can export all critical information that Bio-Rad Technical Support requires to diagnose issues. The log files and data are zipped and saved to a location that you choose. See Exporting Diagnostic Logs on page 275 for more information.

About — displays version and copyright information about ChromLab software.

Toolbar Commands

Save — saves in a data file steps executed during a manual run.

Clear — deletes manual run data from the display.

Autoscale — automatically scales the chromatogram's primary y-axis to the tallest peak height during the run. Autoscaling is enabled by default. When disabled, you can change the value of each individual UV trace The Autoscale mode and the UV trace values are saved when you save the run.

Reset Zoom — resets the view to show the full chromatogram.

Annotate — adds a note to the chromatogram at points on the x-axis during a run or after the run completes.

Context Menu Commands

To access context menu commands

▶ Right-click in the chromatogram and choose a command from the menu that appears.

Undo Zoom — restores immediately previous zoom level.

Reset Zoom — resets the view to show the full chromatogram.

Autoscale UV Trace — automatically scales the primary y-axis to the tallest peak height during the run. While enabled, UV scale in the legend cannot be manually set. When disabled, you can change the value of each individual UV trace The Autoscale mode and the UV trace values are saved when you save the run.

Copy Chromatogram — copies the chromatogram to the clipboard so you can paste it into another application.

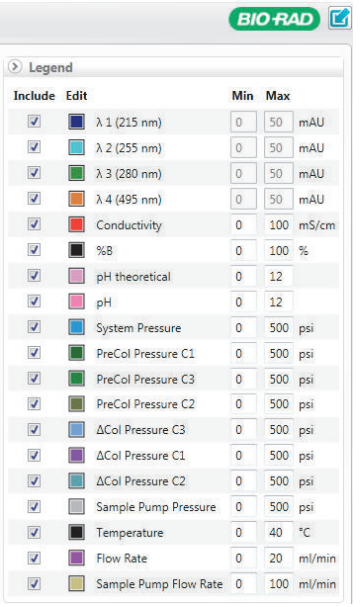
Save Chromatogram As —saves the chromatogram in an image format you choose (.bmp, .gif, .jpeg, .png, or .tiff).

Export as .csv — exports run data as a .csv file, which can be opened in spreadsheet applications.

Chromatogram View

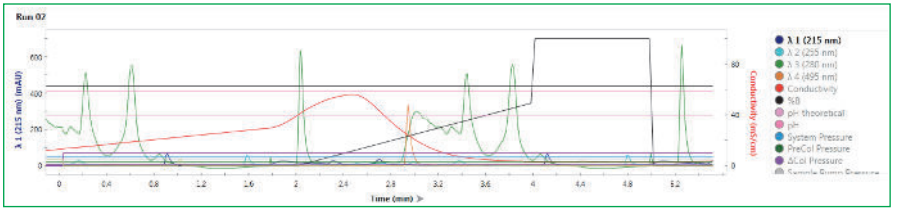
A chromatogram shows the real-time acquisition of data during the run as well as its outcome. It also depicts the quality of the purification. The chromatogram is recorded only when pumps are running and data are being acquired by the detectors in the fluidic scheme.

The chromatogram legend matches each colored trace to its detector type. When you expand the legend, you can hide traces by clearing the checkbox beside the trace, edit trace colors to differentiate among them, and change the y-axis scale for a trace. See Changing Trace Colors on page 211 and Changing the Axes on page 210 for more information.



When a run starts, the chromatogram viewer in the System Control window displays data acquired from the detectors as traces based on time, volume, or column volume. Pausing the pointer on a trace displays a tooltip with the trace x- and primary (left) y-axis values at that location. The legend also displays the wavelength value in nanometers for UV traces.

You can expand the chromatogram or the fluidic scheme by clicking the arrow in the upper right corner of the relevant pane. This is especially useful when viewing a chromatogram on the touch screen. Events that occur during the run, such as valve changes or changes in certain parameters, appear as event markers. Error events such as overpressure appear in red. The view also shows when fractions are collected and their locations in the selected rack.



The chromatogram has two y axes. The left axis is the primary axis. Its default trace is UV absorbance. Conductivity is the default trace for the right axis. The chromatogram also shows the following traces, which are defined in Table 1:

- Single/Multiple UV
- Conductivity
- pH
- Temperature
- Gradient
- Pressure
  - Precolumn pressure
  - Delta-column pressure
- Flow Rate

Table 1. Trace definitions

Trace	Module	Explanation
UV λ 1–4	Single-Wavelength UV and Multi-Wavelength UV/Vis Detectors	UV — with Single-Wavelength UV module, a reading of absorbance MV — with Multi-Wavelength UV/Vis module, up to four readings (λ 1–4)
Conductivity	Single-Wavelength UV and Multi-Wavelength UV/Vis Detectors	The conductivity of the fluid, read by the conductivity monitor (mS/cm)
%B Theoretical	System Pump	Programmed by the user in the method or in the System Pump dialog box ■ Gradient, system pump: % of pump B ■ Gradient, blending valve: % of valve Q4 ■ Buffer blending: % of valve Q4
pH Theoretical	pH	Theoretical pH, as programmed in the method or System Pump dialog box. Available only with the buffer blending valve in buffer blending mode
pH	pH	The measure of pH read from the pH probe, available only when a pH valve is present
System Pressure	System Pump	Measured at the mixer (psi or MPa)
PreCol Pressure	Column Switching Valve	Available when at least one column switching valve is present **Tip:** When two or more column switching valves are present, the traces appear in different shades of green on the chromatogram. The trace number corresponds to the valve number in the fluidic scheme. For example, PreCol Pressure 1 in the legend and the chromatogram corresponds to C1 in the fluidic scheme.

Table 1. Trace definitions, continued

Trace	Module	Explanation
Delta Col Pressure	Column Switching Valve	Available when at least one column switching valve is present **Tip:** When two or more column switching valves are present, the traces appear in different shades of blue on the chromatogram. The trace number corresponds to the valve number in the fluidic scheme. For example, ΔCol Pressure 1 in the legend and the chromatogram corresponds to C1 in the fluidic scheme.
Sample Pump Pressure	Sample Pump	Sample pump pressure (psi or MPa)
Temperature	Single-Wavelength UV and Multi-Wavelength UV/Vis Detectors	The temperature of the fluid, read at the Single-Wavelength UV or Multi-Wavelength UV/Vis module
Flow Rate	System Pump	Current system flow rate (ml/min)
Sample Pump Flow Rate	Sample Pump	Sample pump flow rate (ml/min)

Changing Chromatogram View Settings

You can change trace display attributes and other run view settings in the System Control window. Changes you make to the following settings are saved and used in the display of subsequent runs:

- Trace Show/Hide state
- Trace color
- Trace y-scale range (Min/Max y values)
- Active x-scale units
- Show/Hide state of events, fractions, errors, and annotations

When a manual or method run is saved, the new settings are saved with it. When you open the run in the Evaluation window, it displays the new settings. Some chromatogram view settings that are changed on the computer automatically change in the touch screen chromatogram view and vice versa.

The following display attributes are synchronized:

- Trace Show/Hide state
- Trace color
- Trace y-scale range (Min/Max y values)
- Autoscale (on/off)
- Active x-scale units
- Show/Hide state of events, fractions, errors, and annotations
- Active y-scale (selected trace y-scale)

Showing or Hiding Traces

To show or hide a trace

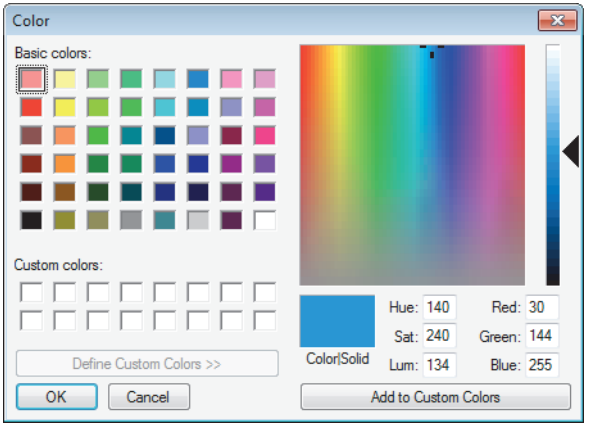
1. Expand the chromatogram legend.
2. Select or clear the trace entry to turn the trace on or off.

Tip: See Trace Settings Tab on page 76 to show all hidden traces.

Changing Trace Color

To change trace color

1. Expand the legend and click the trace color in the Edit column.
2. In the Color dialog box, select a color and click OK.



Tip: See Trace Settings Tab on page 76 to revert trace colors to their factory default settings.

Zooming In and Out

To zoom in on a section of the chromatogram

▶ While clicking in the chromatogram, drag the pointer to mark the zoom region of interest.

To zoom out to the previous zoom level

▶ Double-click the chromatogram or right-click the chromatogram and choose Undo Zoom in the menu that appears.

To zoom out to the full-scale view

▶ Click Reset Zoom on the tab toolbar or right-click the chromatogram and choose Reset Zoom in the menu that appears.

Changing the X-Axis Units and Scale

For runs performed from saved methods, the x-axis unit can be changed to Time (min), Volume (ml), or Column Volume (CV). For runs performed manually, the x-axis unit can be changed to Time (min) or Volume (ml).

To change the x-axis units and scale

▶ Click the x-axis title to toggle among the available options.

Changing the Y-Scale Values

You can change the maximum and minimum y-scale values in the legend to set the chromatogram scale.

To change the y-scale values

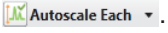
1. Expand the legend in the chromatogram view.
2. Enter maximum and minimum values in the appropriate trace row, ensuring that the maximum value always exceeds the minimum value.

Tip: See Trace Settings Tab on page 76 to revert the y-scale values to their factory default settings.

Autoscaling the UV Trace

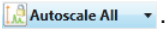
Autoscaling the UV trace scales the chromatogram's primary UV y-axis based on signal intensity. Autoscaling is enabled by default.

When multiple UV absorbance traces are present you can

- Autoscale each UV trace independently 

.

  The y-axis scale of each trace is scaled to the UV signal intensity of the individual trace.

- Autoscale all UV traces to the same scale 

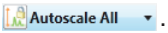
.

  The y-axis scale is based on the signal intensity of the UV trace with the highest intensity and all other traces are normalized to the intensity of that trace.

- Disable autoscaling 

.

When a single UV absorbance trace is monitored with a single-wave UV you can

- Autoscale all UV traces to the same scale 

.

  The y-axis scale is based on the signal intensity of the UV trace with the highest intensity and all other traces are normalized to the intensity of that trace.

- Disable autoscaling 

.

  When disabled, you can change the value of each individual UV trace The Autoscale mode and the UV trace values are saved when you save the run.

To autoscale the UV trace

▶ Do one of the following:

- Click Autoscale on the toolbar to choose a status.
- Right-click the chromatogram, choose Autoscale UV trace, and select a status.

Annotating the Chromatogram

You can add notes to the chromatogram in manual mode at any time during a run or after a run completes to associate observations with data points on the chromatogram. The annotation dialog box contains three fields: Location (in time, volume, or CV), Title, and Description. When the annotation is saved, its title appears at the specified location on the x-axis. The description appears in the run report.

You can add annotations when a method run is in progress. However, if the run ends while the annotation dialog box is open, the annotation is saved and the dialog box closes automatically. When a method run is complete, annotations cannot be added to the chromatogram in System Control. You can annotate completed runs by opening them in Evaluation mode.

To add annotations

1. Click Annotate on the toolbar and drag the icon onto the chromatogram. The green annotation dialog box opens with the Location field automatically filled.

   Tip: You can edit the Location field if necessary.

2. Type a title for the annotation.
3. (Optional) Type a description for the annotation.
4. Click Save to save the annotation.

   Note: Clicking ☒ closes the dialog box without saving the annotation.

To edit an annotation

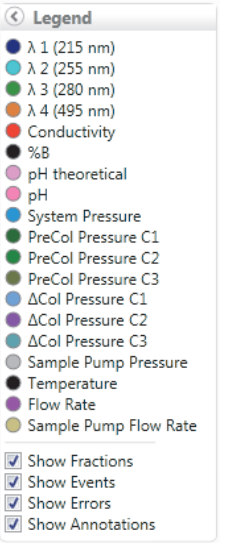
1. Double-click the annotation on the chromatogram to open its dialog box.
2. Edit the annotation and click Save to save the changes.

To delete an annotation

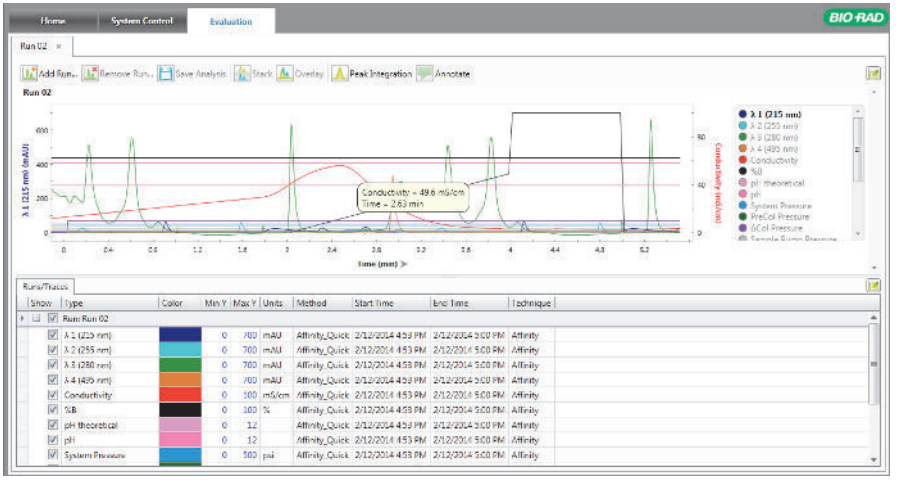
▶ Double-click the annotation to open its dialog box and click Delete.

Showing and Hiding Events, Fractions, Errors, and Annotations

You can choose whether to show or hide fractions, events, errors, and annotations by selecting or clearing the appropriate checkboxes at the bottom of the legend.



When you pause the pointer on a trace, a tooltip displays the real-time reading at the current location in the chromatogram.

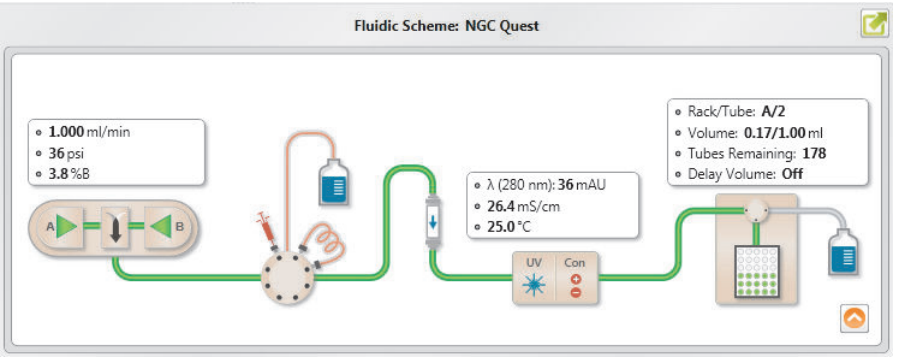


Fluidic Scheme Pane

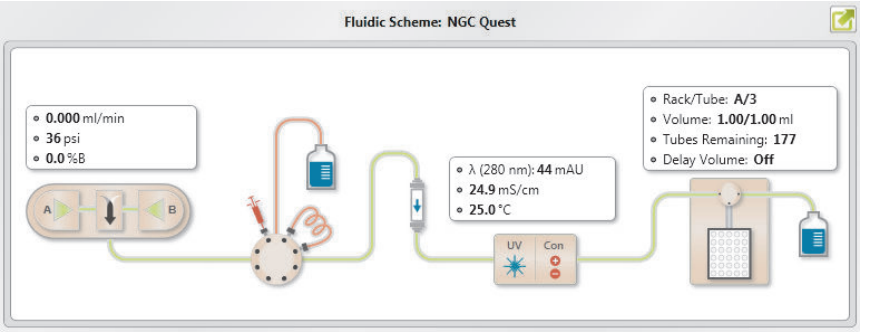
The Fluidic Scheme pane graphically displays each module currently in use on the instrument. Real-time status information includes buffer flow rate, sample flow rate, pressure, and valve position through the system. The fluidic scheme reflects how the instrument is plumbed and the flow path through the various modules on the system.

Important: The selected fluidic scheme must match the system's installed hardware modules.

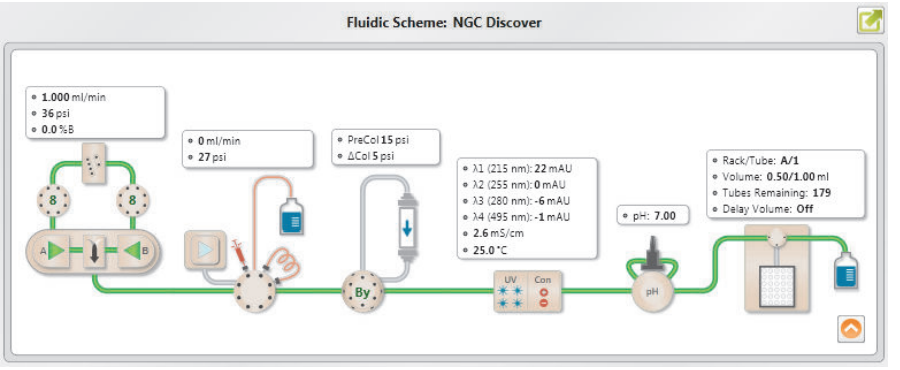
The fluidic scheme shows the real-time flow path (bright green) through the system and, in manual mode, provides access to device settings. The path to fluidic scheme components excluded from the flow path appears in grey.



When pumps are not running, the predicted flow path appears in a lighter color corresponding to the pump flow path (light blue for the sample pump or light green for the system pump).

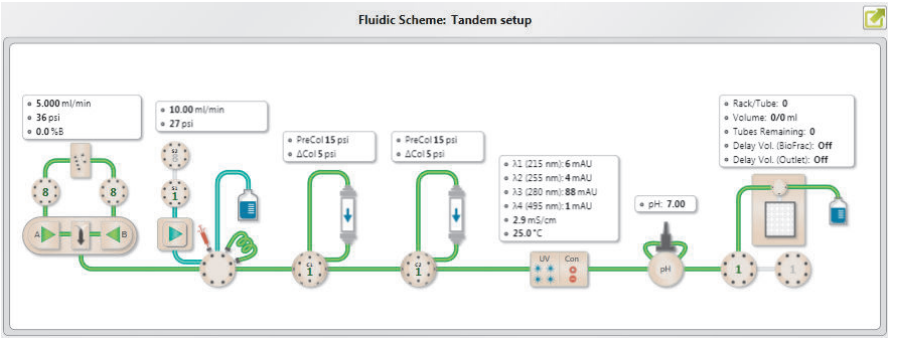


On the NGC Discover system, two flow paths are displayed — one for the system pump (bright green) and another for the sample pump (light blue). In the following screen, the gray path from the column switching valve to the column indicates that the column switching valve is not inline with a pump or injection syringe but is in bypass.



The fluidic scheme displays the number of a valve's active port in bright green. A grey number on a valve indicates that it is not in use. The NGC instrument supports up to four inlet valves (two buffer inlet and two sample inlet valves), three column-switching valves, and two outlet valves. Pausing on a valve displays a tooltip indicating its type.

Tip: If the system includes two sample inlet valves, the fluidic scheme identifies the first sample inlet valve as S1 and displays it as the lower of the two. The second is identified as S2. If the system includes multiple column switching valves or outlet valves, the valves are identified numerically in ascending order.



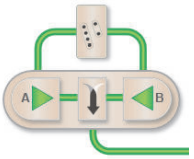
Modules

Fluidic scheme modules are more fully described in Chapter 2, Components, of the Instrument Guide. For ease of reference, Table 2 depicts each module that can appear in a fluidic scheme and describes corresponding dialog box options.

Table 2. Fluidic scheme modules

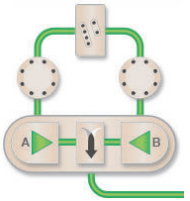
System Pumps and Mixer

This module dialog box controls the NGC system's two gradient pumps and shows the gradient status. It controls flow rate, gradient type (salt or pH), gradient duration, and system pressure limits.



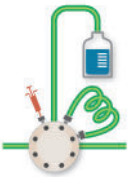
System Pumps with Buffer Blending Valve

This module dialog box controls the NGC system gradient pumps when connected to a buffer blending module and shows the pump and gradient status. It controls flow rate, buffer blending valve priming, buffer recipe, gradient composition, pH, gradient duration, and system pressure limits.



System Pumps with Buffer Blending Valve and Buffer Inlet A and B

This module dialog box controls the NGC system gradient pumps when connected to inlet valves and a buffer blending valve. It controls gradient mode (two-pump gradient or buffer blending valve), flow rate, buffer blending valve priming, buffer recipe selection, gradient composition, pH, gradient duration, and system pressure limits.



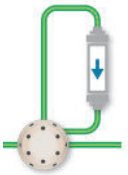
Sample Inject Valve

This module dialog box controls the NGC sample inject valve. It is used to route fluid from the gradient pump, sample pump, and injection port to the loop, column, and waste as required during an experiment.

Table 2. Fluidic scheme modules, continued

Sample Pump

This module dialog box controls the sample pump and displays the sample pump status. It is used to automatically load samples into a sample loop or to directly inject samples onto a column during an experiment. It is used primarily for large volume samples.



Column Switching Valve

This module dialog box controls the column switching valve and displays the valve status, the precolumn pressure, and the pressure drop across the column (ΔCol). It is used to select one of the five columns that can be connected to it, bypass the columns, or reverse the flow through the columns.

UV/Conductivity Monitors

This module dialog box controls the single-wavelength UV or multi-wavelength UV/Vis monitor and displays the UV, temperature, and conductivity monitor status. It is used to turn on the UV monitor and to set the wavelength that will be used for monitoring. It is also used to zero the baseline during a run. The single-wavelength monitor can monitor one wavelength at a time (255 or 280 nm). The multi-wavelength UV/Vis monitor can monitor up to four wavelengths simultaneously (190–800 nm).

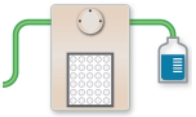
Signal Import Module

This module dialog box controls the signal import module (SIM) and displays the output of the attached external detectors. Up to two external devices can be attached to the SIM. This module displays the output from both devices or output from either SIM1 or SIM2 if one device is turned off.

Table 2. Fluidic scheme modules, continued

pH Monitor and Valve

This module dialog box controls the pH monitor and displays the pH. It is used to place the pH flow cell inline or to bypass it during an experiment. It also enables you to calibrate the pH probe without taking it offline.



Fraction Collector

This module dialog box controls fraction collection. It is used to select racks and rack location and to start and stop fraction collection. It is also used to determine fraction size and the number of tubes to collect.

Fluidic Scheme Configurations

The fluidic scheme must be customized to match your instrument hardware setup. In manual mode, you can access the Fluidic Scheme Selector dialog box to change the detailed settings of most elements. You can also create custom fluidic schemes. See To create a new fluidic scheme on page 60.

Table 3. Fluidic scheme configuration options

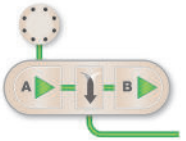
Pumps	Configuration	Explanation
	System pump only	Used to start and stop pumps, change buffer, gradient, and duration, and to set pressure limits.
	System pump and inlet A	Select from up to eight different buffers for A.

Table 3. Fluidic scheme configuration options, continued

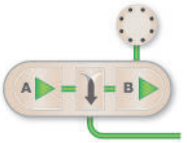
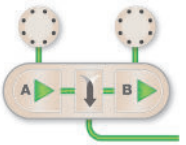
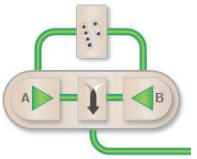
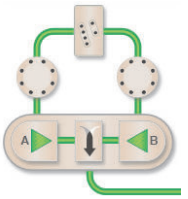
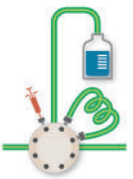
	System pump and inlet B	Select from up to eight different buffers for B.
	System pump and inlet A and B	Select from eight buffers each for A and B (16 total).
	System pump and buffer blending valve	Start and stop buffer blending valve gradient formation and perform buffer blending.
	System pump and buffer blending valve with inlets A and B	Start and stop buffer blending valve gradient formation and perform buffer blending. Select from up to seven additional buffers and solutions.
Sample Inject	**Configuration**	**Explanation**
	Sample inject valve	Load a specific predetermined sample volume onto a column.

Table 3. Fluidic scheme configuration options, continued

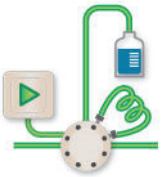
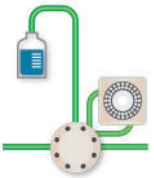
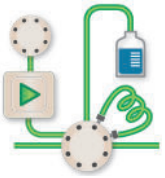
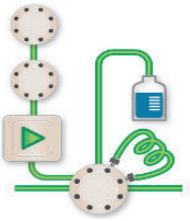
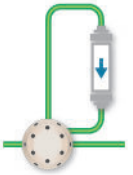
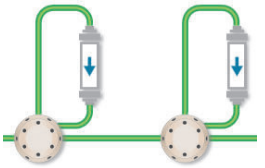
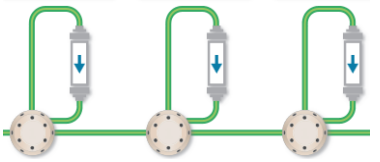
	Sample inject valve with sample pump	Automatically load a specific predetermined sample volume onto a sample loop, or directly onto the column, using the sample pump.
	Sample inject valve with autosampler	Automatically load samples onto a column using an autosampler.
	Sample inject valve with sample pump and a single sample inlet valve	For use as a mini autosampler, can select from eight different samples.
	Sample inject valve with two sample inlet valves	For use as a mini autosampler, can select from 15 different samples when two sample inlet valves are daisy-chained together.

Table 3. Fluidic scheme configuration options, continued

Columns	Configuration	Explanation
	Column	Self-explanatory when column switching valve is absent.
	Column with column switching valve	Used to select from up to five different columns, bypass the columns, or reverse the flow through the columns.
	Two column switching valves, each with a column attached	Used to select from up to 10 different columns, bypass the columns, or reverse the flow through the columns.
	Three column switching valves, each with a column attached	Used to select from up to 15 different columns, bypass the columns, or reverse the flow through the columns.

Detectors	Configuration	Explanation
	Single-wavelength UV with conductivity	For selection of a single-wavelength UV detector with conductivity and temperature.

Table 3. Fluidic scheme configuration options, continued

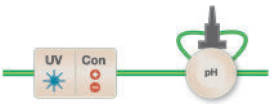
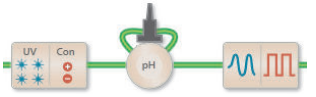
	Single-wavelength UV with conductivity and pH probe	For selection of a single-wavelength UV detector with conductivity, temperature, and pH valve.
	Multi-wavelength UV/Vis detector with conductivity and temperature	For selection of a multi-wavelength UV/Vis detector with conductivity and temperature.
	Multi-wavelength UV/Vis detector with conductivity, temperature, and pH probe	For selection of a multi-wavelength UV/Vis detector with conductivity, temperature, and pH valve.
	Signal import module (SIM) with single-wavelength UV detector with conductivity and temperature	For selection of the SIM with a single-wavelength UV detector with conductivity and temperature.
	SIM with single-wavelength UV detector with conductivity, temperature, and pH probe	For selection of the SIM with a single-wavelength UV detector with conductivity, temperature, and pH valve.
	SIM with multi-wavelength UV/Vis detector with conductivity	For selection of the SIM with a multi-wavelength UV/Vis detector with conductivity and temperature.
	SIM with multi-wavelength UV/Vis detector with conductivity and pH probe	For selection of the SIM with a multi-wavelength UV/Vis detector with conductivity, temperature, and pH valve.

Table 3. Fluidic scheme configuration options, continued

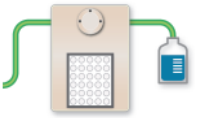
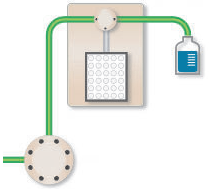
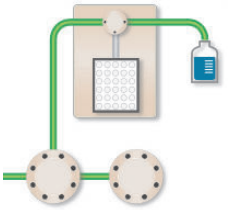
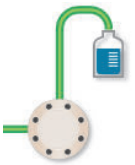
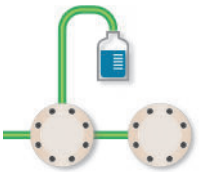
Fraction Collection	Configuration	Explanation
	BioFrac™ fraction collector	For configuring a BioFrac fraction collector. Used for starting and stopping fraction collection to determine fraction size and number of tubes to collect.
	Fraction collector with outlet valve	For collecting a combination of large and small volume fractions. Select outlet ports can be plumbed to divert a fraction eluted from a column onto a sample/storage loop to be subsequently reinjected onto a second column during tandem applications.
	Fraction collector with two outlet valves	For collecting a combination of large and small volume fractions. Select outlet ports can be plumbed to divert a fraction eluted from a column onto a sample/storage loop to be subsequently reinjected onto a second column during tandem applications.
	Outlet valve	For use as a fraction collector, can collect up to 11 large-volume fractions. Select outlet ports can be plumbed to divert a fraction eluted from a column onto a sample/storage loop to be subsequently reinjected onto a second column during tandem applications.

Table 3. Fluidic scheme configuration options, continued

	Two outlet valves	For use as a fraction collector, can collect up to 22 large-volume fractions when two outlet valves are daisy-chained together. Select outlet ports can be plumbed to divert a fraction eluted from a column onto a sample/storage loop to be subsequently reinjected onto a second column during tandem applications.

Working with Fluidic Schemes

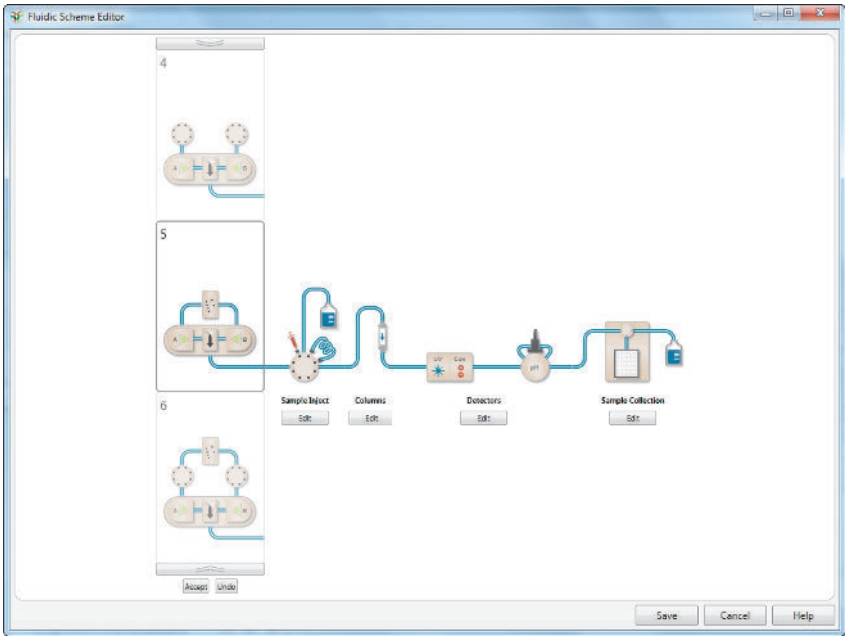
Five sections of the fluidic scheme can be configured based on the available modules and experiment requirements.

- Pumps
- Sample inject
- Columns
- Detectors
- Sample collection

To change a fluidic scheme

1. Do one of the following:
   - In the System Control window, select Tools > Change Fluidic Scheme.
   - On the touch screen, choose Change Fluidic Scheme on the dropdown menu.

   The Fluidic Scheme Selector dialog box appears. A descriptive tooltip appears when you pause the pointer on a module in the fluidic scheme.



2. Click an entry in the Fluidic Scheme Selector pane.

   The right pane displays the fluidic scheme you selected.

3. Click Select.

To create a new fluidic scheme

Note: You cannot save a duplicate fluidic scheme.

1. Do one of the following:

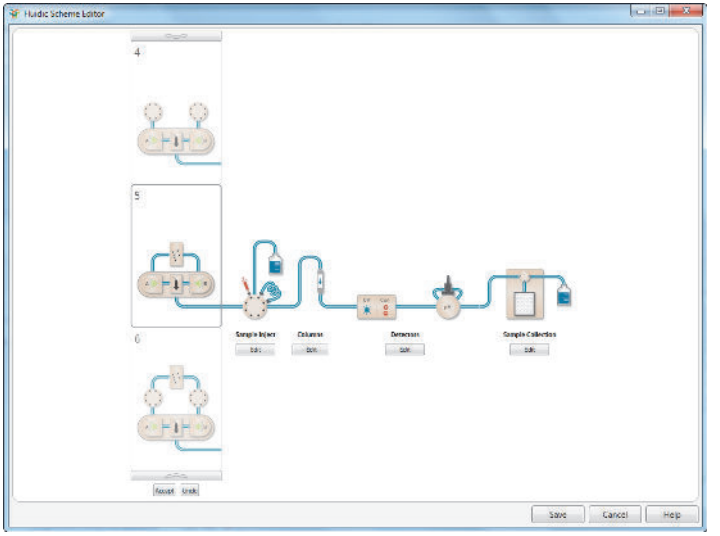
   - In the System Control window, select Tools > Change Fluidic Scheme.
   - On the touch screen, choose Change Fluidic Scheme on the dropdown menu.

2. Click New.

   The current fluidic scheme appears with Edit buttons that enable you to select alternative modules in spin boxes.

3. Click Edit under the group of modules to change.

   A spin box displays a number of modules of the type you selected.



4. Scroll up or down to view module choices.
5. Select a module and click Accept.
6. Repeat steps 3–5 to edit other modules.
7. When you have finished editing modules, click Save and type a name in the New Fluidic Scheme dialog box that appears.
8. Click OK.

To change module settings

1. In the fluidic scheme, double-click a module.
2. In the dialog box that appears, edit module options.
3. Click Apply.

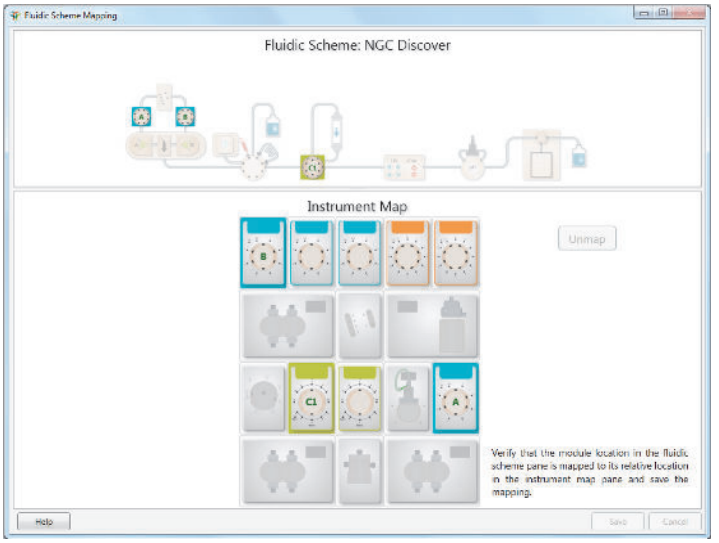
Fluidic Scheme Mapping

The NGC instrument supports up to four inlet valves (two buffer and two sample inlet valves), three column-switching valves, and two outlet valves. When more than one of a specific valve type is installed, ChromLab automatically detects the valves and maps the fluidic scheme to their default positions on the NGC instrument. For example, when two or more inlet valves are present in the fluidic scheme, ChromLab automatically detects the position of the valves on the NGC instrument (starting from the left side of the bottom tier) and assigns the first valve Inlet A.

If the configuration of your NGC instrument is different than the default, or if you changed your fluidic scheme, you must manually map the valves on the instrument to their locations on the fluidic scheme. This is done using the Fluidic Scheme Mapping dialog box.

Note: If you have only two inlet valves on your NGC instrument and both valves are designated as buffer inlets, you do not need to map them. The system automatically determines their locations and function on the instrument.

The Fluidic Scheme Mapping dialog box displays the current fluidic scheme in the upper pane and the current NGC instrument configuration in the lower pane. ChromLab automatically detects which valves in the fluidic scheme can be mapped. These valves appear color-coded in the upper pane. The corresponding valves appear in the same color code on the instrument map in the lower pane, in relative location to the mappable modules on the NGC instrument. All other modules appear inactive in the lower pane.



The color and labeling scheme for the Fluidic Scheme Mapping dialog box is as follows:

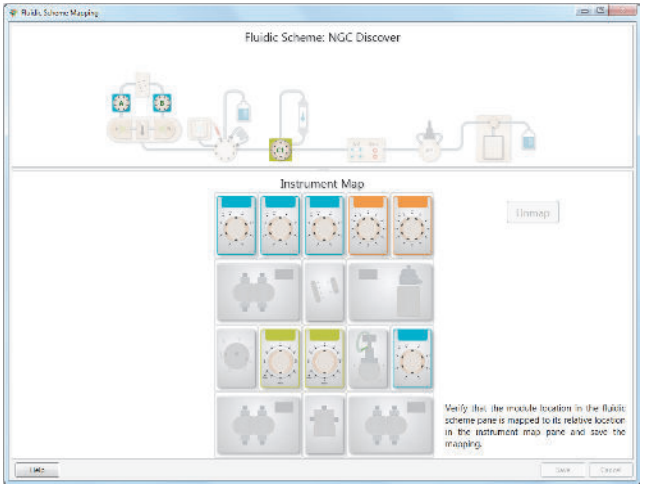
Color	Label	valve
Blue	A and B	Buffer inlet valve
Blue	S1 and S2	Sample inlet valve
Green	C1–C3	Column switching valve
Orange	O1 and O2	Outlet valve

To map a fluidic scheme to the instrument, you first unmap the current settings and then map the valves to the new fluidic scheme.

To unmap valves

1. Do one of the following:
   - In the System Control window, select Tools > Map Fluidic Scheme.
   - On the touch screen, choose Map Fluidic Scheme on the dropdown menu.

   The Fluidic Scheme Mapping dialog box appears with the valves on the fluidic scheme mapped to their default positions on the instrument map.
2. Select a valve on the instrument map. The border of the valve on the fluidic scheme becomes highlighted, indicating that it is selected.
3. Click Unmap. The border of the valve on the instrument map thins and its label disappears, indicating that the valve is no longer mapped to the fluidic scheme.
4. (Optional) Continue unmapping all valves that need to be remapped.



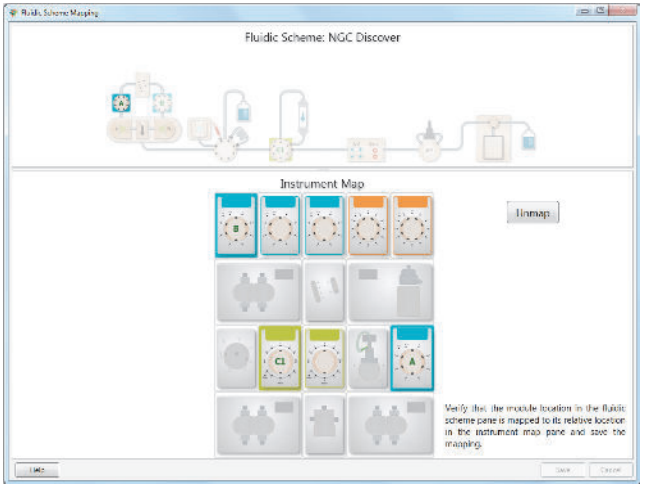
5. To map the valves, proceed to To map valves on page 65.

To cancel unmapping valves

▶ Click Cancel to cancel unmapping the valves. The fluidic scheme and instrument map return to the previous mapping configuration.

To map valves

1. In the Fluidic Scheme Mapping dialog box, select a valve to map in either the upper or lower pane.
2. Select its position on the corresponding scheme. In the instrument map, a label appears on the selected valve and its border thickens, indicating that it is mapped.



3. (Optional) Continue mapping the unmapped valves.
4. Click Save to save the new mapping.

Calibrations

The NGC instrument arrives factory calibrated. For the most part, the instrument will not require further calibration. The pH probe, however, should be recalibrated each day the instrument is used and when there are large fluctuations in temperature because pH probe settings drift with time.

In addition to the pH probe, ChromLab includes settings for calibrating the following:

- Pump flow rate
- Sample pump pressure
- System pressure
- Column switching valve pressure
- Conductivity monitor

Calibrating these modules is optional.

Calibrating a Module

Note: You cannot run a method during calibration.

On the Tools menu, clicking Calibrate displays a Calibration dialog box in which you can select a module to calibrate. The System Control window also appears with the title Calibration in Progress in the title bar.

To select a module to calibrate

▶ Select a module in the Calibrate dropdown list at the top of the Calibration dialog box.

Instructions for calibrating the module you selected appear in the dialog box.

Calibrating Flow Rate of Pumps

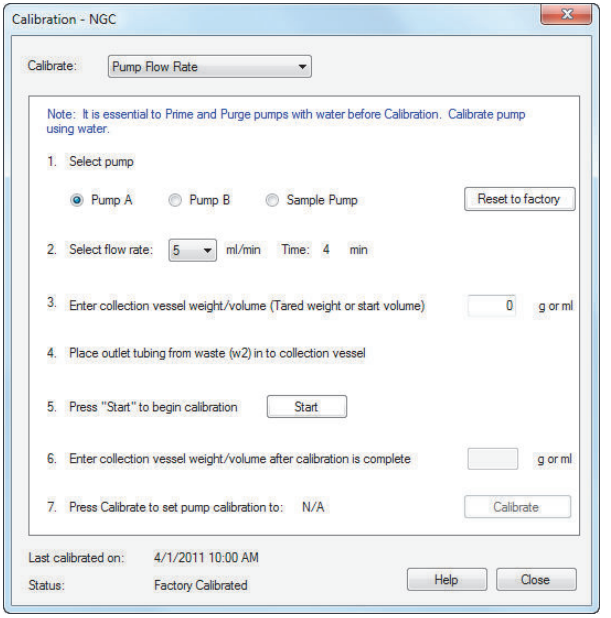
If the buffer flow seems to be incorrect, you might want to recalibrate the pumps.

Important: The system must be primed with water before you calibrate the pumps' flow rate. Otherwise the calibration will be incorrect.

To calibrate the flow rate of the pumps

▶ Select Pump Flow Rate in the Calibrate dropdown list at the top of the Calibration dialog box.

The Calibration dialog box displays instructions and settings for calibrating the pumps.



Calibrating Pressure Settings

WARNING! You must remove the tubing from the pressure sensor before you calibrate it. The sensor must be at atmospheric pressure when you start.

You can calibrate sample pump pressure, system pressure, and column switching valve pressure to zero. If pressure is not reading zero or close to it, you can reset the pressure values to zero.

To calibrate a pressure setting

1. Select a pressure module in the Calibrate dropdown list at the top of the Calibration dialog box.
2. Follow the dialog box instructions to reset the pressure setting to zero.

Note: To calibrate pressures at the upper limit, call Bio-Rad Support.

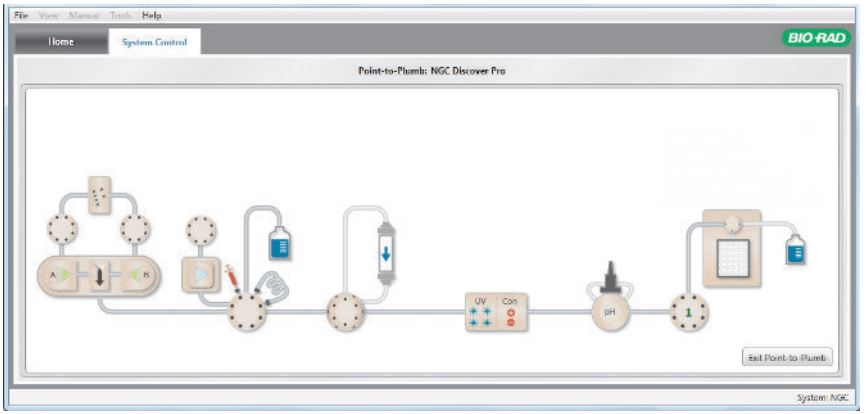
Verifying Plumbing with the Point-to-Plumb Feature

The NGC chromatography system instrument arrives plumbed. The Point-to-Plumb feature enables you to change the plumbing or to verify that the instrument is plumbed correctly for the fluidic scheme you want to use.

You can access the Point-to-Plumb feature on the touch screen menu or on the computer running ChromLab. (Select Tools > Point-to-Plumb.)

Tip: Using the touch screen makes it easy to view the Point-to-Plumb window and the front of the instrument at the same time.

When you select Point-to-Plumb, the current fluidic scheme appears in Point-to-Plumb mode. All LEDs on the instrument turn off. When you select a line between two modules, the LEDs corresponding to the two ports to be connected light up.



In the Point-to-Plumb window, the proper plumbing path is indicated by the color gray. Selected flow paths turn green. Path elements that are not plumbed appear in a paler shade of gray and do not change color when you select them.

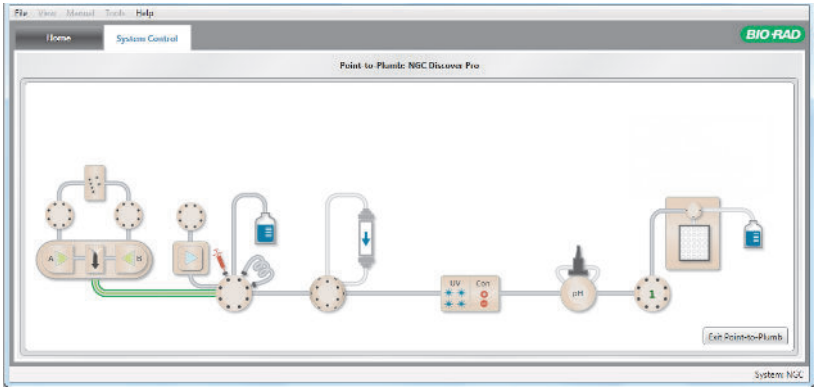
Note: When the fluidic scheme includes a signal import module (SIM), the SIM is also present in the Point-to-Plumb window. In this case, the outlet LED on the module that connects to the external detector (via SIM) turns on when the fluid

path between them is selected. If the fluidic path includes an NGC module after the external detector, the inlet LED on that module turns on.

To verify the plumbing path

1. With both the Point-to-Plumb window and the front of the instrument in view, click a segment of the gray path in the Point-to-Plumb window.

   The segment you selected turns green to indicate the correct plumbing path.



   At the same time, green LED lights flash on the front of the instrument, indicating the ports to be connected to each other.

2. Check the instrument plumbing and the Point-to-Plumb display to verify that the selected segment of the plumbing is correct. If it is not correct, replumb it.
3. Continue selecting segments of the plumbing path and verifying that they are correct.
4. When you have finished verifying the plumbing path, click Exit Point-to-Plumb.

The LEDs on the instrument turn on. Depending on the module, LEDs indicate

- Flow from the system pumps (green LEDs)
- Flow from the sample pump (blue LEDs)

System Settings

The System Settings dialog box enables you to customize your system. From this dialog box you can

- Set the delay volume
- Control the flow rate to prevent overpressure
- Enable remote access to ChromLab from an iPad, Android device, or another PC
- Reset the trace settings, the minimum and maximum y-scale values, and the trace colors in the chromatogram to their factory default settings
- Enable or disable external detectors that are connected to the NGC instrument via a SIM device
- Enable or disable the ability to send UV signals to external devices that are connected to the NGC instrument via a SIM device
- Set air sensors to detect either end of sample or end of buffer
- Enable the system to send email messages about system events to a list of users
- Set a unique name for the NGC system

Note: This dialog box is accessible in manual mode. The settings are saved and the fraction collector is also synchronized in method runs.

Delay Volume Tab

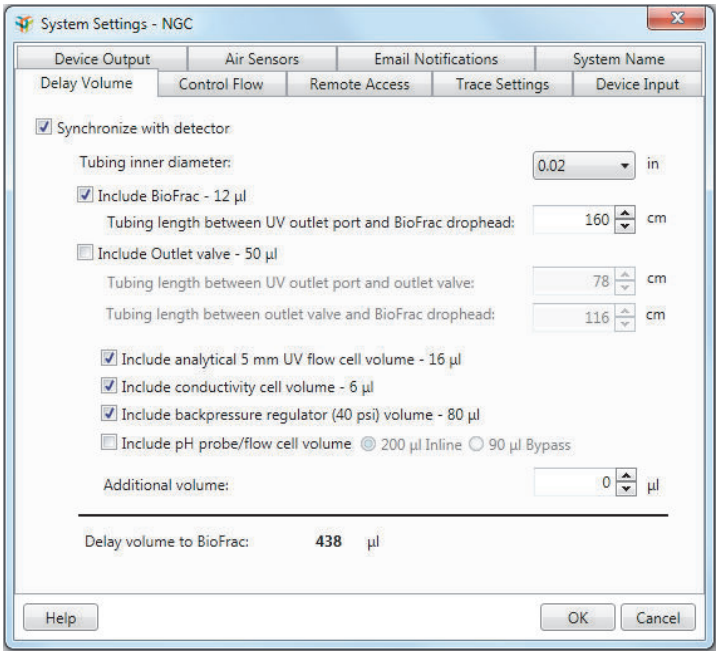
Delay volume is the volume of plumbing between the UV detector and the fraction collector drophead and/or the outlet valve. It indicates the delay between the detection of the fraction's UV peak and the time it takes for this peak to traverse the volume of tubing and flow cells to the fraction tube into which it is collected.

When the delay volume is set, the fraction collector or outlet valves wait for the fraction peak to travel from the detector to the drophead (or port) before collection begins. Fraction collection on the instrument can be delayed relative to the collection information shown in the chromatogram and status panels. When the run reaches the end of rack or the last port on the outlet valve, the chromatogram and status panel might display the start tube and rack or new outlet port before collection actually starts.

Note: If an outlet valve is included in the fluidic scheme and you change the collection port or fraction size during the run, the first container in the new scheme collects the designated fraction size as well as the indicated delay volume. All succeeding fraction containers collect only the designated fraction size.

To set the delay volume

1. Select File > System Settings to open the System Settings dialog box.
2. Choose the Delay Volume tab.



3. Select Synchronize with detector.
4. Specify the inner diameter of the tubing in use.

Tubing Color	Inner Diameter
Orange	0.02" (0.5 mm)
Green	0.03" (0.75 mm)
Clear	0.062" (1.6 mm)

5. If your system includes the BioFrac fraction collector, select the checkbox Include BioFrac – 12 µl and specify the tubing length between the UV outlet port and the fraction collector drophead.
6. If your system includes an outlet valve, select the checkbox Include Outlet valve – 50 µl and specify the tubing length between the UV outlet port and the outlet valve.

7. If your system includes both the BioFrac fraction collector and an outlet valve, select both checkboxes. In the Include Outlet valve – 50 µl section, specify the tubing length between the UV outlet port and the outlet valve as well as the tubing length between the outlet valve and the fraction collector drophead.

8. Select or clear the next four checkboxes, depending on whether these modules are present on your system.

   Note: If a pH detector module is present on your system and you select the checkbox to include its cell volume, determine whether the detector is set to Inline or Bypass mode and select the appropriate radio button.

9. If one or more of the options is not applicable, or if you change the length of the tubing, enter the appropriate values in the Additional volume box.

   The system calculates the delay volume based on your specifications and displays the results in µl at the bottom of the dialog box.

10. Click OK.

Control Flow Tab

Use the option Control Flow to avoid overpressure to prevent the system from exceeding the maximum pressure limit. Control Flow reduces the flow rate by half whenever the system pressure gets within 80% (the default) of the maximum system pressure limit. Control Flow lowers the flow rate multiple times if necessary. However, it will not reduce the flow rate below the minimum user-specified flow rate. For methods, Control Flow resets the flow rate at the start of each phase and then readjusts it if necessary. The pumps are immediately stopped if the pressure exceeds the maximum pressure limit at any time.

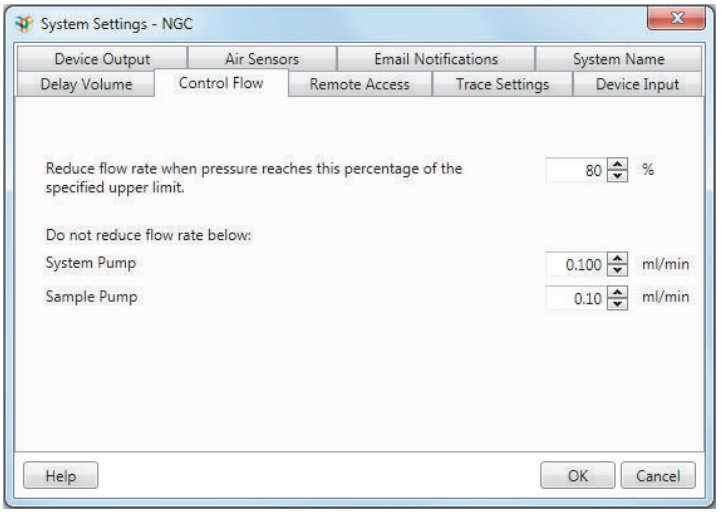
The option Control Flow to avoid overpressure is available in the System Pump and Sample Pump dialog boxes in manual mode as well as in the Method Editor window. You can choose either of these settings before performing a run or running a method to control the flow rate within the pressure limit range using settings in the Control Flow tab of the System Settings dialog box.

When Control Flow to avoid overpressure is selected, the system lowers the flow rate to avoid exceeding the maximum pressure. Typically, Control Flow activates when the pressure reaches 80% of the maximum set pressure from the column. This limit can be changed. The flow rate is lowered to half the set flow rate. Flow rate returns to normal when the phase changes and drops only if the pressure in the next phase continues to exceed the upper pressure limit of the column.

Note: If the pressure increases rapidly and exceeds the specified limits, or the flow rate drops below the specified flow rate, the system shuts down.

To control the flow rate

1. Select File > System Settings to open the System Settings dialog box.
2. Choose the Control Flow tab.



3. Specify a percentage of maximum pressure at which you want Control Flow to activate (the default is 80%).

   The system reduces the flow rate by 50% when pressure reaches the specified percentage of the maximum pressure.

4. (Optional) Specify a minimum flow rate for the system pump and for the sample pump if the sample pump is available.

Tip: When you change the pump heads on the system pumps, ChromLab software checks at instrument startup whether the most recently used flow rate setting in the System Settings dialog box Control Flow tab is compatible with the range that the new pump heads allow. If it is not, the flow rate is set to the minimum that the new pump heads allow.

Remote Access Tab

You can access ChromLab software remotely with an iPad, Android device, or another personal computer utilizing a virtual network computing (VNC) service.

To enable remote access to ChromLab

1. Select File > System Settings to open the System Settings dialog box.
2. Choose the Remote Access tab.
3. Select Enable VNC service for remote screen sharing.

Trace Settings Tab

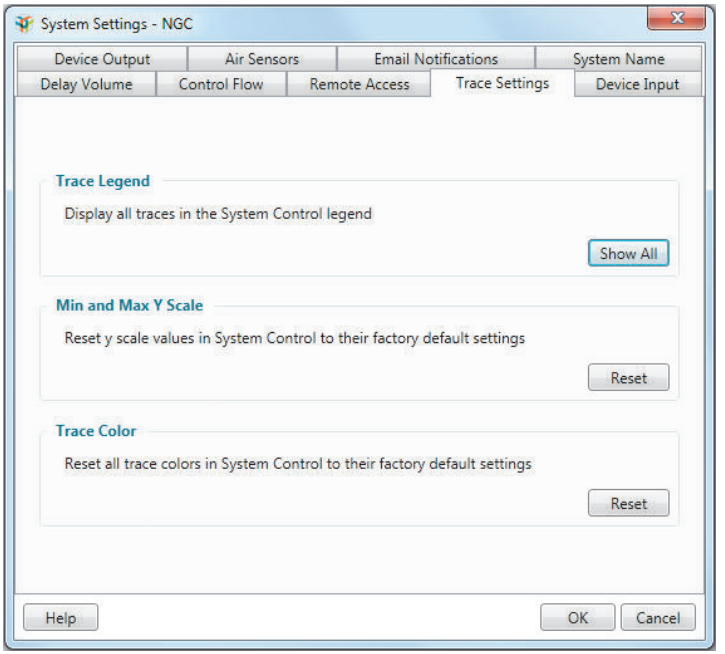
ChromLab saves changes to trace display settings and applies them to subsequent manual or method runs. You can revert the following display settings to their factory defaults:

- Trace Show/Hide state
- Trace y-scale range (Min/Max y values)
- Trace color

Note: When you revert a display setting to its factory default, the change applies to the current and all subsequent manual or method runs. The change does not affect previously saved or evaluated runs.

To revert trace display settings to their factory defaults

1. Select File > System Settings to open the System Settings dialog box.
2. Choose the Trace Settings tab.



3. Do one or more of the following:
   - To show all traces in the System Control legend, click Show All.
   - To reset the y-scale values to their factory default settings, click Reset.
   - To reset all trace colors to their factory default settings, click Reset.
4. Click OK.

Device Input Tab

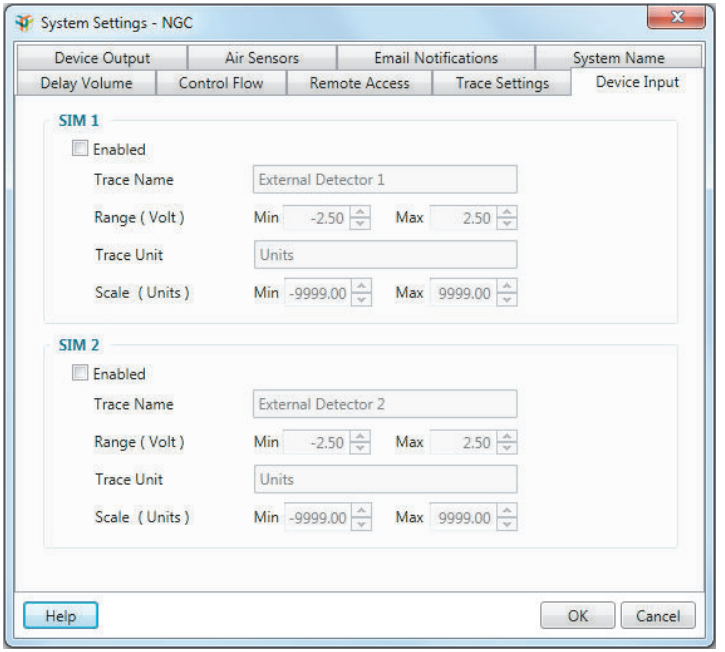
You can import analog (voltage) signal from up to two external devices and convert it to digital NGC data via the signal import module (SIM).The SIM imports and converts the signal to the appropriate trace units through its SIM channels. When enabled, the details appear in the chromatogram's legend, the trace appears on the chromatogram, and the data can be analyzed in the Evaluation window. For each device you can define its

- Trace name
- Minimum and maximum output range (in volts)
- Trace unit type (displayed in the chromatogram's legend)
- Minimum and maximum unit range

To enable trace measurement from external detectors

1. Ensure that the SIM is connected to the NGC instrument.
2. On the back of the SIM, determine which external devices are connected to the SIM 1 and SIM 2 ports.
3. Select File > System Settings to open the System Settings dialog box.

4. Choose the Device Input tab.



5. By default, the fields in this dialog box are disabled. Select the Enabled checkbox for SIM 1 and provide the following details to enable connection to the first detector:

   a. Type a trace name for the detector in the Trace Name field.

      Note: This field has a 20-character limit.

   b. Range (Volt) is the output from the detector. Set the minimum and maximum volt range.

   c. Type a unit type for the trace in the Trace Unit field.

      Note: This field has a 5-character limit.

   d. Scale converts the output to the trace's units. Set the minimum and maximum unit range (y-axis scale).

6. (Optional) Select the Enabled checkbox for SIM 2 and repeat steps 5a—5d for a second external detector.
7. Click OK.

To disable the connection

▶ On the Device Input tab, clear the Enabled checkbox for the device and click OK.

Note: Disabling the SIM connection does not turn off the detector.

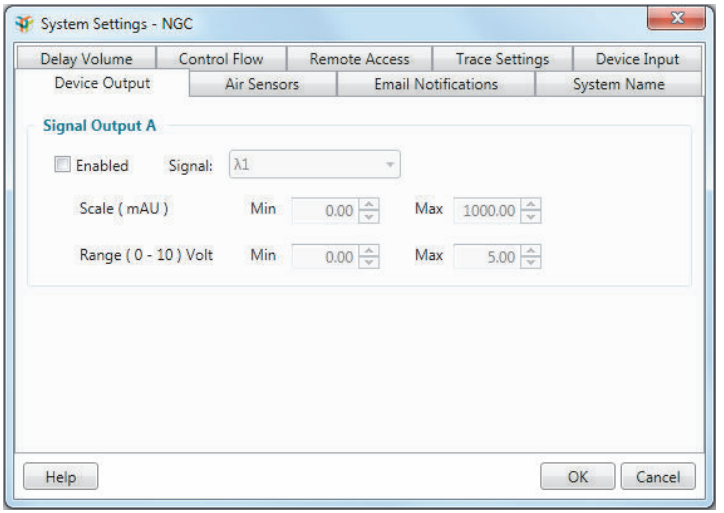
Device Output Tab

You can convert digital NGC data to analog voltage via the SIM and output the voltage to an external recording device, such as a light scattering detector. The SIM converts and outputs NGC trace information as voltage through its digital-to-analog converter (DAC) channels. When enabled, the external device can receive the converted trace information. For the device you can define its

- Signal type
- Minimum and maximum output range (from 0–10 volts)
- Minimum and maximum unit range

To enable output to external devices

1. Ensure that the SIM is connected to the NGC instrument.
2. On the back of the SIM, ensure that the device is connected to DAC A.
3. Select File > System Settings to open the System Settings dialog box.
4. Choose the Device Output tab.



5. By default, the fields in this dialog box are disabled. Select the Enabled checkbox for Signal Output A and provide the following details:

   a. Choose a signal type from the Signal dropdown list.

   b. Scale converts the trace units to the device's input type. Set the minimum and maximum unit range (y-axis scale).

   c. Range is the output to the device. The SIM's DAC channels output 0—10 V. Recommended settings within this range are specific to the external device. Refer to the device's user guide for more information.

6. Click OK.

To disable the connection

▶ On the Device Output tab, clear the Enabled checkbox and click OK.

Tip: Disabling the SIM connection does not turn off the external device.

Air Sensors Tab

The system supports up to two air sensor modules and up to eight air sensors. Each air sensor module can contain up to four air sensors. The system detects the number of air sensors that are attached to the instrument.

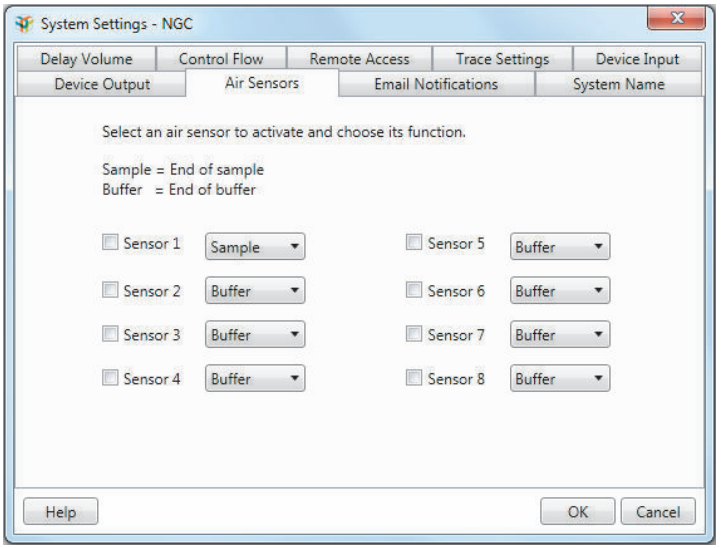
Air sensors can be set to detect air, indicating end of buffer, in lines connected to the system pumps. When the air sensor detects end of buffer, the system stops the system pumps and the run. You can continue the run after replenishing the buffer and purging the lines.

Air sensors can also be set to detect air, indicating end of sample, in lines connected to the sample pump. When the sample pump is used to load sample in manual mode and the air sensor detects end of sample, the system stops the sample pump to keep it from pumping air onto the column. When the air sensor detects end of sample during the sample application phase, the sample pump stops. In this case the run proceeds to the next phase.

To activate air sensors

1. Select File > System Settings to open the System Settings dialog box.
2. Choose the Air Sensors tab.

   By default, only the air sensors that the system detects are enabled. The other sensors are disabled.



3. To activate one or more air sensors, select its checkbox and from the dropdown list choose either Sample (to detect end of sample) or Buffer (to detect end of buffer).
4. Click OK.

To deactivate an air sensor

▶ On the Air Sensors tab, clear its checkbox and click OK.

Email Notifications Tab

You can connect ChromLab to your outgoing email server to send email notifications to a list of users. When this option is enabled, ChromLab sends messages informing users of specific event types such as

- Pumps stopped
- Method run completed successfully
- Method run stopped
- Connection to the NGC system is lost

The email identifies

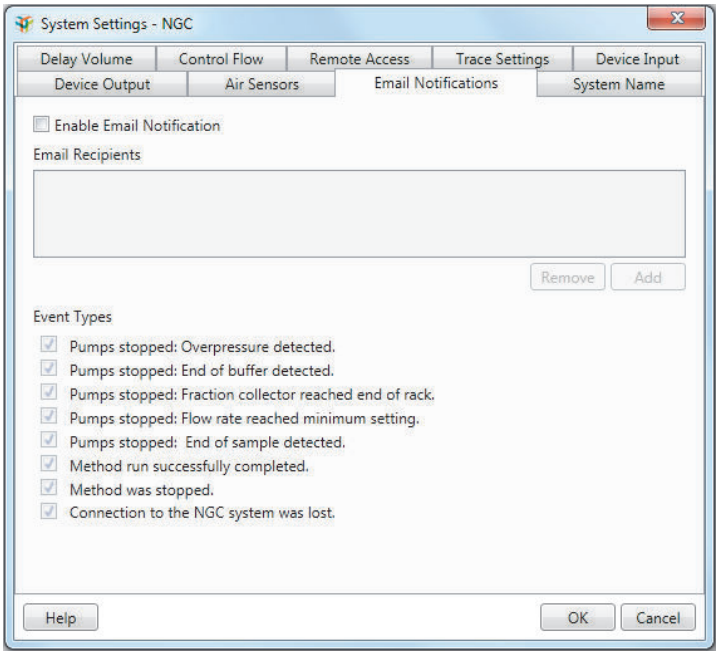
- Type of event
- Time of the event
- Name of the method that was running when the event occurred
- Elapsed time of the method before the event occurred

To set up the connection between ChromLab and your SMTP server see To connect ChromLab to an email server on page 92.

To set up email notifications

1. Select File > System Settings to open the System Settings dialog box.
2. Choose the Email Notifications tab.

   By default, email notification is not enabled.



3. Select Enable Email Notification.

   Note: If you have not set up a valid SMTP connection for ChromLab, an error message appears informing you that ChromLab cannot connect to the email server. Click OK. The Email Server dialog box appears. To set up the connection between ChromLab and your SMTP server see To connect ChromLab to an email server on page 92.

4. Below the Email Recipients box, click Add. The Add Email Address dialog box appears.

5. Type an email address in the text box and click OK.

   Perform this step for each user who wants to receive email alerts from ChromLab. You can also enter a user group email address to email a group of users.

All added users receive an email from DoNotReply_NGC notifying them that they are registered to receive email about NGC system events.

6. In the Event Types section, clear the checkboxes for the events about which you do not to want to receive email notifications.

   Note: All email recipients will receive email about all selected event types.

7. Click OK to save the changes and exit the dialog box.

To edit a name in the Email Recipients section

1. Double-click the name in the Email Recipients list. The Edit Email Address dialog box appears.
2. Modify the email address and click OK.

To remove a name from the Email Recipients list

▶ In the Email Recipients section, select the email recipient and click Remove.

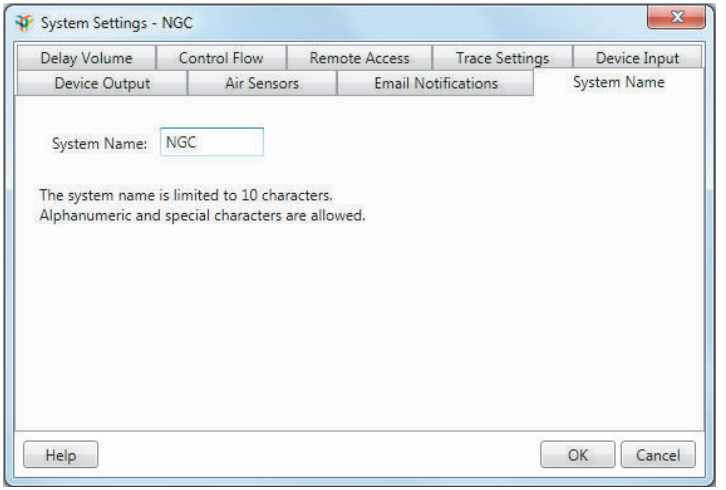
To stop all email notifications

▶ On the Email Notifications tab, clear the Enable Email Notifications and click OK.

System Name Tab

You can specify a unique name for the NGC instrument. ChromLab uses this name to identify the system to which the computer is connected. The system name appears in the Home window when ChromLab connects to the NGC system. It also appears in the formatted run reports to identify the system on which the run was generated.

Note: The system name is limited to any combination of 10 alphanumeric and special characters.



System Information

The System Information dialog box enables you to view general information about your system as well as information about the system components, processes, and UV and UV/Vis detectors.

Note: This dialog box is accessible in manual mode and from the menu on the touch screen.

General Tab

This tab displays details about the NGC instrument, including

- Type of configuration (for example, NGC Discover 10)
- System's serial number
- Name of your NGC system
- Name of the ChromLab computer
- Version of ChromLab software running on the system
- System's IP address
- System's network name
- System's available disk space
- System's BIOS information (including the BIOS version and build date)

Devices Tab

This tab displays details about each module on the NGC instrument, including its version number and serial number. This information is useful when you need to order replacement modules.

Process Tab

This tab displays details about the processes running on the NGC instrument, as well as on the ChromLab computer and the touch screen.

Detector Tab

This tab displays details about UV or UV/Vis lamp usage, from which you can determine whether the lamps need to be replaced. The system determines which detector module is installed and displays information specific to that module.

Single-Wavelength Detector

The system retrieves the lamp status for both lamps (255 nm and 280 nm) and displays the reference voltage, pulse-width modulation (PWM) percentage, and total lamp hours for each lamp on the Detector tab.

The system must have stopped running before lamp status can be determined. If the pumps are running, a warning message appears when you click Get Lamp Status, informing you that the system is unable to acquire the lamp status because the system is busy.

The system turns off the lamp if it is on when the pumps stop running. The system then turns the lamp on, which updates the PWM percentage data. The system sets the wavelength to 255 nm, waits for the reference signal to stabilize, and reads the reference signal data. The system then sets the wavelength to 280 nm and follows the same protocol. The values appear on the Detector tab after the data for both wavelengths have been determined.

You can use the lamp usage hours or the PWM percentage to determine how close the lamp is to its end of life. Higher percentage values indicate lower usefulness.

After you change the lamps, reset the lamp hours to reflect the new lamps' usefulness.

To display the signal and lamp usage time

1. Select File > System Settings to open the System Information dialog box.
2. Choose the Detector tab. The empty detector screen appears.
3. Click Get Lamp Status.
4. Click Close to close the System Information dialog box.

To reset the lamp hours

▶ On the Detector tab, click Reset Lamp Time.

Note: A dialog box appears warning you that this cannot be undone. Click No if you do not want to reset lamp hours.

Multi-Wavelength Detector

The tab displays the measured signal counts at specific wavelengths. It also displays the lamp usage time (in hours) for both the deuterium and tungsten lamps.

The system must have stopped running before lamp status can be determined. If the pumps are running, a warning message appears when you click Get Lamp Status, informing you that the system is unable to acquire the lamp status because the system is busy.

Note: The lamps must be turned on and in Standby mode. If the lamps are off, a warning message appears when you click Get Lamp Status, informing you that the lamps must be turned on and the detector must be in Standby mode. In Manual mode, turn the lamps on and wait for the lamps to display Standby. This might take some time.

When the pumps are idle and the lamps' signal is stable, the system sets the deuterium lamp to 240 nm and the tungsten lamp to 600 nm. After the measurements are complete, the tab displays the measured reference counts and lamp usage data (in hours). After you close the dialog box, the system restores the detector to the wavelength that was set before the procedure started. You can use the lamp usage hours to determine how close the lamps are to their end of life.

To display the reference counts and lamp usage time

1. Select File > System Settings to open the System Information dialog box.
2. Choose the Detector tab. The empty detector screen appears.
3. Click Get Lamp Status.
4. Click Close to close the System Information dialog box.

Preferences

The Preference dialog box enables you to select pressure units for all system and software pressure values. This is a global setting.

This dialog box also enables you to connect ChromLab to your internal email server. ChromLab can then send email alerts about specific system events.

Email Server Tab

Note: See your system administrator to connect ChromLab to the internal email server.

ChromLab can connect to your internal email server and send email notifications about system events to a list of users.

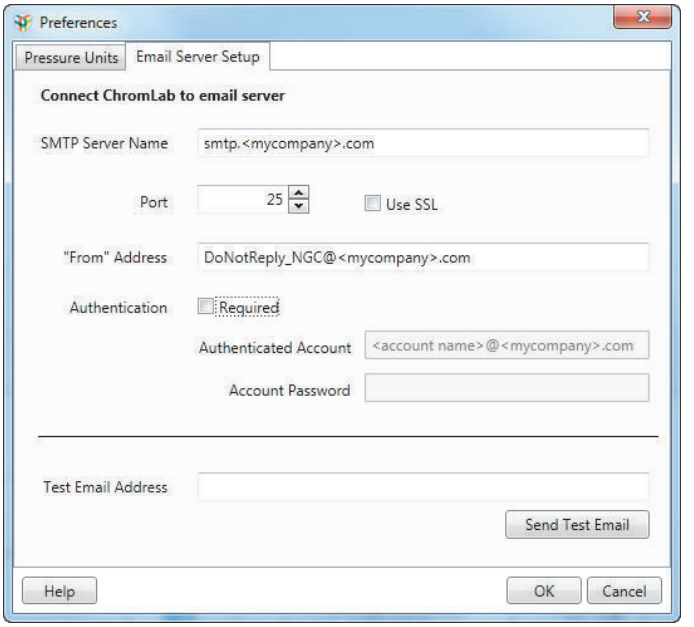
The email identifies

- Type of event
- Time of the event
- Name of the method that was running when the event occurred
- Elapsed time of the method before the event occurred

To enable email notification see To set up email notifications on page 84.

To connect ChromLab to an email server

1. In ChromLab select File > Preferences.
2. Choose the Email Server Setup tab.



3. Provide the following information for your company:
   - **SMTP server name** — the name of the outgoing email server at your company.
   - **Port** — the port number for the SMTP server. The default is 25.
   - **Use SSL** — by default Secure Socket Layer (SSL) is disabled. If you use SSL at your company, select this checkbox.
   - **From address** — the name of the email server at your company.

- **Authentication** — by default, Authentication is disabled. If your site requires account authentication, select this checkbox. The Authenticated Account and Password fields become active.
- **Authenticated Account** — the name of the authenticated account.
- **Account Password** — the password for the authenticated account.

4. To verify that the SMTP server settings are correct, enter a valid email address in the Test Email Address field and click Send Test Email.
5. Click OK to close the dialog box.

4 Performing a Manual Run

The NGC™ chromatography system instrument operates in two modes: manual mode and automated, programmed method mode. In manual mode you have full control of each NGC pump, valve, detector, and fraction collector. This mode is used primarily for nonautomated processes such as priming and cleaning or purging the system, but it can also be used to load samples, optimize chromatography parameters, and run simple experiments.

Method mode enables you to execute preprogrammed steps automatically. See Chapter 5, Method Editor, and Chapter 6, Creating a Method, for information about working in method mode.

When the NGC system is turned on, the instrument touch screen displays the Home window in manual mode. By default the system remains in manual mode unless calibration is in progress or a method is running.

You can enter the required instrument settings by double-clicking the individual module dialog boxes in the fluidic scheme. This chapter describes these settings. See also Chapter 3, Preparing the Instrument, in the NGC Chromatography Systems and ChromLab Software Instrument Guide for more detailed information on tasks described in this chapter.

Manual tasks can be performed in the System Control window using the computer connected to the NGC instrument or on the touch screen. Runs performed manually can be saved to the ChromLab™ database located on the computer.

Note: The NGC system can be operated in manual mode without a connected computer.

Preparing the System

System preparation consists of priming the system to remove air from the pumps, tubing, and valves followed by flushing the fluidics system to remove storage buffer, cleaning solutions, or old buffer. Priming is performed in manual mode, while both purging and cleaning tasks can be performed either manually or through preprogrammed method phases. See Chapter 5, Method Editor for more information about working with methods.

Priming and Purging the System

Perform the following procedures consecutively to prime and purge your NGC systems.

Important: Whenever you add or remove a module or change or upgrade your NGC system hardware configuration, you must replumb and reprime the system.

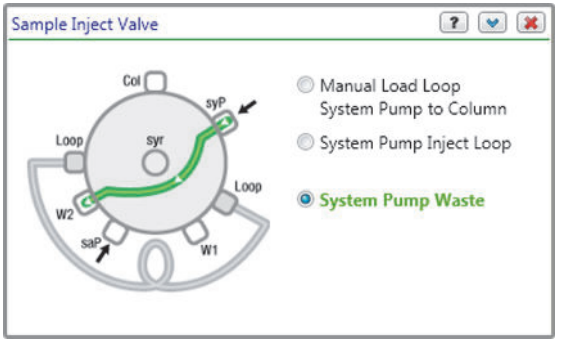
To prime the system

1. Power on the NGC system and its connected computer.
2. To enter manual mode, do one of the following:
   - On the computer, click Manual Run in the ChromLab Home window or click the System Control tab and then click in the fluidic scheme.
   - On the touch screen, click Enter Manual Mode if the application is not already in manual mode.
3. (Optional) If your hardware configuration has changed, select a fluidic scheme that matches your current system configuration.
   a. Select Tools > Change Fluidic Scheme.
   b. Select the appropriate fluidic scheme in the Fluidic Scheme Selector pane.
   c. Click Select to choose the manual mode fluidic scheme.
4. Insert appropriate inlet lines into the buffer, storage solution, and/or cleaning solutions to be primed and flushed through the system.

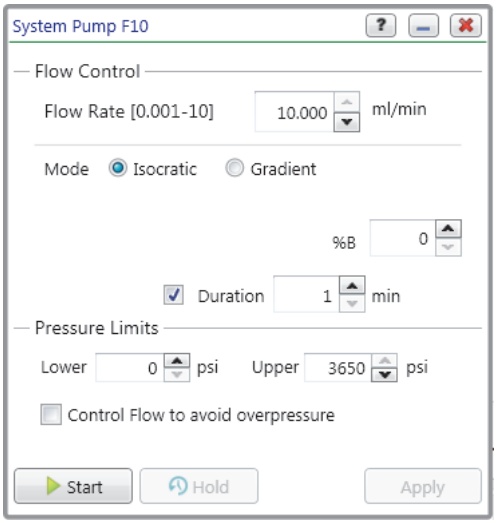
5. Prime the system. (See Priming and Purging the Systems in Chapter 3 of the NGC Chromatography Systems and ChromLab Software Instrument Guide for detailed instructions.)

To purge the NGC Quest system pumps

1. In the fluidic scheme, touch or click the Sample Inject valve module to open its dialog box.
2. Set the sample inject valve to System Pump Waste.



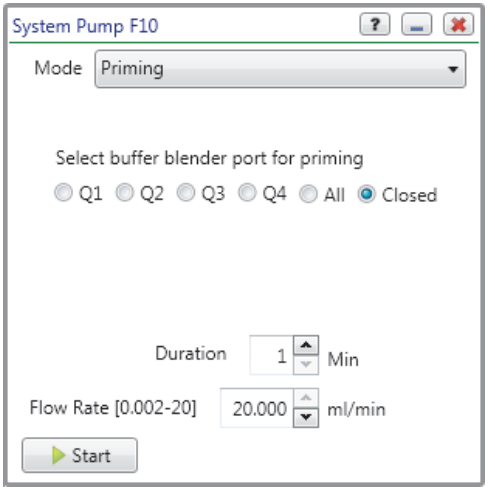
3. In the fluidic scheme, double-click the System Pump module to open its dialog box.



4. In the System Pump dialog box:
    a. Set Flow Rate to 10 ml/min.
    b. (Optional) Set Duration to at least 1 min.
    c. If selected, clear the Enable Air Sensor checkbox.
    d. Click Start.

To purge the buffer blending valve

1. In the fluidic scheme, double-click the System Pump module to open its dialog box.
2. Select Priming in the Mode dropdown list.



- To purge an individual line of air bubbles, proceed to step 3 on page 100.
- To purge all lines of air bubbles sequentially, proceed to step 4 on page 100.

Tip: The available buffer blending valve modes are defined as follows:

Mode	Description
Buffer Blending	Blends stock acid, base, water, and salt solutions to form isocratic or linear buffer gradients at a user-specified buffer concentration and pH using the Buffer Blending module.
Priming	Opens the buffer blending inlet ports for priming and turns the pumps on to flush the inlet line and pumps with the buffer at all four ports.
Gradient via Inlet Valves	Buffers are selected at the inlet valve. Gradients are made by pumps A and B using the selected buffers.

Mode	Description
Gradient via Blending Valve (High Flow)	Buffer blending valve ports Q1 and Q4 are used as inlets for buffers A and B. Gradients are formed by the buffer blending valve rather than by the pumps. This enables each pump to run at their full flow rate capacity which doubles the total flow rate, for example to 20 ml/min for F10 pumps.

3. To purge an individual line of air bubbles and fill it with buffer:
   a. Select a port to open.
   b. (Optional) Change the default flow rate. The default flow rate is set to 20 ml/min.
   c. Click Start.
   d. Repeat steps 3a–c for any other port to purge.
4. To purge all lines of air bubbles and fill them with buffer:
   a. Click All.
   b. (Optional) Change the default flow rate. The default flow rate is set to 20 ml/min.
   c. Click Start.
5. Monitor the pump pressure and outlet flow to ensure that the fluid is flowing properly.

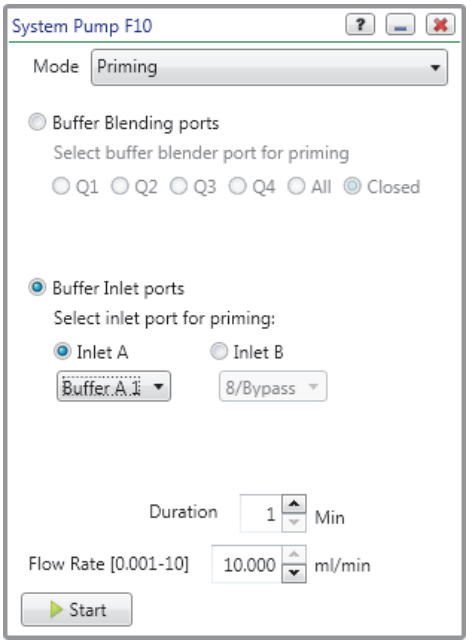
   The system pumps and the buffer blending valve start operating at the specified flow rate, exchanging the solution in the inlet lines and expelling any trapped bubbles, cycling sequentially through the ports Q1 to Q4. After the specified duration, the pumps stop momentarily while the blending valve switches to port Q3 in order to flush the lines with water before closing the valve. No change is allowed during this process for the specified duration.

   Note: You can stop the cycle before it completes by clicking Stop. Before stopping the purge cycle, ensure that all buffer lines are filled with the appropriate buffers before starting a run.

To purge the buffer inlet valves (if available)

1. Open the System Pump dialog box and select Priming on the Mode dropdown list.

   Note: When in Priming mode, the sample inject valve switches the system pump flow to waste (W2). After the process is completed, the sample inject valve switches the system pump to manually load the column through the loop.



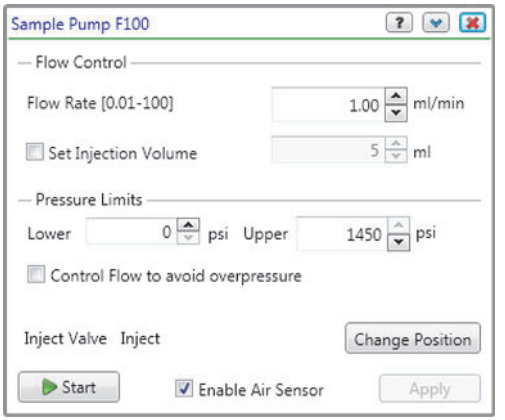
2. Select the first buffer port to purge, for example Buffer A 1.
3. (Optional) Change the default flow rate.

   The default flow rate is set to 10 ml/min.

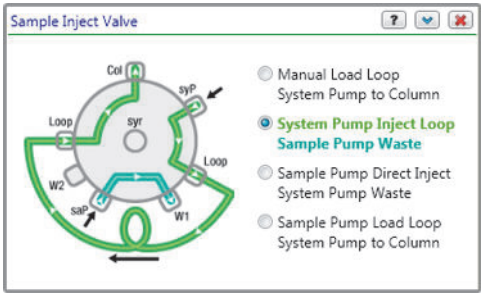
4. Click Start.
5. Repeat steps 2–4 for the remaining buffer inlet ports.

To purge the sample pump (if available)

1. In the fluidic scheme, double-click the Sample Pump module to open its dialog box.



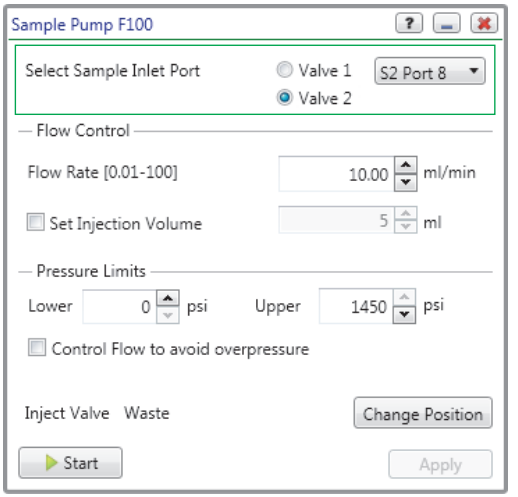
2. Set Flow Rate to 10 ml/min.
3. Click Change Position to set the sample inject valve to Sample Pump Waste and close the dialog box.



4. (Optional) When an air sensor is present, in the Sample Pump dialog box, select Enable Air Sensor to use end-of-sample detection to stop the pump.
5. Click Start.
6. Monitor the pump pressure and outlet flow to ensure that no air is in the lines and that the fluid is flowing properly.

To purge sample inlet valves (if available)

1. In the fluidic scheme, double-click the Sample Pump module to open its dialog box.



2. Select a port to purge.
   - If only one sample inlet valve is present, select S1 Port 8 from the dropdown list.
   - If two sample inlet valves are present, click Valve 2 and select S2 Port 8 from the dropdown list.
3. Set Flow Rate to 10 ml/min.
4. Click Change Position to set the sample inject valve to Sample Pump Waste and close the dialog box.
5. (Optional) When an air sensor is present, select Enable Air Sensor in the Sample Pump dialog box to use end-of-sample detection to stop the pump.
6. Click Start.
7. Monitor the pump pressure and outlet flow to ensure that no air is in the lines and that the fluid is flowing properly.

Cleaning the System

System cleaning consists of rinsing the system to remove buffers, washing with base to hydrolyze any adsorbed proteinaceous material from the fluidic system, and rinsing again to remove the base. The system can also be cleaned in method mode. See Chapter 5, Method Editor for details.

To clean the system manually

1. Take the column offline.
2. Place both pump inlet lines in water, set %B to 50%, and flush the system while simultaneously switching valves through each position.
3. Place both pump inlet lines in 1 M sodium hydroxide, set %B to 50%, and flush the system while simultaneously switching valves through each position. Monitor system cleaning using the single- or multi-wavelength conductivity monitor. Flush until conductivity monitor reads max conductance and is stable.
4. Place both pump inlet lines in water, set %B to 50%, and flush the system thoroughly to remove all sodium hydroxide while simultaneously switching valves through each position. Monitor the system rinsing using the conductivity monitor. Rinse until conductivity monitor reads zero.
5. (Optional) Place both pump inlet lines in storage solution (for example, 20% ethanol), set %B to 50%, and flush the system with the solution while simultaneously switching valves through each position.

Running an Experiment

Important: When running an experiment manually, ensure that the valves are set in the correct positions before starting the pump.

Clicking Start in the System Pump dialog box starts the run. You can change valve positions while the pump is running and your manual run is in progress. You can stop a run at any point and save it. You can then restart the pumps and continue the run with a different name.

Note: Complete the following procedures consecutively to perform a manual run.

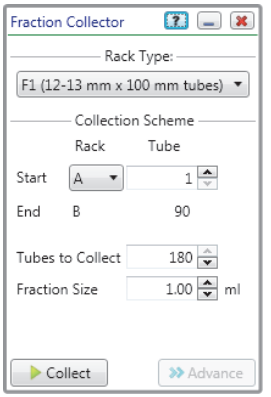
To select a fluidic scheme and install accessories

1. (Optional) If your hardware configuration has changed, do one of the following:
   - Enter manual control mode on the touch screen.
   - On the computer, start ChromLab software and click Manual Run in the Home window.
2. Select Tools > Change Fluidic Scheme and choose the fluidic scheme that matches the configuration of the devices connected to your NGC system.

   Note: If your NGC system includes multiple valves of the same type and this is the first time you are using the fluidic scheme, the Fluidic Scheme Mapping dialog box appears. Use this dialog box to map your NGC system to the fluidic scheme.
3. Install the accessories required to run the experiment:
   a. Connect a column.
   b. Install fraction collector racks and tubes.

To set up the experiment in the fluidic scheme

1. Double-click the Fraction Collector module and choose the following settings in the dialog box that appears.



   a. In the Rack Type dropdown list, select the installed rack type.

   b. Under Collection Scheme, select the fraction collection Start Rack and Tube, number of Tubes to Collect, and Fraction Size.

   c. Press Collect.

2. (Optional) Double-click the pH module, if available, and set the valve position:

   - Select Bypass to bypass the pH electrode.
   - Select pH to direct flow through the pH flow cell and monitor the pH during the run.

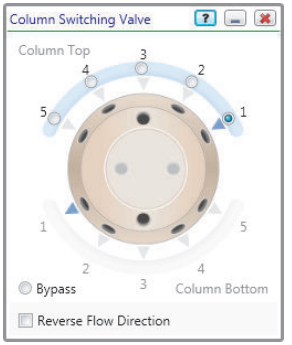
3. Double-click the Single- or Multi-Wavelength UV Detector plus Conductivity Monitor module, choose the following settings, and then close the dialog box.

   a. Select the wavelengths for monitoring the experiment: 280 nm for proteins containing tryptophans and tyrosines; 255 nm for nucleic acids; and, for multi-wavelength detection only, 215 nm for the amide bond of peptides.

   b. Turn the lamp On if it is off.

   c. Click Zero Baseline to zero the UV lamp.

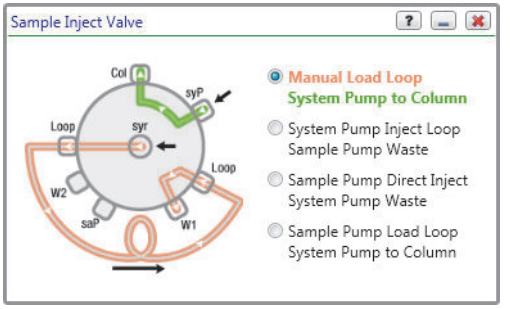
4. (Optional) Double-click the Column Switching Valve module, if installed, choose the following settings, and close the dialog box.



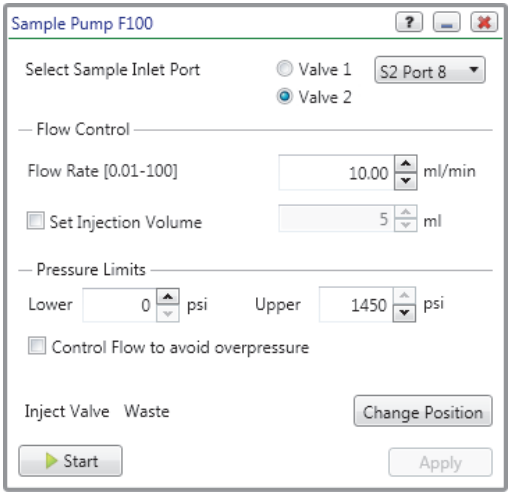
   a. Select the column position or click Bypass.

   b. (Optional) Select the Reverse Flow Direction checkbox to cause the buffer to flow from the column bottom to its top instead of from top to bottom.

5. Double-click the Sample Inject Valve module and set the valve to Manual Load Loop.



6. Double-click the Sample Pump module, if installed, and choose the following settings in the dialog box that appears:



   a. If one or two sample inlet valves are in line, select a port from which to draw sample.

   b. Under Flow Control, select the flow rate.

   c. Under Pressure Limits, select the lower and upper pressure limits.

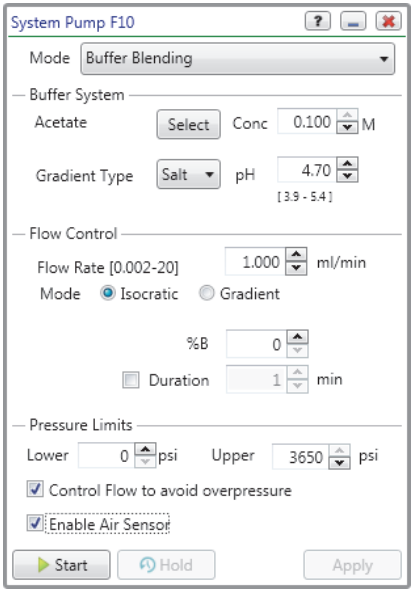
   d. (Optional) Select Control Flow to avoid overpressure. This setting causes the flow rate to decrease automatically if the system exceeds set pressure limits.

   e. If not already set, click Change Position and select the position for the inject valve.

   f. (Optional) Select Enable Air Sensor if air sensors are in line and set to detect end of sample.

Important: Select flow control settings for the run before you click Start. Clicking Start starts the run.

7. Double-click the System Pump module and choose the following settings in the dialog box that appears:



   a. If a buffer blending module is in line, select a Mode (Isocratic or Gradient) under Flow Control.

   b. Under Pressure Limits, select the lower and upper pressure limits.

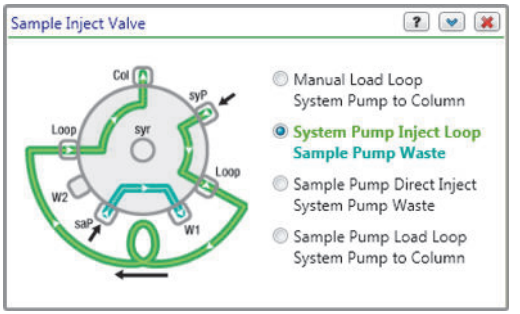
   c. (Optional) Select Control Flow to avoid overpressure. This setting causes the flow rate to decrease automatically if the system exceeds set pressure limits.

   d. (Optional) Select Enable Air Sensor if air sensors are in line and set to detect end of buffer.

Important: Select flow control settings for the run before you click Start. Clicking Start starts the run.

To run an experiment step

1. Isocratic or gradient step:
    a. In the System Pump module dialog box, under Flow Control, set the Flow Rate, Mode (Isocratic or Gradient), %B value, Start and End values (in Gradient mode), and Duration (optional for Isocratic mode) for the run.
    b. Click Start to begin the run.
    c. Minimize the Sample Inject Valve dialog box.
2. Sample injection step:
    a. With the valve in the Manual Load Loop position, use a syringe to inject sample through the sample inject port into the sample loop.



    b. Change the valve position from Manual Load Loop to Inject Sample.
    c. When the sample finishes injecting, switch the valve back to Manual Load Loop.

Changing Module Settings

Tip: For some modules, you must stop the pumps before you can change the settings.

To change module settings

1. In the fluidic scheme, double-click a module.
2. In the dialog box that appears, edit module options.
3. Click Apply (where necessary).

Stopping a Manual Run

To stop a manual run

▶ Click Stop in the System Pump dialog box.

Clearing Run Data

A manual run must be cleared before you can run a method. You can clear the data and start a new manual or method run.

To clear run data

▶ Click Clear in the System Control window toolbar.

Saving a Manual Run

Saving a manual run saves the chromatographic data and run log. You can open the saved chromatogram in the Evaluation window.

To save a manual run

▶ Click Save on the System Control window toolbar to save manual run data.

Important: If you do not save the manual run before you switch to a method run, the manual run data are lost.

Saving a Manual Run on the Touch Screen

To save a manual run on the touch screen

1. Click Save on the touch screen toolbar.

   The Save Manual Run dialog box appears.

2. Do one of the following:

   - In the Projects pane, select a project or subproject.
   - In the toolbar, click the appropriate button to create a new project, subproject, or root project and type a name for the project in the box that appears above the Projects pane.

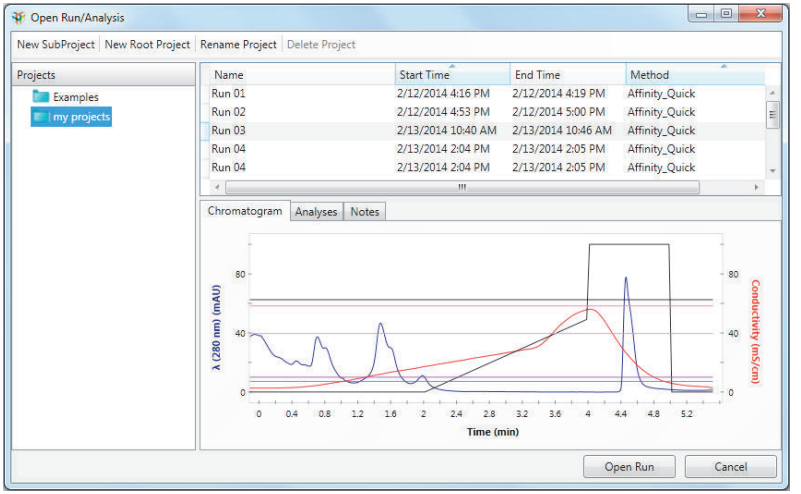
3. Type a name for the run in the Manual Run Name box.
4. (Optional) In the Notes box, type information about the run.
5. Click Save.

Viewing Run Data

Note: See Chapter 7, Evaluating Results, for detailed information about analyzing a run.

When you select a single saved run in the Open Run/Analysis dialog box, a read-only image of its chromatogram appears in the pane at the bottom of the dialog box.

Clicking the Analysis tab displays saved analysis data, if they are available. Clicking the Notes tab displays the text entered when the run was saved.



To view saved run data in the Evaluation window

1. In the Home window, click Open Run/Analysis in the Evaluation pane. The Open Run/Analysis dialog box appears.

   By default, runs are saved in the My Projects folder which appears in the left pane. All saved run files appear in the upper right pane.

2. Highlight the file to view a read-only chromatogram of the saved run in the Chromatogram tab.

See Managing Runs on page 221 for information on renaming and deleting runs.

5 Method Editor

This chapter introduces ChromLab™ software Method Editor concepts and functionality and prepares you to create a method, the subject of Chapter 6.

A method consists of a list of standard or custom phases that are executed in a sequence. Each phase consists of a list of method steps. All of these steps and phases are associated with elements of the fluidic scheme. When the fluidic scheme changes, Method Editor settings change to match it.

The Method Editor automates chromatography runs. Create a method outline using standard chromatography phases such as equilibrium, sample application, and elution, and adjust the parameters to meet experiment needs.

The Method Editor makes it easy to create a method quickly, and it is flexible and powerful enough to create complex methods. Method Editor features include the following:

- Standard phases for fast method creation
- Fluidic scheme that depicts the devices present in the system and the flow path between them
- Gradient graph that displays the method's elution profile as a function of %B
- Ability to edit method steps editing and to create custom phases
- Ability to save custom phases for specific applications
- Ability to save method templates for reuse
- Ability to export methods and print method reports

Before You Begin

Before you work with Method Editor features, it is important to set as the default the fluidic scheme that matches the plumbing and configuration of your NGC™ system. When you run a method, ChromLab expects the system to be plumbed exactly like the fluidic scheme. The fluidic scheme selected also determines the phase parameter settings that appear in the Method Settings view.

See Fluidic Scheme Configurations on page 53 for more information.

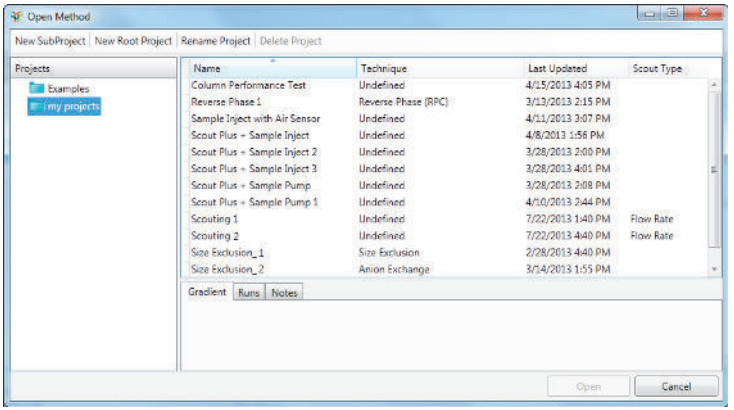
Accessing Method Editor Features

In the Home window, the Method Editor pane displays commands that access the following:

- Opening a method template
- Reviewing, editing, or running a method
- Creating a new method
- Selecting a method from a list of recent methods

Opening a Method

When you select Open Method, the Open Method dialog box appears.



This dialog box lists saved projects in the left pane. Saved methods in the selected project are listed in the upper right pane. The lower-right pane displays information about the selected method in three tabs:

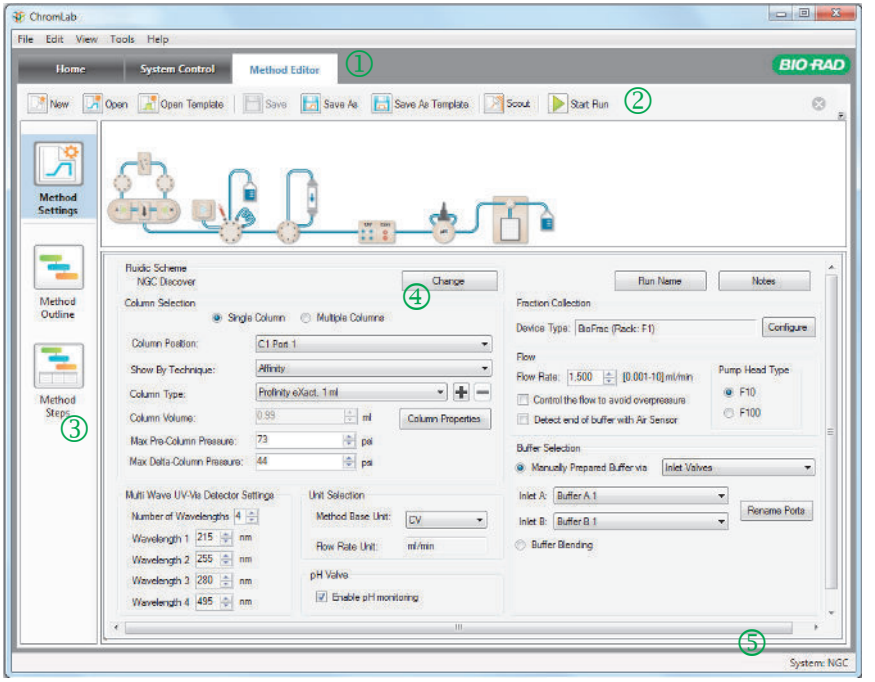
- The Gradient tab displays the gradient graph for the selected method.
- The Runs tab displays a list of runs in the selected method.
- The Notes tab displays text entered when the run was saved.

To open a method

1. Do one of the following:
   - On the Home window, click Open Method.
   - In the Method Editor, select File > Open Method.
2. In the Open Method dialog box, select a method and click Open to launch it in the Method Editor window.

Method Editor Window

The Method Editor enables you to create, open, import, export, review, edit, and run a method or a redefined method template. The Method Editor window presents several panes, which differ depending on the selected view. By default, the Method Editor opens displaying the method settings for the selected method.



LEGEND

1	Tabs access main functional areas.
2	Toolbar commands provide quick access to menu options.

LEGEND

3	The left pane displays the method view buttons: ■ **Method Settings** — (default view) displays the fluidic scheme and method settings for the selected method. ■ **Method Outline** — displays the method outline, gradient graph, and phase parameters for the selected method. ■ **Method Steps** — displays the method outline, gradient graph, and method steps for the selected method.
4	The main pane displays the method details, depending on the selected view.
5	The status bar displays the connected NGC instrument.

File Menu Commands

New Method — opens the Method Editor window in which you can create a method.

Open Method — opens a dialog box in which you can select a project and open a method.

Open Template — opens a dialog box, which lists all method templates and their details along with the displayed method's gradient.

Save — saves the current method.

Save As — saves the current method with a new name that you supply.

Save As Template — saves the current method as a template.

Rename — opens the Rename Method dialog box in which the current method name appears. You can rename the method using up to 50 characters.

Delete — deletes only the currently open method or the method and its associated runs. Does not delete an associated run that is open in the Evaluation window or is part of a multi-run analysis.

Start Run — sends the programmed method to the instrument to be run once or many times.

Close Method — closes the current method and returns you to the Home window.

Export Method — exports a saved regular or scouting method to the desktop or to a directory and file name you choose and appends the file extension .ngcMethod. Exported methods can be imported into another NGC system.

Export Method with Runs — exports a saved method with runs to the desktop or to a directory and file name you choose and appends the file extension .ngcMethodRuns. Exported methods and their runs can be imported into another NGC system. If the method has been saved multiple times, this command exports the most recently saved method and its associated runs.

Import — opens a dialog box in which you can select a project and a method file (including a scouting method file), select a unique name for the method file, and then import the file into the project.

Method Report — generates a formatted report of all information about the current method. You can print the report and save it in .pdf, .ppt, and .doc file formats.

Preferences — opens dialog boxes in which you can do the following:

- Select pressure units for all system and software pressure values. This is a global setting.
- Set up an SMTP server to send email messages about system notifications from the ChromLab computer.

Exit — closes ChromLab.

Edit Menu Commands

Delete Phase — opens a dialog box in which you can verify deleting the phase and delete it.

Save As New Phase — opens a dialog box in which you can specify a name for the new custom phase and save the phase.

Rename Phase — opens a dialog box in which you can rename the phase.

View Menu Command

Show Gradient Graph — displays the gradient graph of the current method. Clearing this command hides the gradient graph from view.

Tools Menu Command

Flow Rate Converter— opens the Flow Rate Converter tool, which enables you to determine the flow rate to use for each column in the method based on the column size and the initial rate entered. A rate entered in ml/min is converted to cm/h and L/h; a rate entered in cm/hr is converted to ml/min and L/h. You can copy the result in the converter and paste it into your method.

Help Menu Commands

Help — displays screen-level help topics and links to installed manuals.

Export Diagnostic Logs — opens the Export Diagnostic Logs dialog box in which you can export all critical information that Bio-Rad Technical Support requires to diagnose issues. The log files and data are zipped and saved to a location that you choose. See Exporting Diagnostic Logs on page 275 for more information.

About — displays version and copyright information about ChromLab software.

Toolbar Commands

New — opens the Method Editor window in which you can create a method.

Open — opens a dialog box in which you can select a project and open a method.

Open Template — opens a dialog box, which lists all method templates and their details along with the displayed method's gradient.

Save — saves the current method.

Save As — saves the current method with a new name that you supply.

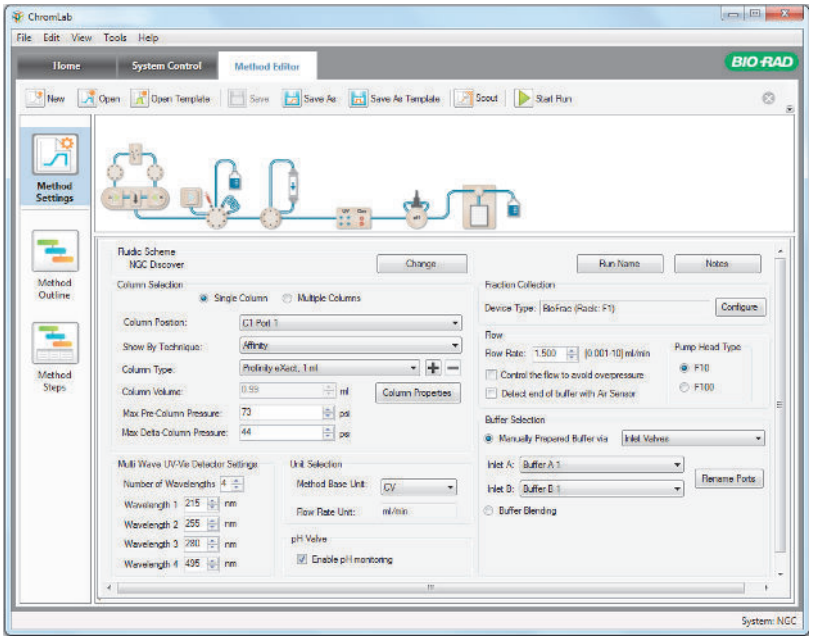
Save As Template — saves the current method as a template.

Scout — opens the scouting wizard, from which you can create scouting runs based on the phases of the currently displayed method. For more information about using the scouting wizard, see Scouting on Parameters within a Method on page 184.

Start Run — sends the programmed method to the instrument to be run once or many times.

Method Settings View

The default view, the Method Settings view displays the fluidic scheme and method settings for the selected method. When you create a new method, the Method Settings view displays the fluidic scheme of the NGC instrument.



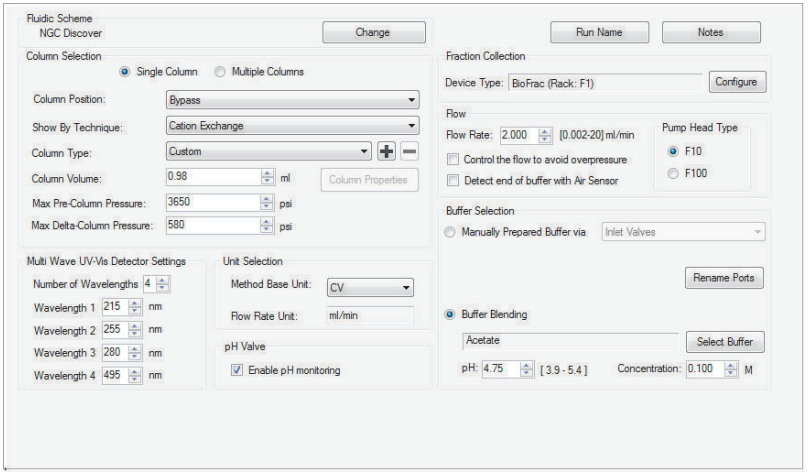
Fluidic Scheme Pane

The fluidic scheme, for visual reference only, displays the current instrument configuration and flow path. Parameters available in each phase are specific to the devices in the fluidic scheme that participate in the phase.

For information about changing the fluidic scheme, see Fluidic Scheme on page 133.

Method Settings Pane

The Method Settings pane displays detailed settings for the selected method.

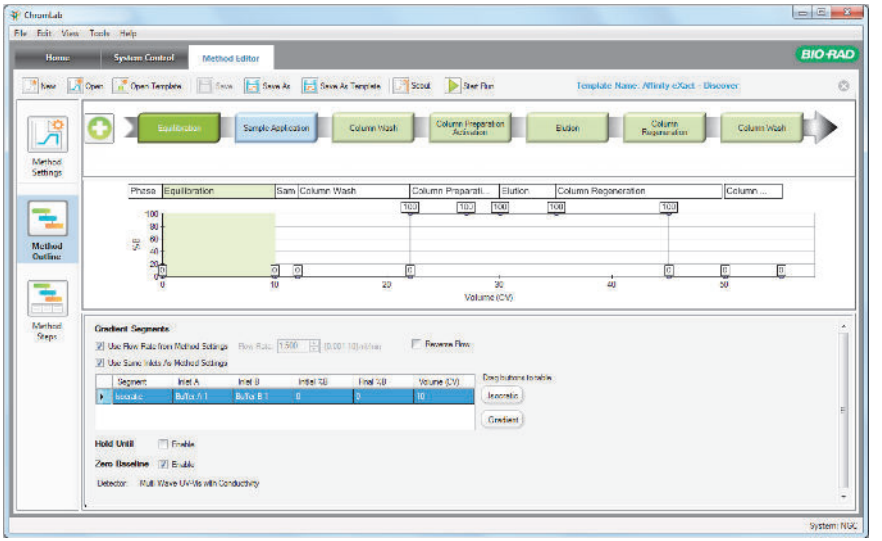


In this pane you can define the parameters of each method phase. Parameters vary depending on the phase and the devices available on the system that is to participate in that method phase. Some global method parameters defined in the Method Settings pane can be altered for each phase. Such parameters include flow rate, buffer selection, pH, and fraction volume.

For detailed information about method settings, see Method Settings Parameters on page 133.

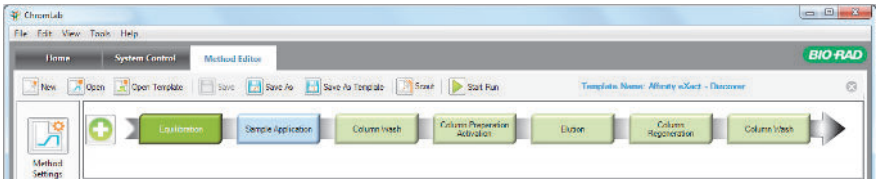
Method Outline View

When selected, the Method Outline view displays the method outline, gradient graph, and phase parameters for the selected method. When you create a new method, the Method Outline view displays the Phase Library, from which you can drag phases onto the method outline to create a custom method.



Method Outline Pane

Visible when you select either Method Outline or Method Steps in the left pane, the Method Outline pane displays phases in the order in which they are executed.



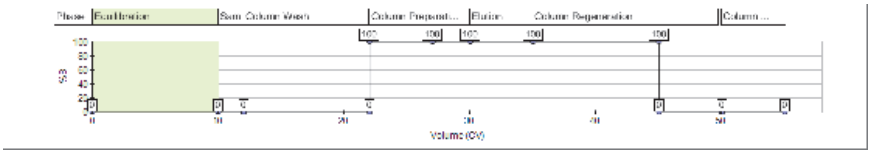
As you drag additional phases from the Phase Library into the Method Outline pane, they appear in the order in which you add them. You can reorder phases by dragging them to a new location. You can remove a phase from the method outline by right-clicking it and choosing Delete in the menu that appears or by selecting the phase and pressing Delete on your keyboard. You can also save a phase as a new custom phase or rename the phase by right-clicking it and choosing the appropriate command in the menu that appears.

Selecting a phase in the method outline highlights the same phase in the gradient graph pane. When Method Outline is selected in the left pane, the parameters for the selected phase appear in the bottom pane. When Method Steps is selected in the left pane, the selected phase is highlighted in the method steps table in the bottom pane.

Tip: Use the slider that appears below the method outline to quickly advance or return to phases that might not appear within the boundaries of the pane.

Gradient Graph Pane

Visible when the method outline is present, the gradient graph displays the programmed gradient and break points for flow segments with a gradient step.



The segments on the graph correspond to the phases in the method outline. You can edit gradient duration and slope by dragging black dots to new locations. The changes are reflected in the phase parameter settings in the Method Outline pane.

Hiding the Gradient Graph Pane

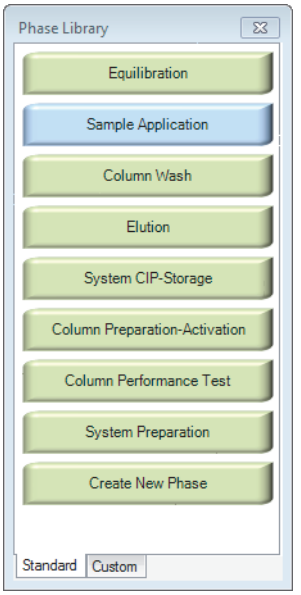
You can hide the gradient graph pane when more space is needed to view phase parameters or method steps.

To hide the gradient graph pane

- ▶ Choose View > Show Gradient Graph to clear the checkbox and hide the gradient graph.

Phase Library

Visible when you select New Method in the Method Editor window or click the Add Phase button in the Method Outline pane, the Phase Library comprises both standard and custom phases that you can use to create methods.



Each phase consists of a series of programmed steps that represent a process in chromatography. You can add or remove steps from a phase to customize it for a specific application. You can save modified phases as custom phases in the Custom tab where they will be available to all ChromLab software users. Standard phases are further described in Standard Phases on page 146.

Table 4. Standard phases

Phase	Explanation
Equilibration	Equilibrates the column before or after elution.
Sample Application	Applies sample to the column. Defines mode of application, either direct or through sample loop, sample volume, flow rate, and buffers used for sample loading.
Column Wash	Washes out unbound proteins (flow through) after sample application. When used after an elution, the conditions defined would remove proteins bound strongly to the media under conditions used for elution.
Elution	Elutes the sample from the column.
System CIP (Clean in Place)/ Storage	Rinses the system with a cleaning solution. This phase pulls from a single cleaning or storage solution. **Note:** System CIP must be run as the only type of phase in the method. You can add several System CIP phases to the method if multiple cleaning solutions are required.
Column Preparation-Activation	Before column use, removes the storage solution and equilibrates the column. By repeating the phase multiple times, several preparation solutions can be used one after another.
Column Performance Test	Tests the efficiency of a packed column in terms of height equivalent to a theoretical plate (HETP) and the peak asymmetry factor (A_s).

Table 4. Standard phases, continued

Phase	Explanation
System Preparation	Before a run, removes storage solution and fills the system and inlets with buffer solution. This phase pulls from a single buffer solution each time the phase is used. **Note:** System Preparation must be run as the only type of phase in the method. You can add several System Preparation phases to the method if cycling between multiple solutions is required.
Create New Phase	Adds steps from the Step Library to create a phase and store it in the custom phase library.

Adding a Phase to a Method

To add a phase to a method

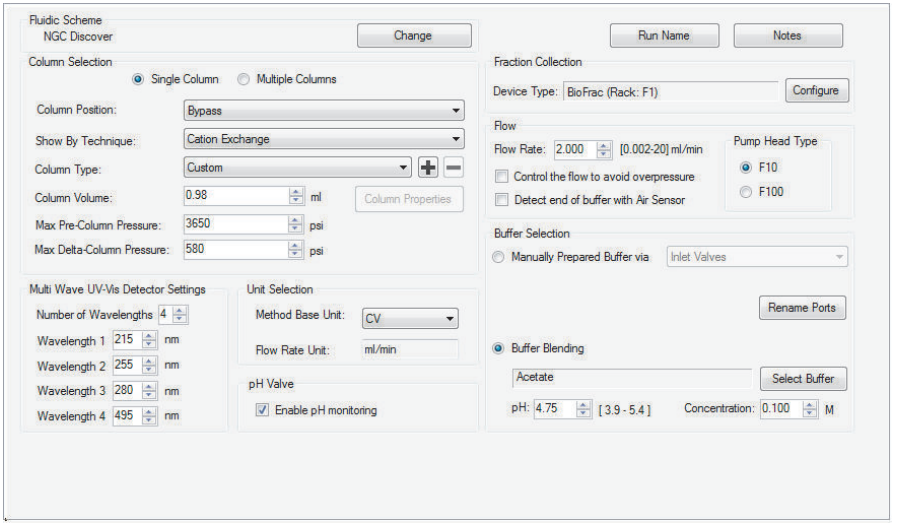
1. In the Method Outline view, click the Add Phase button to display the Phase Library if it is not open.
2. Do one of the following:
   - Drag the phase to the appropriate location in the method outline.
   - Double-click the phase to append it after the currently selected phase in the outline.

     Tip: Ensure that the phase is appended after the selected phase, not at the end of the method outline. If necessary, reposition the phase by dragging it into place.

Phase Parameters Pane

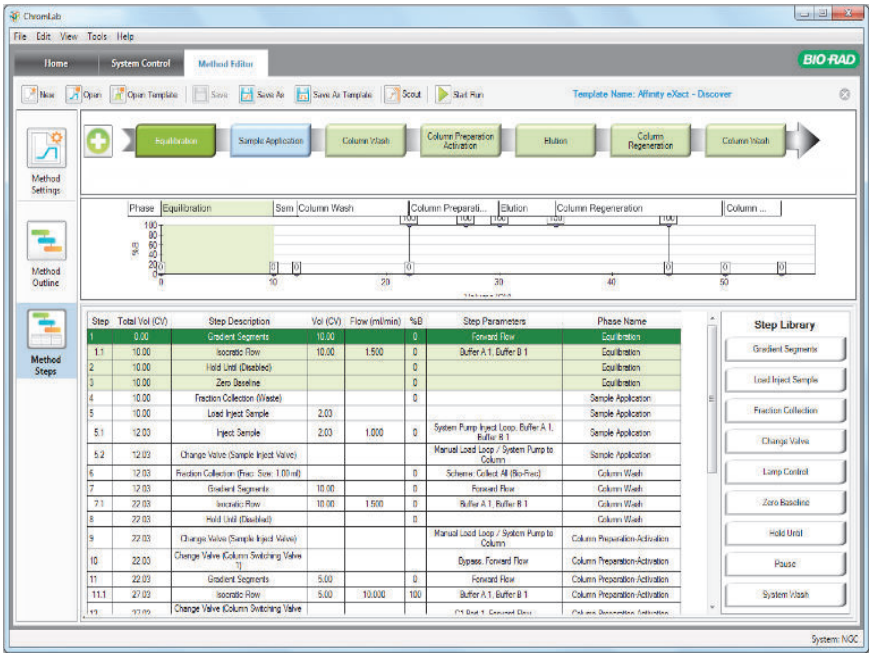
The Phase Parameters pane displays details of the phase selected in the method outline. A phase is a method segment composed of a discrete block of steps created to accomplish a specific task. Use this pane to modify the parameters of each phase as you add it to the method outline.

For detailed information about phase controls and parameters, see Phase Controls and Parameters on page 144.



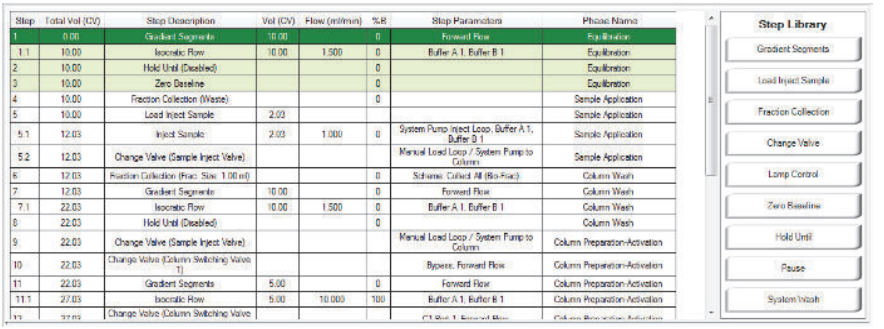
Method Steps View

When selected, the Method Steps view displays the method outline, gradient graph, and method steps for the selected method. The Method Steps view also displays the Step Library, from which you can select specific steps to add to individual phases within the method.



Method Steps Pane

The Method Steps pane displays a table of all steps associated with the method and their detailed settings. Use this pane to modify phases and to create custom phases.



When a SIM is present in the fluidic scheme with an autosampler, SIM control appears in the Step Description column when the autosampler is activated. When a SIM is present in the fluidic scheme without an autosampler, SIM Control appears in the Step Description column and the output name and pulse type for the device appear in the Step Parameters column. The pulse type changes to from High to Low when sample injection starts and returns to High when the injection stops.

When the method is a scout, the table includes a Scout column. The scouted steps are identified with a check mark in the Scout column.

Step Library

The Step Library comprises all the necessary steps to create a phase. Steps determine individual events that occur on the system; for example, changes in valve positions. Double-clicking a step name adds the step to the current phase. You can add multiple steps of the same type to a single method.

The Step Library includes the following steps:

- Gradient Segment
- Load Inject Sample

- Fraction Collection
- Change Valve
- Lamp Control
- Zero Baseline
- Hold Until
- Pause
- System Wash

To get more information about method steps

▶ In the Method Editor window, select Help > Method Steps and then select a step on the dropdown list that appears.

Viewing Details of a Step

To view step details

▶ Right-click the step in the step table and choose Show Step Details on the menu that appears.

Adding, Modifying, and Deleting Steps

To add a step to the step table

1. Select a step in the step table.
2. Double-click a step in the Step Library.

   The step appears in the table immediately below the step you selected.

To modify a step in the step table

▶ Double-click the step to open its dialog box in which you can change the step settings.

To delete a step from the step table

▶ Right-click the step and choose Delete Step on the menu that appears.

Method Settings Parameters

Method settings are general parameters that affect the entire method. These settings include fluidic scheme, column selection, monitor settings, unit selection, pH valve, fraction collection, and buffer selection.

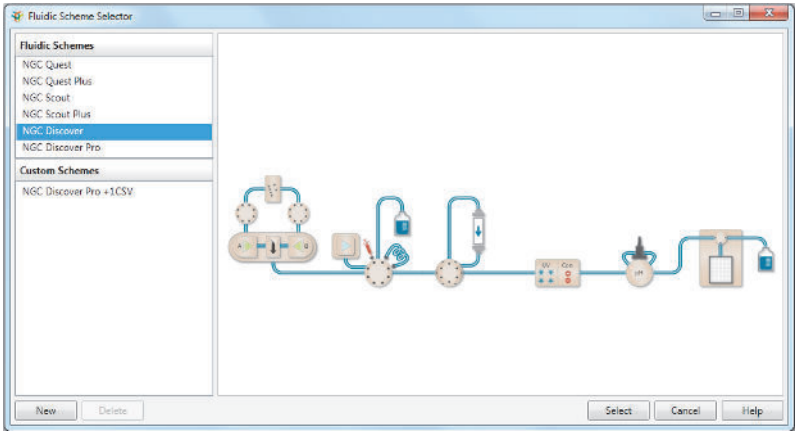
Fluidic Scheme

The current instrument configuration appears in the upper pane of the Method Settings view. Parameters available in each phase are specific to the devices in the fluidic scheme that participate in the phase. See Fluidic Scheme Configurations in Chapter 3.

To change the fluidic scheme selection

1. In the Method Settings pane under Fluidic Scheme, click Change to open the Fluidic Scheme Selection dialog box.
2. Change or edit the current fluidic scheme selection to match the devices present on your system.
3. Click Save to save the configuration.

Important: Changing the fluidic scheme of a method that includes phases might invalidate some phases. Bio-Rad recommends selecting the fluidic scheme before adding phases to a method.



Column Selection

Under Column Selection you select the column and the column parameters for the method. The column's volume and pressure parameters are set automatically when you select a column type from the list of predefined columns.

If you have one or more column switching valves in the fluidic scheme, you can choose whether to set the parameters for a single column or for multiple columns. When multiple columns are used in a method, the method's phases use the pressure parameters for any columns that are in line for that phase. You can use method steps to place two or three columns within the same method. In these cases, a high-pressure event is triggered when the delta pressure for one of the column switching valves reaches the high-pressure limit as defined in Method Settings for the columns that are in line.

You can configure the properties of multiple columns, one for each port on each column switching valve, and add them to the method using the Configure Ports dialog box. One column is set as default when you select its checkbox. The method uses the default's value for column volume throughout the method regardless of any other columns the method uses. In addition, the method uses the default's maximum pre- and delta-column pressure until other columns are switched in line. If two or more columns are switched in line, the method uses the maximum pre-column pressure of the first column.

You can remove a column from the list if it is no longer used, clearing the assigned port. You can then add a different column to the port. You can add a port with no assigned column to the method using the Change Valve step from the Step Library. When you save the method, a message appears prompting you to assign pressure limits to that port. You can use the Configure Ports dialog box to assign pressure limits without assigning a column to the port. This is useful when pressure-sensitive loops are used for certain multicolumn purifications. If the port does not have an assigned pressure limit, then the default system pressure limit applies.

Note: If all column switching valves are in bypass mode or if there are no column ports in line, the system uses the Max Pre-Column Pressure (Bypass All) value that you set in the Configure Ports dialog box.

You can add columns to the column library using the Add User Defined Column dialog box. Added columns appear as unique column types under the User Defined category. The added columns also appear in the column selection list in the scouting wizard if you scout for columns.

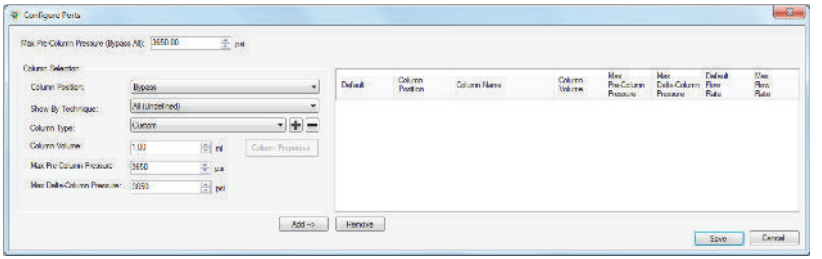
You can also remove user-defined columns from the column library using the Remove User Defined Column dialog box. If you remove a column that is used in a saved method, the method retains the column details. However, once removed from the column library the column no longer appears in the column selection list and is no longer available for running methods. You can reuse the name of the removed column when you create another user-defined column.

To configure parameters for multiple columns

1. In the Column Selection section, select Multiple Columns. The Configure Ports button appears.

   Note: This button appears only after you select Multiple Columns.

2. Click Configure Ports. The Configure Ports dialog box appears.



3. Select a column position to configure from the dropdown list.

4. (Optional) Show the columns that are available for a specific technique from the dropdown list.

5. Select a column type. Do one of the following:

   - Choose a column type from the dropdown list. The volume and pressure parameters are set automatically for that column type.

- Choose Custom from the dropdown list and set the volume and pressure parameters for the column at that port.

6. Click Add.
7. Repeat steps 3–6 for remaining ports.

   Note: The first column added is set as the default column. The default column defines the column volume used as the base in the entire method and defines the initial pressure limits for the method. To change the default to another column, select its checkbox.

8. Click Save.

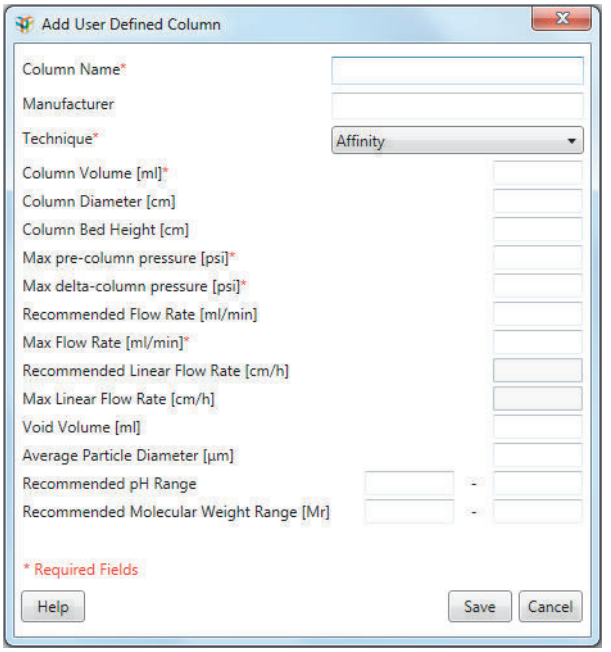
To clear an assigned column position from a method

▶ In the Configure Ports dialog box, select the column to remove, click Remove and then click Save.

To add user-defined columns

1. In the Column Selection section, do one of the following:
   - Select Single Column and click  .
   - Select Multiple Columns, click Configure Ports, and click  in the Configure Columns dialog box.

The Add User Defined Column dialog box appears.



Tip: The pressure units are based on the pressure values set in File > Preferences.

2. Provide the requisite information for the column. A red asterisk (*) indicates the field is required.

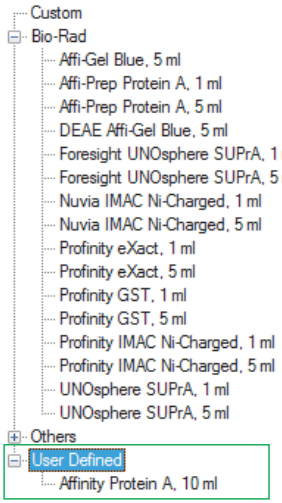
   Note: The Column Name field has a 45-character limit.

   The Column Volume, Column Diameter, and Column Bed Height are connected. When you enter or change values for any two fields, the system automatically calculates the value for the third field.

   The Recommended Linear Flow Rate and Max Linear Flow Rate fields are read-only. These fields are automatically calculated when you enter values in the Recommended Flow Rate and Max Flow Rate fields, respectively.

3. Click Save.

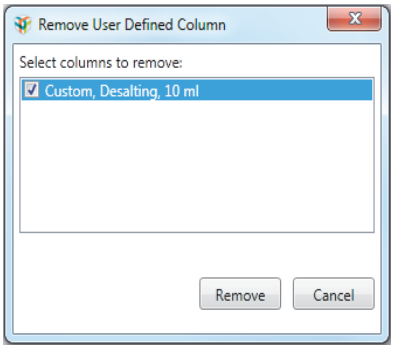
   The new column appears in the Column Type dropdown list under the User Defined category.



To remove user-defined columns

1. In the Column Selection section, do one of the following:
   - Select Single Column and click ➖.
   - Select Multiple Columns, click Configure Ports, and click ➖ in the Configure Columns dialog box.

   The Remove User Defined Column dialog box appears.



2. Select one or more columns to remove.
3. Click Remove.

Detector Settings

Under Detector Settings, set single UV or multi UV/Vis wavelengths that will be used to monitor the run. For the single-wavelength detector, choose either 280 nm or 255 nm. (The most commonly used wavelength for proteins is 280 nm.) For the multi-wavelength detector, choose any four wavelengths from 190–800 nm.

Unit Selection

Method Base Unit — sets the default x-axis unit for the chromatogram.

Flow Rate Unit — the system's default flow rate unit.

pH Valve

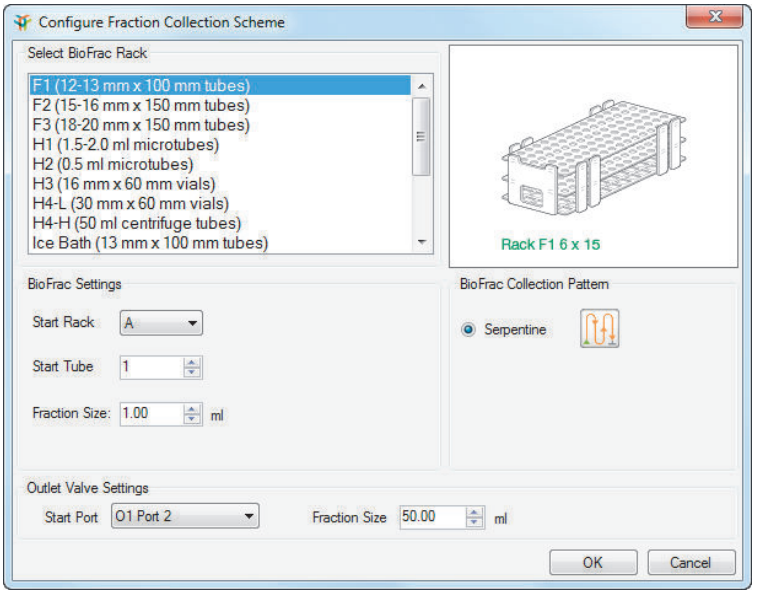
Enable pH monitoring — if a pH valve is present, this checkbox is selected by default. To bypass the pH valve, clear this box.

Fraction Collection

Device Type — displays the currently selected fraction collector device. If your fluidic scheme has

- Only a fraction collector, this dialog box displays BioFrac and the rack type
- Only outlet valves, this dialog box displays Outlet Valve
- Both a fraction collector and outlet valves, this dialog box displays Outlet Valve and BioFrac and the rack type

Click Configure to configure the collection device and pattern.



Important: The minimum recommended fraction size for NGC systems is 10% of the flow rate.

BioFrac Settings — by default, fraction collection begins in the first tube of the first rack. The fraction size is the default for the rack type.

BioFrac Collection Pattern — by default, Serpentine is selected. For microplates, fractions can also be collected by rows or columns.

Outlet Valve Settings — by default, fraction collection begins from Outlet valve 1 (O1) Port 2. O1 Port 1 connects to the BioFrac™ fraction collector if it is present or directs flow to waste if the fraction collector is absent. The default fraction size is 50 ml.

Flow

Flow Rate — shows the default rate of flow for the method.

Control the flow to avoid overpressure — monitors the pressure and reduces the flow rate by 50% when the pressure gets within a certain percentage of the maximum (default is 80%, see Control Flow Tab in Chapter 3 for more information).

Detect end of buffer with air sensor — stops the system pumps if air is detected in any of the lines connected to air sensors set to detect end of buffer. Air sensor settings are determined on the Air Sensors tab in the System Settings dialog box. (See Air Sensors Tab in Chapter 3 for more information).

Buffer Selection

Note: When the configuration does not include a buffer blending valve, buffer settings are inactive.

You can choose one of three Buffer Selection modes:

- **Manually Prepared** — buffers are user prepared and fed directly into the pumps or selected by means of buffer inlet valves.
- **Manually Prepared via Blending Valve** — buffers are user prepared and fed to the pump through buffer blending valve ports Q1 and Q4. Gradients are formed by the blending valve rather than the pumps. Because of this, both pumps can run at their full capacity simultaneously, which doubles the available flow rate range.

- **Buffer Blending** — buffer acid, buffer base, water, and salt are mixed together to form a buffer of a specified pH, buffer concentration, and salt concentration. A buffer system (recipe) is selected for use, after which the acid, base, water, and salt solutions are prepared and fed to the pumps through the buffer blending valve ports, Q1–Q4. Gradients are formed by the blending valve rather than the pumps. Because of this, both pumps can run at their full capacity simultaneously, which doubles the available flow rate range.

Clicking Rename Ports makes it possible to rename the ports on the column switching valves, buffer inlet valves, and sample inlet valves. For example, you can rename the ports on the column switching valve to display the name of the columns connected to each specific port. Once the ports are renamed, the new names appear in the method in place of the valve port names.

Use Table 5 to select the default buffer mode for the method. The options available depend on the fluidic scheme selected and the valves connected to your system. To set the delay volume see System Settings on page 71.

Table 5. Buffer selection modes and buffer inlet types

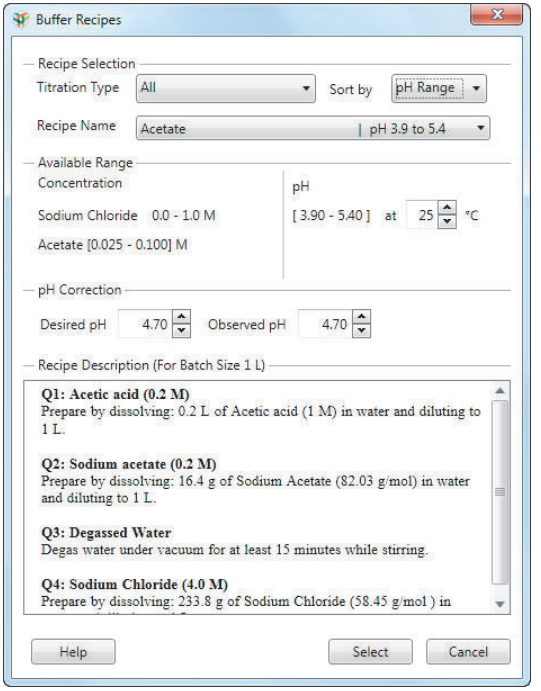
	Buffer Selection Modes		
Buffer Inlet Type	**Manually Prepared**	**Manually Prepared via Blending Valve**	**Buffer Blending**
Pump Inlets	✓		
Pump + Inlet Valves	✓		
Pump + Buffer Blending		✓	✓
Pump + Buffer Blending + Inlet Valves	✓	✓	✓

If you are working with a fluidic scheme that has multiple inlet valves, you can switch between buffers.

Note: Buffer blending requires a buffer blending valve.

Buffer blending can be used to dilute buffers. Define the pH of elution and select buffers to achieve this pH.

Select Buffer — opens the Buffer Recipes dialog box in which you can choose a recipe for the buffer and name the buffers.



To select a buffer

1. In the Buffer Recipes dialog box under Recipe Selection, you can filter the list of recipes by selecting a titration type on the dropdown list.
2. On the Sort by dropdown list, choose Name or pH to view the list of recipe names in the order you prefer.
3. Choose a recipe on the Recipe Name dropdown list.

Available Range — available ranges for the recipe concentration and pH values vary depending on the recipe you choose. The ranges provided are recommendations. You can choose values above the upper end of the range, but doing so might require other adjustments to ensure accuracy. For most recipes you can change the buffer concentration by reducing the stock concentration by 1/2 to 1/8 on Conjugate Acid/Base Pair titration and up to 1/4 on Acid or Base titration. Stock concentration for most recipes is 0.2 M.

Tris and Phosphate are the most commonly used recipes, depending on the type of chromatography being performed.

pH Correction — used to correct the observed pH so it matches the pH set for the run (Desired pH) in the System Pump dialog box or Phase Parameters pane in the Method Outline view. pH correction uses the difference between the desired pH and observed pH to make the correction.

Tip: Calibrate the pH probe regularly for accurate pH reading.

Phase Controls and Parameters

The Method Editor includes several standard phases. See Standard Phases on page 146 for details.

A phase is a method segment composed of a discrete block of steps created to accomplish a specific task. Each phase comprises controls and parameters specific to the task it is designed to accomplish.

The controls and parameters used in individual phases are as follows:

- **Gradient Segments** — determine the length and buffer composition (%B) for isocratic or gradient steps.

  Tip: Each phase runs at a single flow rate. To change the flow rate, select a different phase.

  In isocratic steps, initial %B and final %B must be equal. In linear segments, initial %B and final %B are independent of each other.

In gradient segments, lower numbers yield increasing gradients. Higher numbers yield negative gradients.

- **Load/Inject Sample** — controls sample loop loading, sample injection, and loop washing.
- **Fraction Collection** — turns on fraction collection for the phase, sets the fraction collection scheme, enables you to select the primary collector (BioFrac fraction collector or outlet valve), sets the start tube or port, and sets the fraction size.
- **Change Valve** — changes the selected valve to the selected position.
- **Lamp Control** — sets the wavelength and turns the lamp on/off for the detector selected in the fluidic scheme.
- **Zero Baseline** — sets zero as the baseline for the UV detector selected in the fluidic scheme.
- **Hold Until** — keeps the phase at the current composition until a button is pressed, timeout occurs, or a threshold value is reached.
- **Pause** — pauses the method until a button has been pressed or a timeout time has been reached.
- **System Wash** — sequentially switches through pumps, valves, and sample loops and flows a defined volume of cleaning solution through each position at a defined flow rate.

  Note: This step is not supported when either Gradient Segments or Load Inject Sample steps are included in the method.

Standard Phases

Some standard phases use identical parameters. For example, the column wash phase and the elution phase display the same phase parameters. An example of each phase's parameters is included in its description.

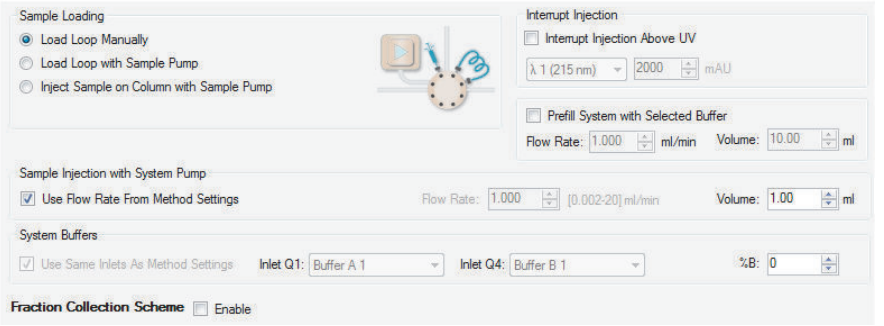
Note: The images in this section display the parameters and settings for the NGC Discover Pro fluidic scheme. Your parameters and settings might vary depending on the fluidic scheme and method in use.

Sample Application Parameters

In sample application phase, you define how a sample gets injected onto a column. Depending on your system's configuration, you can apply a sample to the column manually via a sample loop or directly via the sample pump or autosampler (if connected).

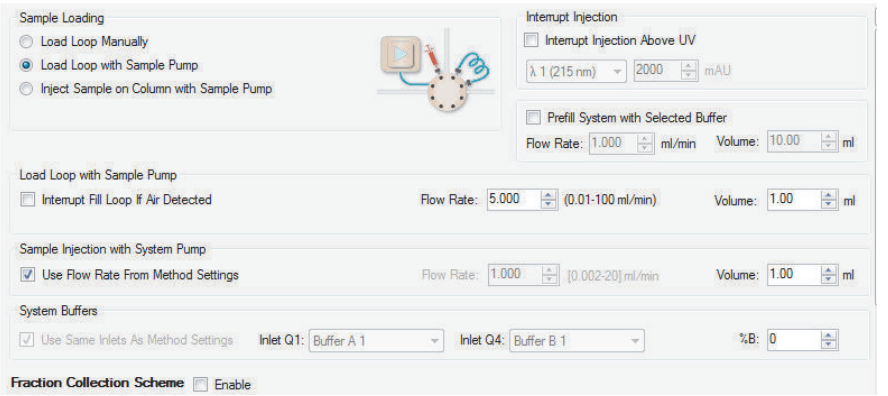
For all configurations, you have the option to continue injecting a sample until a particular UV reading is reached. You also have the option to prefill the lines with the specified volume of buffer when the sample loading buffer is different from the buffer used in the previous phase.

If your fluidic scheme includes a buffer blending valve and you choose to manually prepare buffer via inlet valves in Method Settings, you have the option to use the same inlets as defined in Method Settings or to select specific buffers from each port.



Loading the Loop with a Sample Pump

When a sample pump is used to load the loop, you can define the flow rate, the volume to load into the loop, and, subsequently, the volume of sample to be injected onto the column. You also have the option to interrupt loading the loop if air is detected. If you select this checkbox and air is detected in any of the lines connected to air sensors set to detect end of sample, the system stops the sample pump and the method continues to the next step.



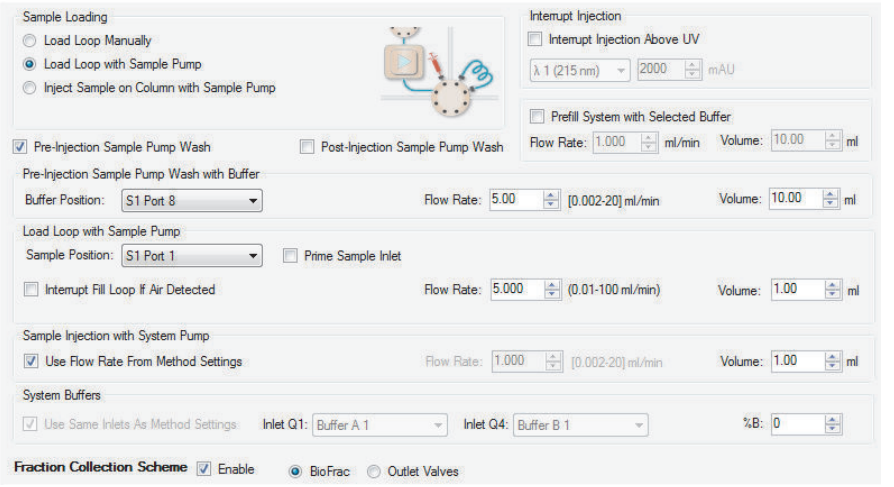
Fluidic Scheme Includes a Sample Inlet Valve

If your fluidic scheme includes at least one sample inlet valve and you choose to load the loop through the sample pump, you have the option to perform a pre-injection wash with buffer to flush the sample pump and lines and the loop. If you select this checkbox, you can choose the port on the sample inlet valve from which to draw buffer solution. You can define the flow rate and the volume of buffer for the preinjection wash.

You also have the option to flush the sample pump and related connections after sample injection. Again, you can choose the port on the sample inlet valve from which to draw buffer solution and define the flow rate and the volume of buffer for the postinjection wash.

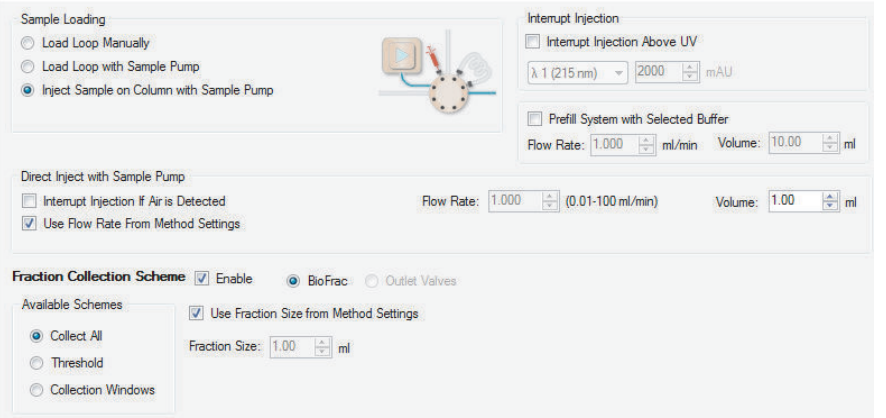
You can change the port on the sample inlet valve from which to load sample (the system defaults to Port 1). You can also choose to prime the tubing with sample before application. If you select this checkbox, you can define the flow rate and sample volume with which to prime.

Finally, you can choose to interrupt loading the loop if air is detected. If you select this checkbox and air is detected in the line connected to the air sensor set to detect end of sample, the system stops the sample pump and the method continues to the next step.



Injecting Sample Directly with a Sample Pump

When a sample pump is present in the fluidic scheme and the sample is injected directly onto the column, you have the option to interrupt the injection if air is detected. If you select this checkbox and air is detected in the line connected to the air sensor set to detect end of sample, the system stops the sample pump and the method continues to the next step.



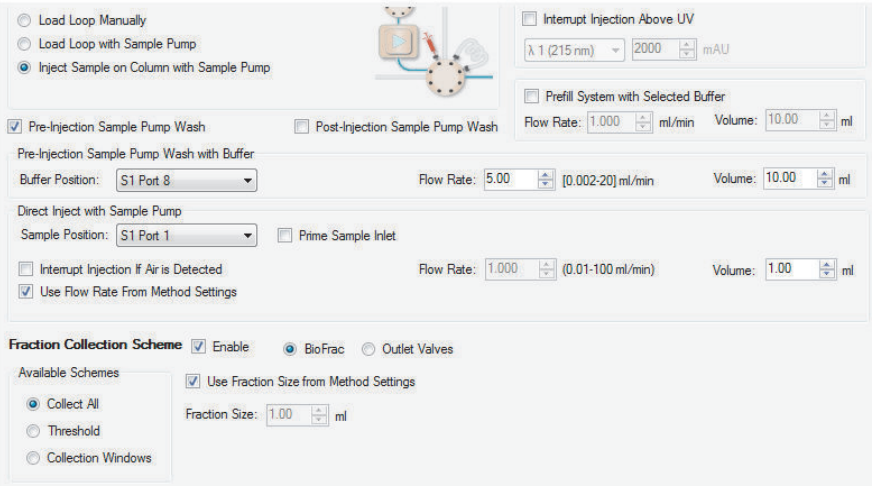
Fluidic Scheme Includes a Sample Inlet Valve

If your fluidic scheme includes at least one sample inlet valve and you choose to inject the sample directly onto the column, you have the option to perform a preinjection wash with buffer to flush the sample pump and lines and the loop. If you select this checkbox, you can choose the port on the sample inlet valve from which to draw buffer solution. You can define the flow rate and the volume of buffer for the preinjection wash.

You also have the option to flush the sample pump and related connections after sample injection. Again, you can choose the port on the sample inlet valve from which to draw buffer solution and define the flow rate and the volume of the buffer for the postinjection wash.

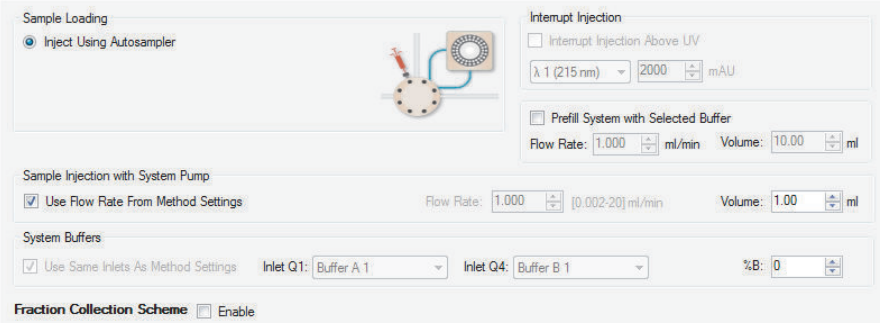
You can change the port on the sample inlet valve from which to load sample (the system defaults to Port 1). You can also choose to prime the tubing with sample before application. If you select this checkbox, you can define the flow rate and sample volume with which to prime.

Finally, you can choose to interrupt injection if air is detected. If you select this checkbox and air is detected in any of the lines connected to air sensors set to detect end of sample, the system stops the sample pump and the method continues to the next step.



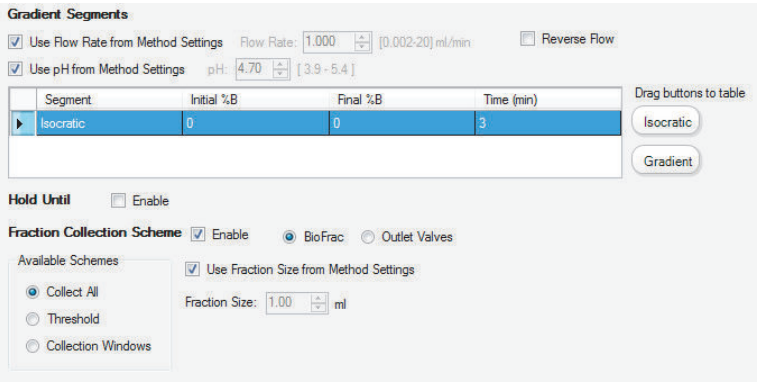
Injecting Sample with an Autosampler

If your fluidic scheme includes an autosampler and you choose to use manually prepared buffer via inlet valves in Method Settings, you have the option to use the same inlets as defined in Method Settings or to select specific buffers from each port.



Column Wash Parameters

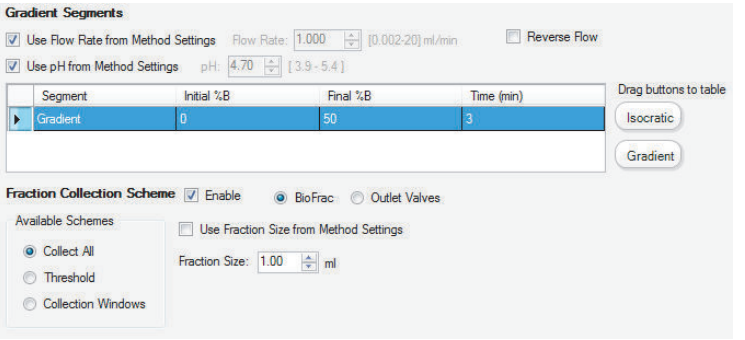
This phase washes the column of impurities and unbound sample. The wash can be programmed to occur for a defined time or volume or to continue until a certain UV or conductivity value has been attained. You can also activate fraction collection during this phase.



Although it is typically used after the sample application or elution phase, this phase can be used on its own or at any point that column washing is wanted.

Elution Parameters

In this phase, the sample is eluted from the column using a gradient or isocratic flow at defined salt concentrations (%B) over a defined volume, column volume, or time. You can edit the parameters in the gradient segment or add more isocratic or gradient steps to this segment. This phase can use the flow rate defined globally in the Method Settings phase or a flow rate different from that of the other phases.



Fraction collection is enabled by default during this phase and offers options to collect all, to collect when a certain threshold of UV or conductivity has been reached, or within defined collection windows. When collecting fractions by threshold, you can opt to collect the fractions that fall outside the defined threshold range using fraction volumes different from the volumes set for the threshold fractions.

You can choose an elution technique from the following options:

- **Isocratic** – define the length of elution (CV, cm/hr, time, volume) and buffer composition (%B).
- **Gradient** – define a linear gradient (%B; default = 0–100%) and gradient slope/duration (CV, time, volume). In this case, the pumps stop at the end of the set duration.

System CIP (Clean in Place) — Storage Parameters

Note: System CIP must be run as the only type of phase in the method. You can add several System CIP phases to the method if multiple cleaning solutions are required. Bio-Rad recommends that you save System CIP as a separate method.

If the system has been exposed to hazardous biological material, run System CIP and Column CIP to flush the entire system tubing with sanitizing solution (for example, NaOH) followed by neutral buffer and finally distilled water before service or maintenance.

This phase cleans the system after purification runs by rinsing the system with cleaning solution. Perform System CIP when required; for example, between runs where different samples and buffers are used or before storing the system. This helps prevent cross-contamination between runs and prevents bacterial growth in the instrument during storage.

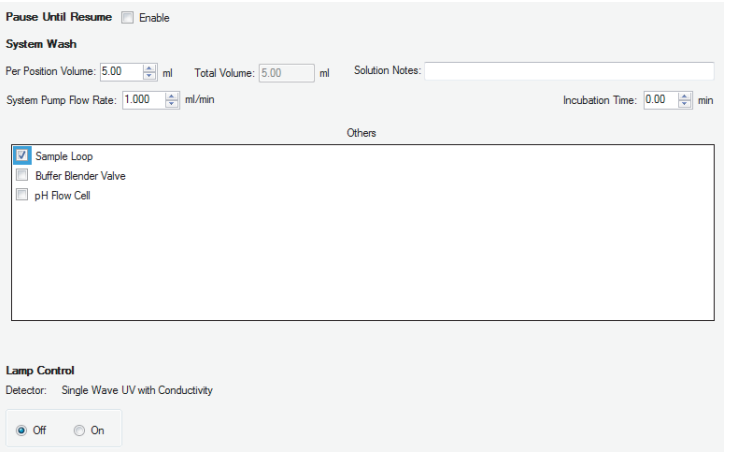
Depending on the modules in the fluidic scheme, choose the scope of cleaning by selecting checkboxes for the different valves, ports, pumps, column positions, modules, and loops to clean and fill with cleaning solution. The System CIP method includes two System CIP phases to facilitate the use of two different solutions; for example, NaOH, buffer solution, or distilled water. Select values for flow rate, volume per position, and incubation time.

Note: Each phase uses one cleaning solution. All inlet lines selected in one phase should be immersed in the same cleaning solution.

Special Considerations

When the fluidic scheme includes a buffer blending valve, NGC systems perform System CIP differently depending on whether the buffer selection is manually prepared via the buffer blending valve, blended through the buffer blending valve, or manually prepared through buffer inlet valves. Note the following special considerations regarding System CIP with a buffer blending valve in the fluidic scheme.

NGC Scout Systems



Buffer selection: Manually Prepared Buffer via Blending Valve

- Components to clean
  - Sample loop
  - Buffer blending valve
  - pH flow cell
- Wash solution is delivered through Q1 for the sample loop and pH flow cell.
- Ports Q1 and Q4 are washed individually with 5 ml.

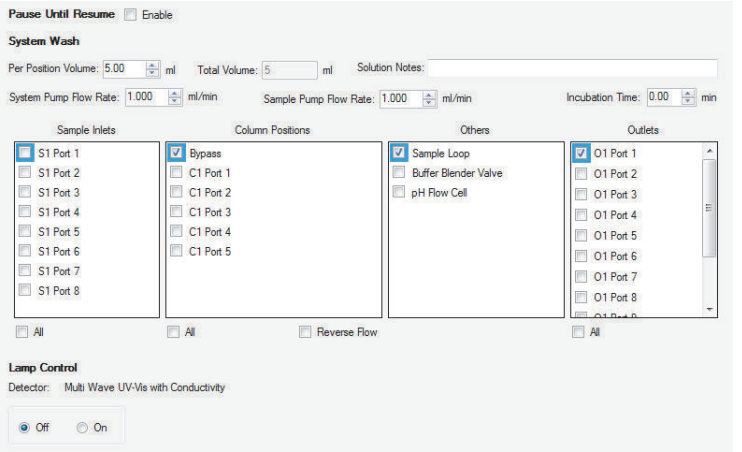
Buffer selection: Buffer Blending

- Components to clean
  - Sample loop
  - Buffer blending valve
  - pH flow cell
- Wash solution is delivered through Q1 for the sample loop and pH flow cell.
- Ports Q1, Q2, Q3, and Q4 are washed individually with 5 ml.

NGC Discover and NGC Discover Pro Systems

Note: To perform CIP for the buffer blending valve and buffer inlet valves you must create separate methods, which you can queue in the Run Scheduler.

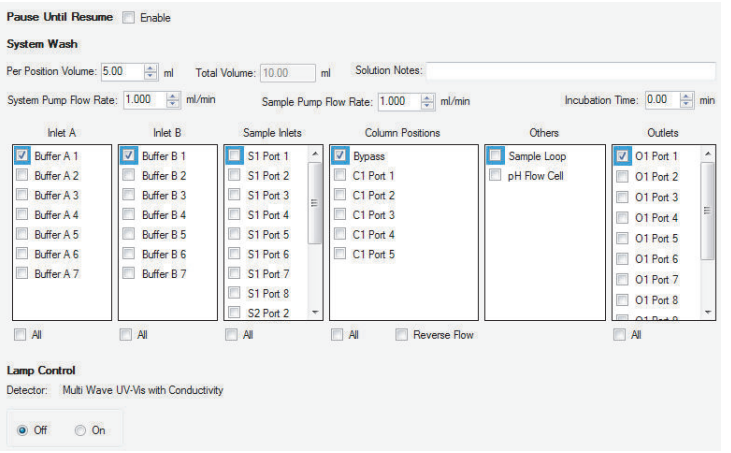
Buffer selection: Manually Prepared Buffer via Blending Valve



- Components to clean:
  - Sample loop
  - Buffer blending valve
  - pH flow cell
  - Sample pump
  - Column switching valves
  - Outlet valves
  - Sample inlet valves
- Wash solution is delivered through Q1 for the sample loop, pH flow cell, column switching valve, and outlet valve.

- Sample inlet valves are washed by using the sample pump to pull wash solution through ports 1–8 on S1 (if only one sample inlet valve is present) or ports 1–7 on S1 and ports 1–8 on S2 (if two sample inlet valves are present).
- Ports Q1 and Q4 are washed individually with 5 ml.
- Buffer inlet ports A1–7 and B1–7 are not washed.

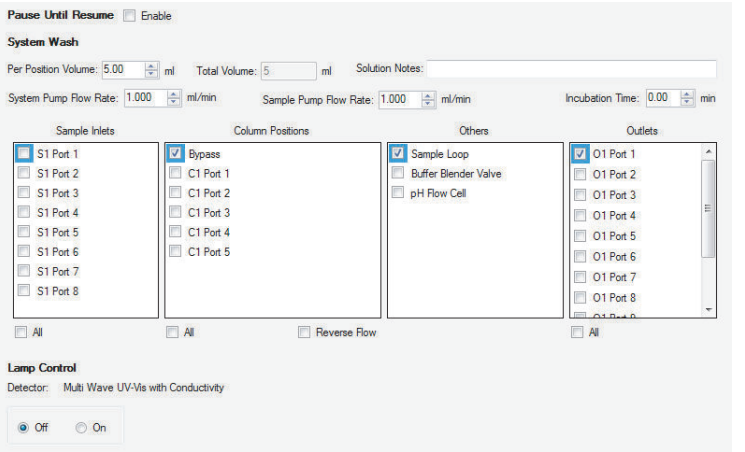
Buffer selection: Manually Prepared Buffer via Inlet Valves



- Components to clean
  - Sample loop
  - pH flow cell
  - Sample pump
  - Column switching valves
  - Buffer inlet valves
  - Outlet valves
  - Sample inlet valves

- Wash solution is delivered through buffer inlet ports for the sample loop, pH flow cell, and column switching valve.
- Sample inlet valves are washed by using the sample pump to pull wash solution through ports 1–8 on S1 (if only one sample inlet valve is present) or ports 1–7 on S1 and ports 1–8 on S2 (if two sample inlet valves are present).
- System pumps each run at 0% B and 100% B from buffer inlet ports A1–7 and B1–7.
- Ports Q1, Q2, Q3, and Q4 are not washed.

Buffer selection: Buffer Blending



- Components to clean
  - Sample loop
  - Buffer blending valve
  - pH flow cell
  - Sample pump

  - Column switching valve
  - Outlet valve
  - Sample inlet valves
- Wash solution is delivered through Q1 for the sample loop, pH flow cell, and column switching valve.
- Sample inlet valves are washed by using the sample pump to pull wash solution through ports 1–8 on S1 (if only one sample inlet valve is present) or ports 1–7 on S1 and ports 1–8 on S2 (if two sample inlet valves are present).
- Ports Q1, Q2, Q3, and Q4 are washed individually with 5 ml.
- Buffer inlet ports A1–7 and B1–7 are not washed.

To clean the flow paths completely

▶ Clean the manual inject port and pH valve manually.

Ensure that the pH probe is removed from the pH valve and that all column ports are connected to bypass tubing.

To clean the pH valves

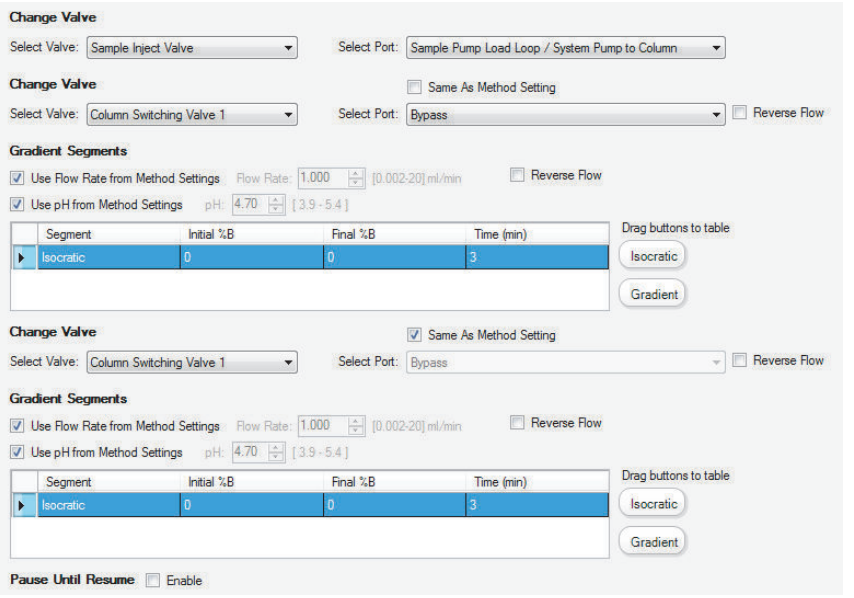
1. Open the System Control window.
2. Select Tools > Calibrate to open the Calibration dialog box.

   Note: No calibration is performed in this procedure.
3. Choose pH in the Calibrate dropdown list.
4. Click Start to set the valve flow path.
5. Fill a syringe with 1 M NaOH, connect the syringe to the pH valve Cal In port, and inject the solution.

6. Fill a syringe with distilled water, connect the syringe to the pH valve Cal In port, and inject the distilled water.
7. Click Close in the Calibration dialog box to leave calibration mode.
8. Switch the valve back to the default position.

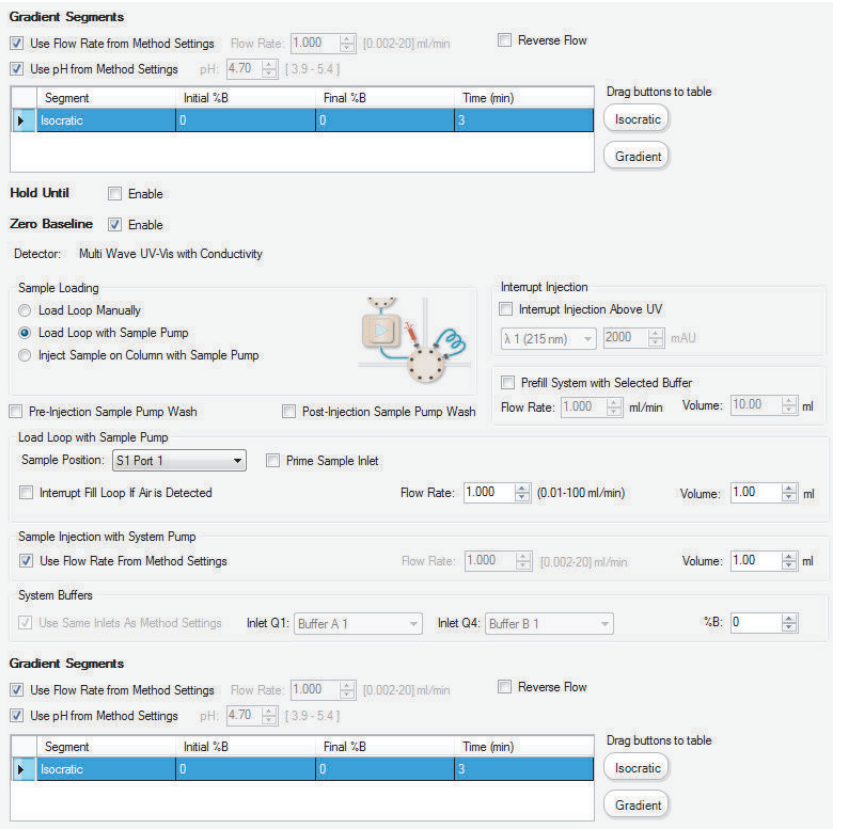
Column Preparation-Activation

This phase is used to prepare a column before use by removing the storage solution or to activate a column to bind the sample before applying it. If activation requires the column to incubate in the activation buffer, select Pause Until Resume and define the time required for the column to incubate.



Column Performance Test Parameters

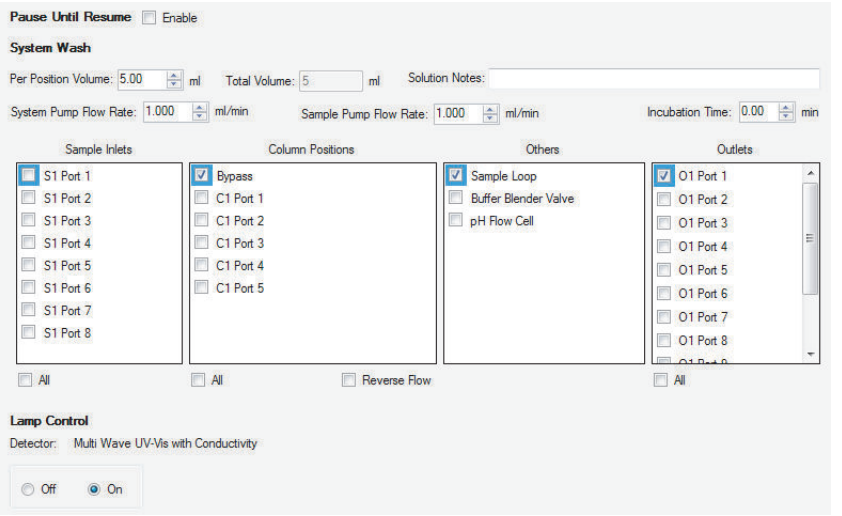
This test combines the three basic phases necessary for a run (equilibration, sample application, and elution). After equilibrating the column, a non-adsorbing sample, such as acetone or salt, is injected via the sample loop and eluted under isocratic conditions. After the run, calculate the column efficiency in terms of height equivalent to a theoretical plate (HETP) and the peak asymmetry factor (As).



System Preparation Parameters

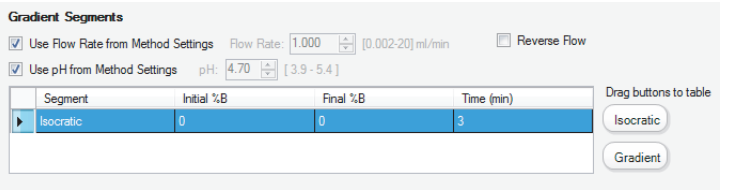
This phase removes storage solution and fills the tubing and system inlet line with buffer solution before a run. The inlets, outlets, and column positions to be prepared are selected, and the system is filled with the appropriate buffer solution. Because each inlet port can be plumbed to a different buffer or sample, multiple buffers and samples can be used in each phase.

Note: System Preparation must be run as the only type of phase in the method. You can add several System Preparation phases to the method if cycling between multiple solutions is required. Bio-Rad recommends that you save System Preparation as a separate method.



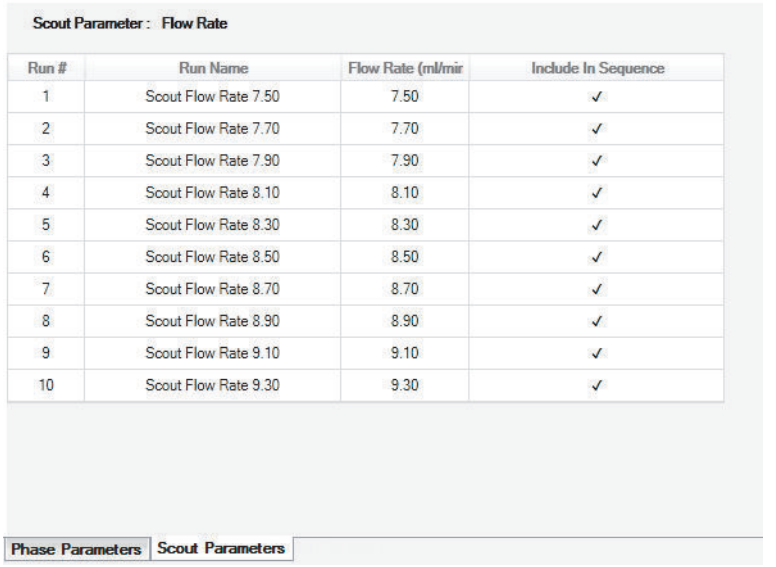
Create New Phase Parameters

This phase enables you to create a new phase by adding steps from the Step Library to the current phase in the Method Steps view. Phases that you create can be saved in the custom phase library for reuse in other methods.



Scout Parameters Tab

Available in the Method Outline view after the scout parameters are set, the Scout Parameters tab displays a table of all scouting runs associated with the scout method and their settings. This pane is read-only and cannot be modified.



6 Creating a Method

In the NGC™ chromatography system, methods are used to encapsulate an entire process to be run. Methods consist of phases. A phase consists of a sequence of steps in a chromatography run and the properties associated with each step. ChromLab™ software includes standard methods as well as tools for creating, editing, and managing methods. You can edit the phases within a method and define the settings within each phase.

Methods are created in the Method Editor window. A method can be created in the following ways:

- From standard phases, the steps of which are edited
- From a standard template
- From another method already defined

For detailed information about Method Editor features, see Chapter 5, Method Editor.

Standard Method Templates

The workflow for standard method templates consists of the following phases:

- Equilibration
- Sample application
- Column wash
- Elution
- Column wash
- Re-equilibration for the next run

ChromLab includes the following eight standard method templates.

Table 6. Standard method templates

Method Template	Explanation
Affinity	Affinity chromatography is the separation of biomolecules based on highly specific interactions. For example: antibody/antigen or antibody/protein A, chelation (polyhistidine-tag/nickel), enzyme/substrate (glutathione-s-transferase/glutathione).
Anion Exchange	Anion exchange chromatography uses a positively charged column matrix to bind negatively charged protein molecules. Proteins are eluted from the matrix using a gradient of increasing ionic strength (typically NaCl). Select a buffer system with a pH lower than the pI of the target protein to enhance protein binding.
Cation Exchange	Cation exchange chromatography uses a negatively charged column matrix to bind positively charged protein molecules. Proteins are eluted from the matrix using a gradient of increasing ionic strength (typically NaCl). Pick a buffer system with a pH higher than the pI of your target protein to enhance protein binding.

Table 6. Standard method templates, continued

Method Template	Explanation
Chromatofocusing	Chromatofocusing chromatography uses a charged matrix to bind protein molecules. A pH gradient is used to elute the bound proteins, which elute when the pH is the same as the pI of the protein of interest (overall charge = 0). Special buffer systems are required to perform the pH gradient over a large range.
Desalting	Desalting is usually used for buffer exchange. Proteins do not bind to the column matrix and are typically eluted isocratically in the void volume of the column. Select a buffer system that maximizes the stability of the target protein.
Hydrophobic Interaction	Hydrophobic interaction chromatography uses high salt buffers to adsorb target proteins to a hydrophobic column matrix. Decreasing salt concentrations are then used to elute and separate bound proteins.
Mixed Mode	Mixed mode chromatography uses a column matrix with hydrophobic and charged ionic interactions. Proteins can be eluted using a gradient of pH (eluting when the pH = pI of the target protein) or salt (increasing salt to elute from the charged moiety or decreasing salt to elute from the hydrophobic moiety of the column matrix).
Multicolumn Sequential	Multicolumn sequential purification uses these templates when multiple samples must be purified on multiple columns. The samples are injected sequentially either by using a sample pump with sample inlet valve or through sample loops. Each sample is loaded onto a column and washed to remove contaminants that can cause sample degradation. The columns are then eluted using either step or linear gradient protocols in a sequence. The fractions are collected with the BioFrac™ fraction collector or an outlet valve.

Table 6. Standard method templates, continued

Method Template	Explanation
Multicolumn Tandem	Multicolumn tandem purification uses these templates when two different chromatography techniques are combined into one method. First, samples are injected, bound to a column and washed to remove any main contaminants. Next, the target fractions are eluted and either applied directly onto another column in tandem or stored temporarily in a sample loop or container. If stored, they are then re-injected onto a second column. In either case, the second column is then eluted and the purified fractions are collected with the BioFrac fraction collector or an outlet valve.
Reverse Phase	Reverse phase chromatography utilizes hydrophobic regions on target proteins to bind to a hydrophobic column matrix. A gradient of increasing organic solvent concentration/ratio is used to elute the proteins from the column matrix.
Size Exclusion (Gel Filtration)	Size exclusion/gel filtration chromatography separates proteins based on their molecular size or weight. Proteins do not bind to the column matrix and are typically eluted isocratically. Select a buffer system that maximizes the stability of the target protein.
System Test	These methods test the performance of NGC systems that have a UV detector. They are intended to assess the flow rate and gradient accuracy of the system pumps, the functionality of the mixer, and the responses of the UV and conductivity detectors. In most cases, a visual examination of the resulting chromatogram can confirm proper performance.

Creating a Method from a Template

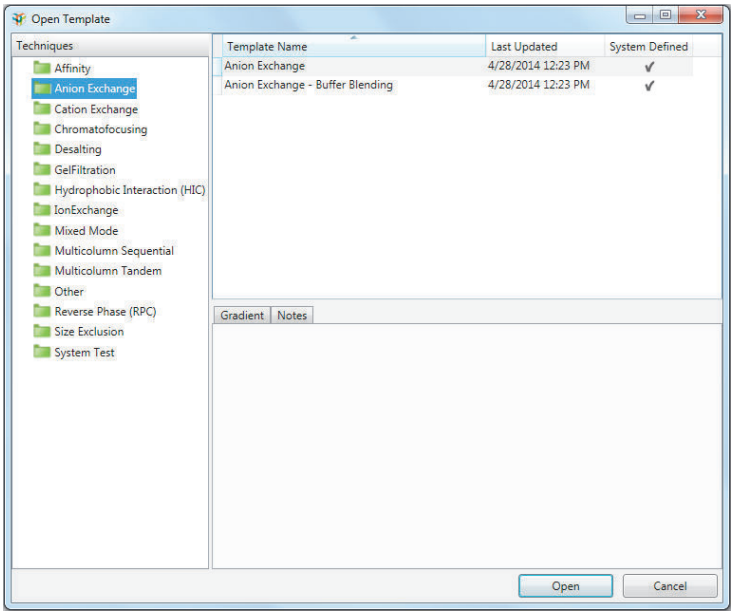
Standard methods are supplied as templates. You must save a template as a method before you can use it.

To select a method template and save it as a method

1. Do one of the following:

   - In the Home window, click Open Method Template.
   - In the Method Editor window, click Open Template on the toolbar.

   The Open Template dialog box appears. Standard method templates are organized in folders by technique.



2. In the left pane, click a technique folder and select a template in the list that appears in the upper right pane.

   In the lower-right pane, the Gradient tab displays the gradient graph of the selected template. The Notes tab displays explanatory text that you can edit in the Phase Parameters pane in the Method Outline view. When available, the Overview tab displays a graphic representation of the purification steps.

   Note: The Overview tab is available only when a multicolumn purification template is selected.

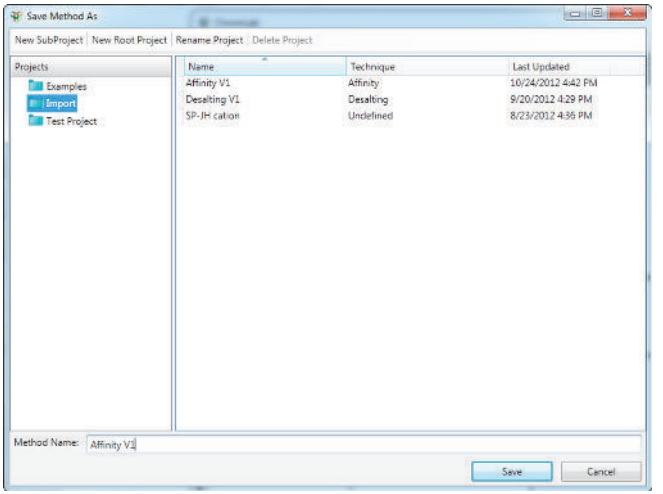
3. Double-click a template name to open the template.

   By default, the method opens in the Method Settings view of the Method Editor.

4. In the Method Settings pane, edit general settings like column type, method base unit, and wavelength.

   ChromLab automatically calculates correct settings for volume, flow rate, and pressure.

5. On the Method Editor window, select File > Save As to open the Save Method As dialog box.



6. Do one of the following:
   - Select a project folder. In the Method Name box, type a name for the method, and then click Save.
   - Click New SubProject or New Root Project and type a name for the new project in the Enter Project Name box that appears above the Projects pane. Click Save.

Creating a Method from Standard Phases

When you create a method from standard phases, you specify in the Method Settings view the general settings that will apply to the entire method. These settings include column selection, pressure limits, flow rate, method base, pH, fraction collector rack type, and fraction volume. The available parameters vary depending on the devices present in the fluidic scheme. For example, pH is available only when the pH valve is present.

The workflow for creating a method from standard phases is as follows:

- Create a method.
- In the Method Settings view, select a fluidic scheme.

  Important: The fluidic scheme must match the configuration of the NGC instrument. If it does not, change the fluidic scheme or create a new one to match the NGC instrument configuration. See To change the fluidic scheme on page 170 in this section.
- In the Method Outline view, add phases to the method by dragging them from the Phase Library to the Method Outline pane in the order to be executed.
- Edit the properties of the phases.
- Save the method.

Each element of the fluidic scheme is associated with settings in the Method Settings view. When the fluidic scheme changes, the method settings change to match it.

To create a method

▶ Do one of the following:

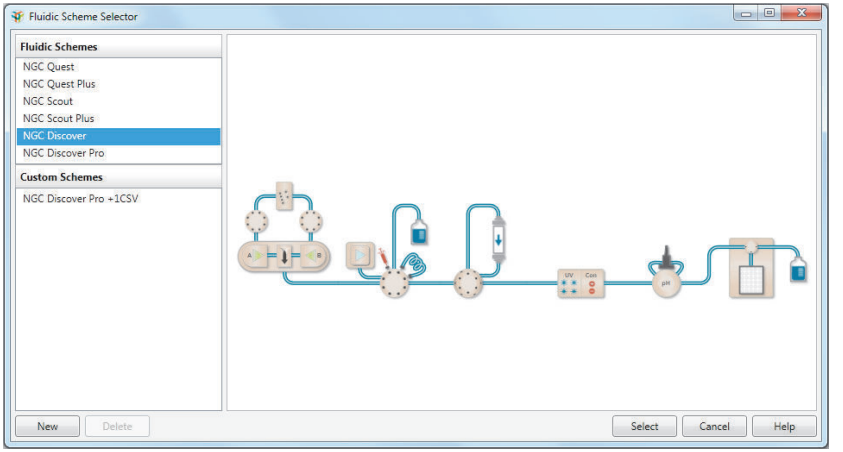
- In the Home window Method Editor pane, select New Method.
- If the Method Editor tab is already in view, click New in the Method Editor toolbar.

The new method opens in the Method Settings view. Its settings appear in the Method Settings pane and the current fluidic scheme appears in the Fluidic Scheme pane.

To change the fluidic scheme

1. Under Fluidic Scheme in the Method Settings pane, click Change.

   The Fluidic Scheme Selector dialog box appears, in which you can select another fluidic scheme or click New to create a new one. For more information, see To create a new fluidic scheme on page 60.



2. Choose a fluidic scheme that matches your NGC instrument configuration and click Select.

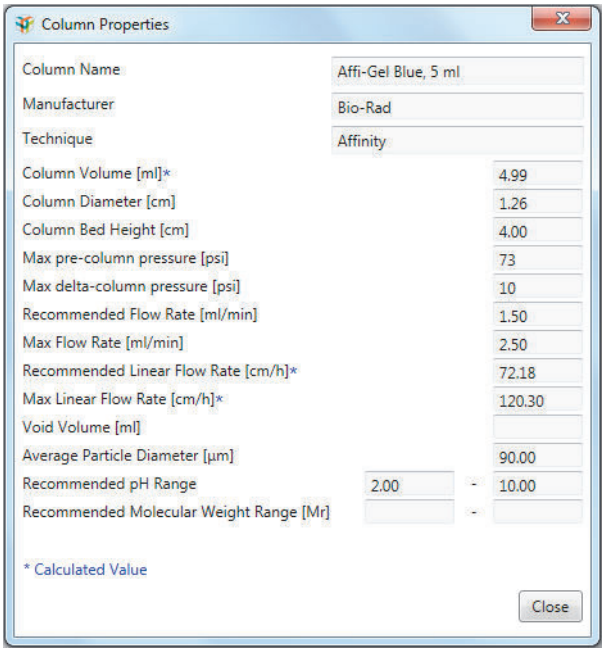
Note: General settings for the fluidic scheme appear in the Method Settings pane. General settings vary depending on the fluidic scheme selected. For more information, see Fluidic Scheme on page 133.

To specify general settings

1. In the Method Settings pane, click Run Name and specify a name in the dialog box for the run that will result, and then click OK.
2. (Optional) Click Notes and enter or edit content in the Notes dialog box that appears. Once the new method is saved, text entered here appears in the Notes tab of the new method's Open Method dialog box.
3. Under Column Selection, select a technique on the Show By Technique dropdown list. This filters the list of column types.
4. Select a column type on the Column Type dropdown list.

   ChromLab automatically fills in the column's volume and pressure settings.

   Tip: Click Column Properties to view a complete list of the selected column's properties and values.



5. Depending on the method, either Single Wave UV Detector Settings or Multi-Wave UV/Vis Detector Settings can be selected. Choose the appropriate UV absorbance wavelength values to monitor the run.

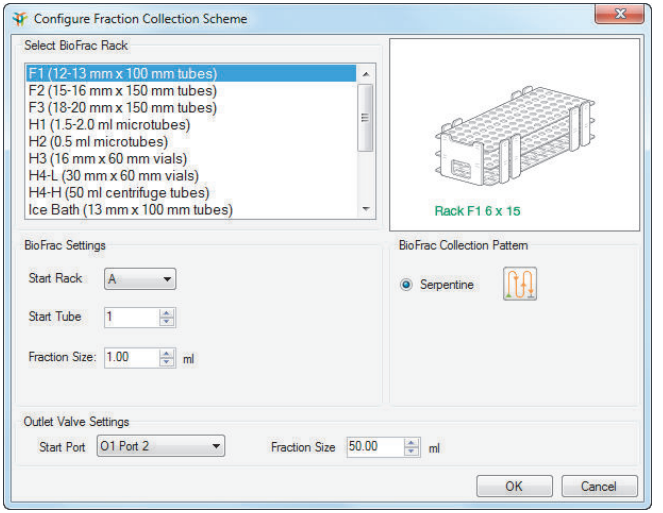
   Tip: The single-wavelength UV detector monitors UV absorbance one wavelength at a time. You can select either 255 nm or 280 nm as the default. The multi-wavelength UV/Vis detector monitors up to four wavelengths in the UV/Vis range of 190–800 nm.

6. Under Unit Selection, select the Method Base Unit: volume (ml), time (min), or column volume (CV).

7. Under Fraction Collection, click Configure to select a rack from images in the Configure Fraction Collector Scheme dialog box and choose an available collection pattern and fraction size.

   Serpentine is selected by default. Fractions can also be collected by rows or columns for microplates. Possible choices appear for the rack you select.

If you have outlet valves in the fluidic scheme, choose a starting valve and port and modify the fraction size.



8. Under Flow, specify the default flow rate for the method. You can change the default flow rate within individual phases if needed.

   Select the Control the flow to avoid overpressure checkbox if you want the flow rate to decrease and the method to continue running in the event that the system reaches high pressure limits. If you do not select this checkbox the system pumps will stop, pausing the method.

   If you have an air sensor installed and you want to monitor and stop the system pumps if air is detected in the lines, select the Detect end of buffer with Air Sensor checkbox.

   Note: If the method pauses due to an end-of-buffer signal, the fluidic lines must be primed before resuming the method. Because the system pump's Purge button is inactive in Method mode, you must reprime the pumps manually. See Priming and Purging the System on page 96 for information about priming your system.

9. Select a pump head type. To set the control parameters see Control Flow Tab on page 74.
10. Under Buffer Selection, select buffers for each inlet line or select the buffer system and pH if you are using the buffer blending module. See also Buffer Selection on page 141.
11. Click Rename Ports to change the names Buffer A and Buffer B to more descriptive terms.

    Names you select here appear in the method report.

Adding Phases

When you add a phase to a method, the parameters for the added phase appear in the Phase Parameters pane.

To add phases to a method

1. Select Method Outline in the left pane.
2. Click the Add Phase button in the Method Outline pane to open the Phase Library.
3. Drag a phase from the Phase Library to a position in the Method Outline pane.
4. Continue adding phases until the method is complete.
5. Save the method.

Editing Methods and Phases

Changes you make in the Method Settings view affect the entire method. Changes you make to specific phases apply only to that phase.

To edit phases in the Phase Parameters pane

1. With Method Outline selected in the left pane, select a phase in the method's Method Outline pane.

   The settings for the selected phase appear in the Phase Parameters pane.
2. Edit the phase parameter settings.

3. Repeat steps 1 and 2 until you have finished editing phase settings for the method.
4. Save the method to preserve your changes.

   Note: Changes you make in the phase parameters to the duration of flow steps and salt gradients are graphically depicted in the Gradient Graph pane. Conversely, changes you make to the gradient graph are reflected in the phase parameters.

Renaming Phases

You cannot rename phases in the Phase Library. Renaming a phase in the Method Outline pane changes its name only for the current method. However, renamed phases can be saved as custom phases for use in other methods.

To rename a phase in the Method Outline pane

1. In the Method Outline pane, select the phase to rename.
2. Do one of the following:
   - Select Edit > Rename Phase.
   - Right-click the selected phase and select Rename Phase.

   The Rename Phase dialog box appears.
3. Edit the name of the phase and click Save.

Rearranging Phases

To rearrange phases in a method

1. Select a phase in the Method Outline pane.
2. Drag the phase to a new position.

Deleting Phases

To delete a phase from a method

1. In the Method Outline pane, select the phase to delete.
2. Do one of the following:
   - Select Edit > Delete Phase.
   - Right click the selected phase and select Delete <phase_name>.
3. To confirm deleting the selected phase, click Yes in the dialog box that appears.

Opening a Method

To open a method

1. Do one of the following:
   - In the Home window, click Open Method.
   - In the Method Editor window, click Open on the toolbar.

   The Open Method dialog box appears. It lists all user methods by default.
2. Click the name of the method to open.

   The method you selected opens in the Method Settings view. Its fluidic scheme appears in the Fluidic Scheme pane and its general settings appear in the Method Settings pane.

Running a Method and Collecting Fractions

You can schedule a method to start immediately or add it to the run queue to run at a later time. You can start a run immediately only if there are no scheduled methods in the run queue. If there are methods in the queue, new methods are added to the end of the queue. Queued methods run sequentially. You can remove a scheduled method from the queue if it is not running, but you cannot reorder the queue. You start queued methods in Manual mode from either the touch screen or ChromLab.

When you schedule multiple runs of a method, you can specify to append or overlay fractions.

Collecting Fractions Using the BioFrac Fraction Collector

Append Fractions — the collection skips a tube between runs and goes to the next available tube.

Overlay Fractions — the collection for all runs uses the same set of fraction tubes.

Collecting Fractions Using Outlet Valves

Append Fractions — the collection goes to the next available port after each run and does not skip a port. When the last port is used, collection restarts at the port defined in the method.

Overlay Fractions — the collection starts at the port defined in the method and uses the same ports for all runs. When the last port is used, collection restarts at the port defined in the method.

Collecting Fractions in Run Queues

When you schedule queued methods, collection begins with the tube or port defined in the first method in the queue.

Collecting Fractions Using the BioFrac Fraction Collector

After the first method is completed, fraction collection skips a tube and continues with the next available tube (as if the run queue is set to Append mode). For example, if Run 01 collects fractions in tubes A1–A10, fraction collection for Run 02 skips tube A11 and begins in tube A12. This process of skipping a tube continues for each run in the queue.

If a subsequent method in the queue is a multiple run in overlay mode, fraction collection for the first run skips a tube and starts repeating tubes from the first tube of the first run in that multiple run.

Note: If a currently running queued method is paused and then continued, fraction collection continues with the next available tube. If a currently running queued method is stopped, the queue begins again with the next method. In this case, fraction collection skips a tube and continues from the next available tube.

Collecting Fractions Using Outlet Valves

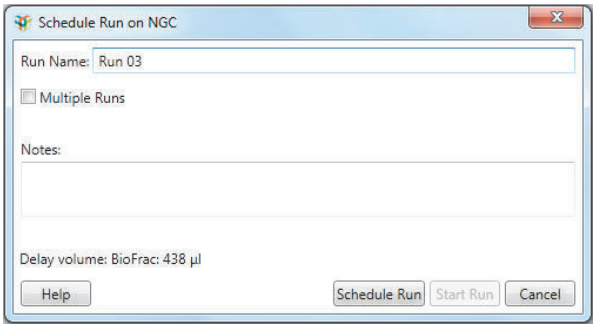
After the first method is completed, fraction collection continues with the next available port (as if the run queue is set to Append mode). For example, if Run 01 collects fractions from ports P2–P10, fraction collection for Run 02 begins from P11. This process continues for each run in the queue.

When the last port is used, collection starts at the port defined in the current method.

Note: If a currently running queued method is paused and then continued, fraction collection continues with the next available port. If a currently running queued method is stopped, the queue begins again with the next method. In this case, fraction collection starts from the port defined in this new method.

To run a method

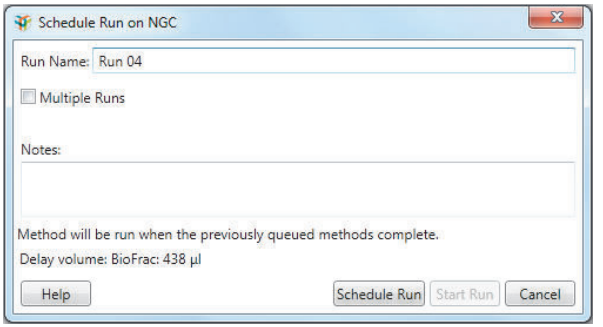
1. Open the method to run.
2. On the toolbar, click Start Run. The Schedule Run dialog box appears.



3. (Optional) Type a name for the run. The run name can consist of up to 85 characters.
4. To begin the run immediately, click Start Run.

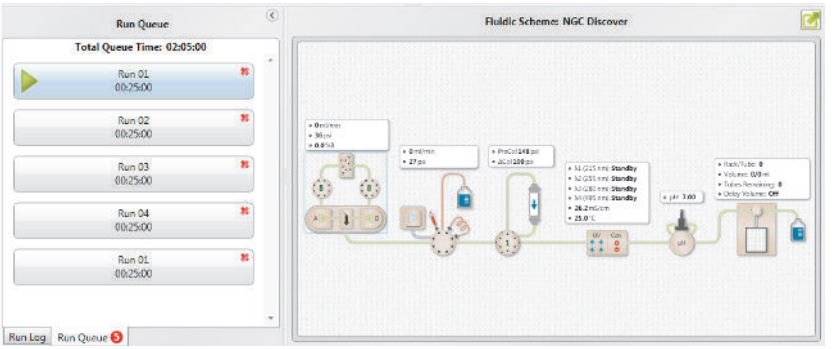
To add runs to the run queue

1. In the Method Editor click Start Run. The Schedule Run dialog box appears displaying a message that the method will be run after all runs currently in the queue are completed.



2. Click Schedule Run. The method is added to the end of the run queue in System Control.

3. Repeat steps 1–2 to add as many methods to the queue as needed.
4. If the first run was not selected to start immediately, click the green start arrow on the first run in the Run Queue to start running the methods in the queue.



Tip: To remove a run from the Run Queue, click .

To run a method multiple times in a sequence

1. In the Schedule Run dialog box, select Multiple Runs.
2. Specify the number of times to run the method and whether to append or overlay fractions.
3. Do one of the following:
   - Click Start Run to begin running the method immediately.
   - Click Schedule Run to place the method in the Run Queue so you can run it later.

Saving a Method

You can modify a method — whether or not you have run it — and save it under the same name. The modifications will not apply to previously saved runs of that method. Saved runs will display the parameters that were used by the method to perform that run before it was modified.

To save a method

▶ Click Save on the toolbar.

The method is saved in the current project.

To save a modified method or to save a template as a method

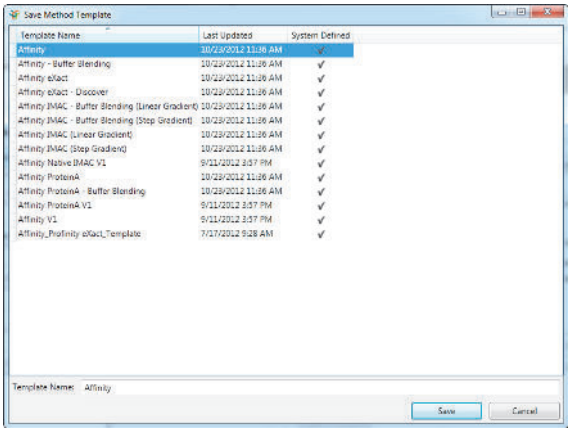
1. Click Save As on the toolbar.
2. The Save Method As dialog box opens with the current project name selected in the Projects pane.
3. (Optional) Select a different project in the Projects pane.
4. Type a name for the method in the Method Name box and click Save.

To save a method as a template

1. Click Save As Template on the toolbar.

   The Save Method As Template dialog box opens. The current method's template type appears in the Template Name box.

   Tip: It is good practice to include the template type as a prefix to the template name.



2. Type a name for the new template and click Save.

Renaming a Method

You can rename an open method. While Save As retains the original method and saves the modified method under a new name, renaming a method saves the original method under a new name.

To rename a method

1. Open the method to rename.
2. Select File > Rename.

   The Rename Method dialog box opens.
3. Type a new name for the method and click Rename.

Deleting a Method

You can delete an open method. If the method is associated with runs, you can choose to delete only the method or both the method and its associated runs.

To delete a method

1. Open the method to delete.
2. Select File > Delete.
3. (Optional) If runs are associated with the method, choose one of the following options in the dialog box that appears:
   - Delete Method Only — the system hides the method from view. If you subsequently open a method through a run associated it, the system displays the deleted method. You can save the deleted method with a new name.
   - Delete Method and Runs — the system deletes the method and its associated runs.

Scouting on Parameters within a Method

Scouting is a procedure used to systematically locate parameters that most impact peak resolution and to optimize on these parameters to achieve your protein purification goals (for example, high purity, yield, stability, or activity). Molecules differ from one another in their charge, hydrophobicity, solubility, reactivity, and substrate specificity, and in their intermolecular interactions. A purification protocol that is satisfactory for one type of molecule might not work for a different molecule type. Several factors influence the quality of separation in a purification procedure. These factors include buffer composition (pH, ionic strength, cosolutes), elution type (gradient slope and gradient duration), flow rate, column chemistry, and sample composition. In principle, each of these can be adjusted to produce the most efficient and effective purification strategy for a molecule. In practice, only a few of these are generally tested due to time and cost considerations. By performing a series of automated scout runs, the time and resources required for protocol optimization can be significantly reduced.

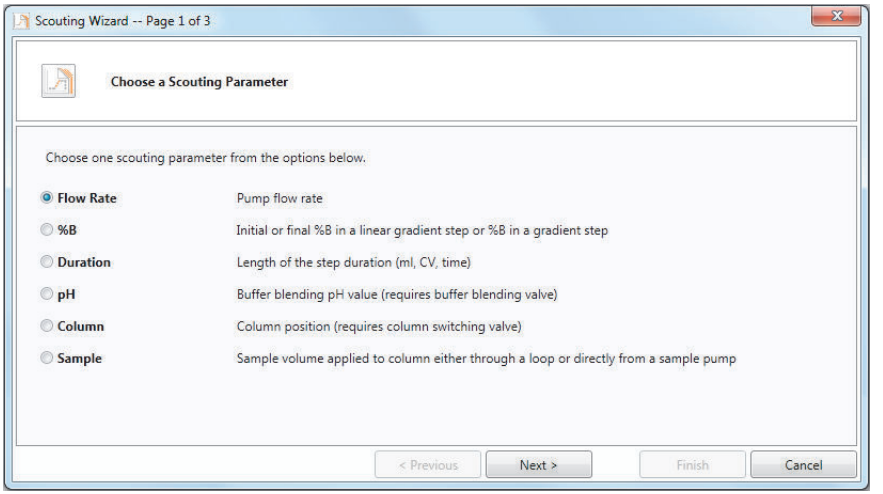
Using ChromLab's scouting wizard you can easily generate a series of methods that scout on a parameter within certain sections of the method. Methods used in scout experiments can be generated from an existing method, created from scratch, or loaded from the Bio-Rad method templates.

Scouting methods can be saved using the Save As option. After you create a scouting series of methods, changing the method outline will invalidate the scouting series and you will be prompted to scout again and save the method as a new scouting method.

The Scouting Wizard

The scouting wizard guides you through three pages: Choose a Scouting Parameter, Select Method Steps to Scout, and Generate Scout Sequence. This section explains how to use each page in detail.

Page 1: Choose a Scouting Parameter



On this page you select the parameter on which to scout. You can select only one parameter per method to scout.

- **Flow Rate** — optimize the pump flow rate for adsorption and elution steps.
- **%B** — vary the buffer composition in isocratic gradient steps or the initial or final buffer composition in linear gradient steps.
- **Duration** — vary the length of the elution step in volume.
- **pH** — find the optimal pH for the method in buffer blending mode (requires a buffer blending valve).

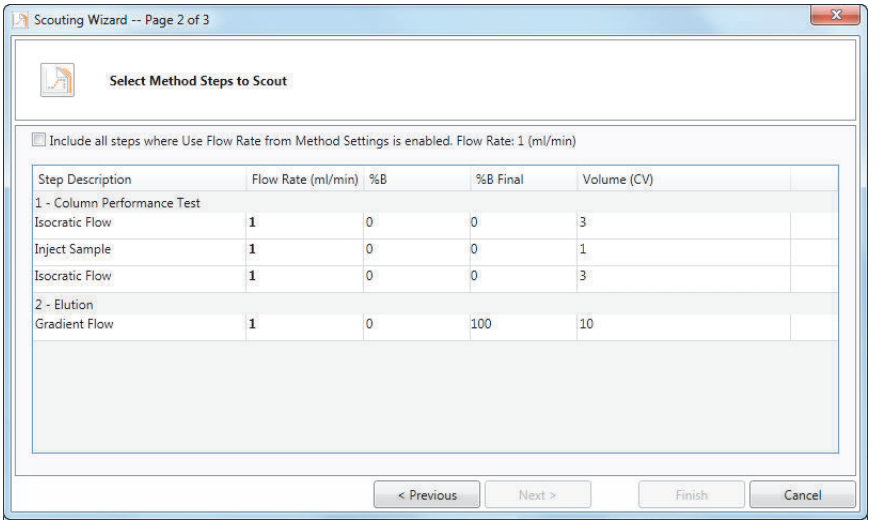
- **Column** — test up to five column types for each column-switching valve in use. This parameter requires at least one column-switching valve in the fluidic scheme.

  Note: Pages 2 and 3 of the scouting wizard differ when scouting for columns and samples. See Using the Scouting Wizard to Scout Columns on page 189 or Using the Scouting Wizard to Scout Samples on page 191 for more information.

- **Sample** — test up to seven different samples when one sample inlet valve is used or up to 14 different samples when two sample inlet valves are used (Port 8 is reserved for wash). This parameter requires at least one sample inlet valve in the fluidic scheme.

  Tip: This option is available if, in the Sample Application phase, you chose to load the loop through the sample pump or inject sample directly onto the column. It is not available if you chose to load the loop manually.

Page 2: Select Method Steps to Scout



On this page you select the method steps to be scouted. The method steps that include the parameter you selected on Page 1 of the scouting wizard are shown.

Depending on the parameter you chose on Page 1, you can select individual steps to scout or you can select the checkbox to scout all steps in the method in which the properties for that parameter from the method settings are enabled. All steps that you select are highlighted.

If you select **Include all steps where <parameter> from Method Settings is enabled**, the following rules apply:

- Steps that have the parameter **Use <parameter> from Method Settings is enabled** selected are automatically included in the scout.
- Steps that do not have the parameter **Use <parameter> from Method Settings is enabled** selected are not included.
- Steps that are not included can still be selected. Steps that are included can be canceled from the scout. In both cases, the checkbox **Include all steps where <parameter> from Method Settings is enabled** is cleared.

If you do not select **Include all steps where <parameter> from Method Settings is enabled**, the following rules apply:

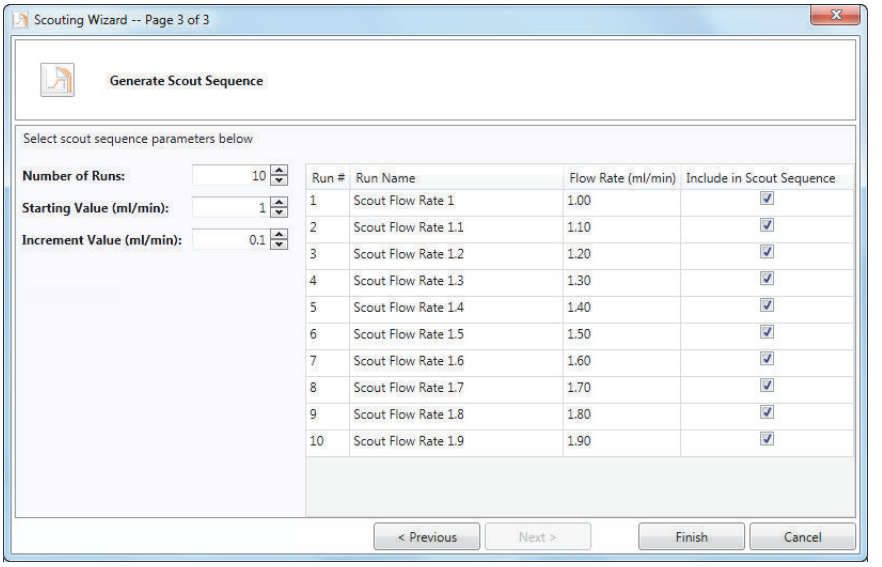
- Only steps that include the scouted parameter can be selected.
- Multiple steps might share the same parent setting. In these cases, selecting one step selects all steps in the group.
- Steps that cannot be selected are grayed out.
- If the scouted parameter values do not match in all steps that you select, the following message appears below the table: “You have selected steps with different starting values. Please deselect these steps or change the starting value to proceed.”

You can clear previously selected steps. The following rules apply:

- You can clear individual steps or groups of steps.
- If you clear a step when the parameter **Include all steps where <parameter> from Method Settings is enabled** is selected, the

checkbox is also cleared. The other steps remain selected and will be included in the scout.

Page 3: Generate Scout Sequence



On this page you set the number of runs to be performed, name each run, and set the parameters for the scouted steps.

- **Number of runs** — sets the number of runs to be performed as part of the scout experiment.
- **Starting Value** — sets the starting value for the parameter chosen to be scouted. The default is the starting value in the base method.
- **Increment Value** — sets the scout run increment values. The scout increment value can be positive or negative.

The scout sequence table comprises the following columns:

- **Run #** — the scout run number. This field is not editable.

- **Run Name** — the scout run name. The autogenerated name is based on the parameter you chose on Page 1 of the scouting wizard and the scout starting value and increment value. This field is editable.

  Tip: To edit the run name column easily, create a list in Microsoft Word or Excel. Copy and paste the list into the column to replace the autogenerated names.

- **<Parameter>** — the value of the scouted parameter for that run based on the starting value in the base method and the increment value. This field is editable.

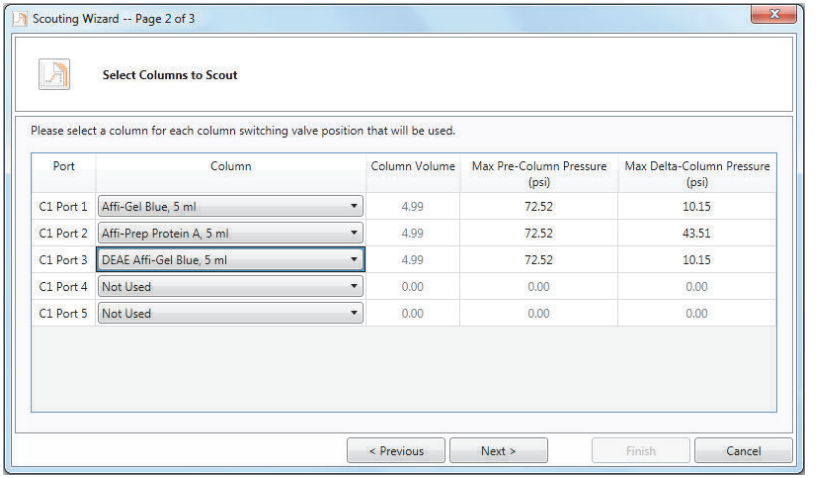
- **Include in Scout Sequence** — determines whether the run is included in the scout method. By default, all runs are included. This field is editable.

Using the Scouting Wizard to Scout Columns

This section explains how to set up pages 2 and 3 of the scouting wizard when scouting columns.

Note: This section applies only if you chose to scout columns on Page 1. Refer to the images in the previous section if you chose another parameter to scout.

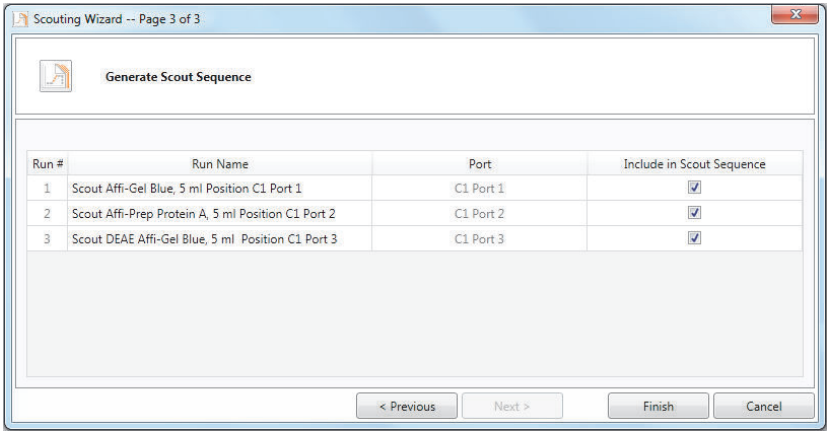
Page 2:



On this page you can select up to five columns for each column-switching valve to scout. The column dropdown list comprises all columns in the column library, including user-defined columns (see the section To add user-defined columns on page 136 for information about user-defined columns). The wizard displays the values for column volume, maximum precolumn pressure, and maximum delta-column pressure for each selected column.

Alternatively, you can choose Custom from the dropdown list and modify these values to create a custom column to scout.

Page 3:



On this page you can name each run and include or exclude a column in the scout sequence. The scout sequence table comprises the following columns:

- **Run #** — the scout run number. This field is not editable.
- **Run Name** — the scout run name. The autogenerated name is based on the column's position on the column switching valve module and the name of the column chosen on Page 2. This field is editable.

  Tip: To edit the run name column easily, create a list in Microsoft Word or Excel. Copy and paste the list into the column to replace the autogenerated names.

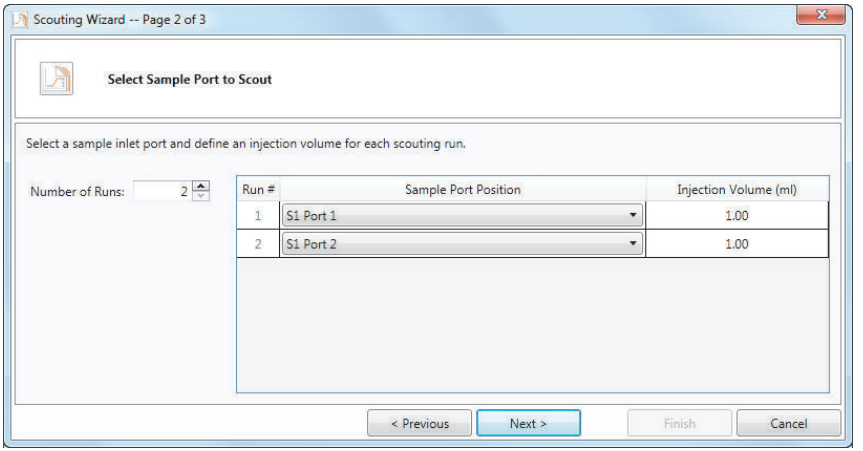
- **Port** — the column's position on the column switching valve module. This field is not editable.
- **Include in Scout Sequence** — determines whether the run is included in the scout method. By default, all runs are included. This field is editable.

Using the Scouting Wizard to Scout Samples

This section explains how to set up pages 2 and 3 of the scouting wizard when scouting samples.

Note: This section applies only if you chose to scout samples on Page 1. Refer to the images in the previous sections if you chose another parameter to scout.

Page 2:

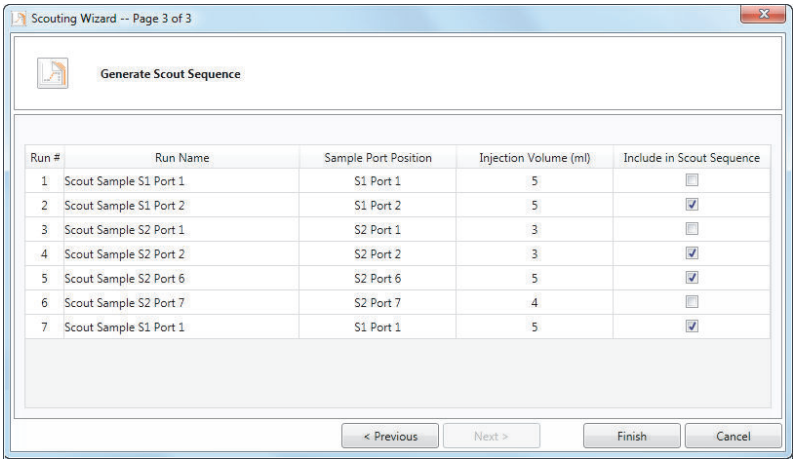


On this page you can select up to 14 samples to scout. For each run, choose a sample inlet valve and port number from the dropdown list and modify the sample injection volume for that port. You can select the same port multiple times if you want to scout different values for that sample.

Tip: You might see a message alerting you that you did not choose to include a preinjection sample pump wash in the method. The wash ensures that any remaining sample is flushed from the flow path before injecting the next sample in order to avoid cross contamination. If you click Yes in the message box to

include a preinjection sample pump wash, the method is updated and you proceed to Page 3. If you click No in the message box (if, for example, you are using the same sample), the method is not updated and you proceed to Page 3.

Page 3:



On this page you can name each run and include or exclude a sample in the scout sequence. The scout sequence table comprises the following columns:

- **Run #** — the scout run number. This field is not editable.
- **Run Name** — the scout run name. The autogenerated name is based on the sample inlet valve module number and the port number on that valve. This field is editable.

  Tip: To edit the run name column easily, create a list in Microsoft Word or Excel. Copy and paste the list into the column to replace the auto-generated names.
- **Sample Port Position** — this field is not editable.
- **Injection Volume (ml)** —this field is not editable.

- **Include in Scout Sequence** — determines whether the run is included in the scout method. By default, all runs are included. This field is editable.

Creating a Scouting Method

Note: Before starting the scouting wizard, ensure that the method settings and protocol are correct. After creating a scouting method series, any changes will require you to run the wizard again.

To create a scouting method

1. Create a new method, copy an existing method or use a Bio-Rad method template.
2. Click Scout in the Method toolbar. The scouting wizard starts.
3. On the first page of the wizard, choose one parameter to scout.
4. On the second page, select the steps in the method to scout.
5. On the last page, set the sequence parameters for the scout:
    - Change the values of the sequence parameters.
    - Double-click the run name to edit the value in that field.
    - Clear the checkboxes for runs that you do not want to include in the scout method.
6. Click Finish to complete the scout setup and close the wizard.

Special Considerations

Rules for generating scout sequences can vary depending on the parameter chosen to scout. This section provides a list of considerations to take into account when generating a scout sequence.

Generating %B Scout Sequences

- You can scout on two or more steps with the same starting %B values. If you select two or more steps with different starting values, you must clear the steps with the different values.
- When a linear gradient is selected for scouting and the value for the next or previous %B step is the same as the selected gradient, that step is automatically selected for scouting. Clear that step if it is not required.

  Note: If an isocratic step is selected for scouting, no other steps are automatically selected. You can choose others manually.
- When the starting value for %B is 100, the increments (on Page 3) default to a negative value.

Generating pH Scout Sequences

- This option is available only when the fluidic scheme includes the buffer blending valve and buffer blending is selected in Method settings.
- The minimum and maximum pH scouting range is defined by the buffer selection made in Method settings.

Generating Duration Scout Sequences

- Choosing two or more steps that have different starting values or that have different units is not supported. Clear the steps with different starting values or units to proceed.

Generating Column Scout Sequences

- This option is available only when the fluidic scheme includes the column switching valve.
- If you chose Custom as the column type and changed the default values for column volume, maximum precolumn pressure, and/or maximum delta-column pressure, your custom settings must be within the system limits defined by the method settings. If your custom values are outside of this range, a red box appears around the number. The wizard prevents you from continuing until you enter a valid number.

Generating Sample Scout Sequences

- This option is available only when the fluidic scheme includes at least one sample inlet valve and you chose to load the loop through the sample pump or inject sample directly onto the column in the Sample Application phase. It is not available if you chose to load the loop manually.
- If your method contains multiple Sample Application phases, scouting is performed only on the first sample loading step in the method.
- If you select Load Loop with Sample Pump in the method, the injection volume in the scout refers to the system pump injection volume, the initial injection volume is the same as the system pump injection volume set in the method, and the minimum injection volume is 0.01 ml.
- If you select Inject Sample on Column with Sample Pump in the method, the injection volume in the scout refers to the sample pump injection volume, the initial injection volume is the same as the sample pump injection volume set in the method, and the minimum injection volume is 0.01 ml.
- The maximum injection volume is 1,000 L.

Viewing a Summary of the Scouting Method

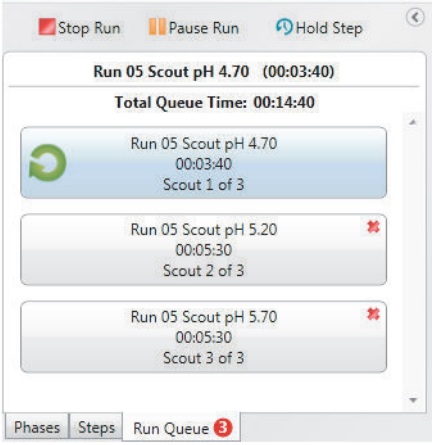
When you complete the scouting wizard, the Method Editor table displays a summary of the scouting parameters in a read-only table in a new pane, Scout Parameters. See Scout Parameters Tab on page 162 for more information.

Tip: When you open the scouting wizard in a saved scouting method, the values for that scouting method persist in the wizard as well as in the method.

Running a Scouting Method

You can schedule scouting methods to run immediately or add them to the queue to run at a later time. As with regular methods, you can start scout runs immediately only if there are no scheduled methods in the run queue. If methods are in the queue, the scout runs are added to the end of the queue.

Each scouting method appears in the Run Queue individually. Each run is identified as a scout run on the run label, for example:



You can remove from the queue any scout methods that are not running, but you cannot reorder the scout run queue.

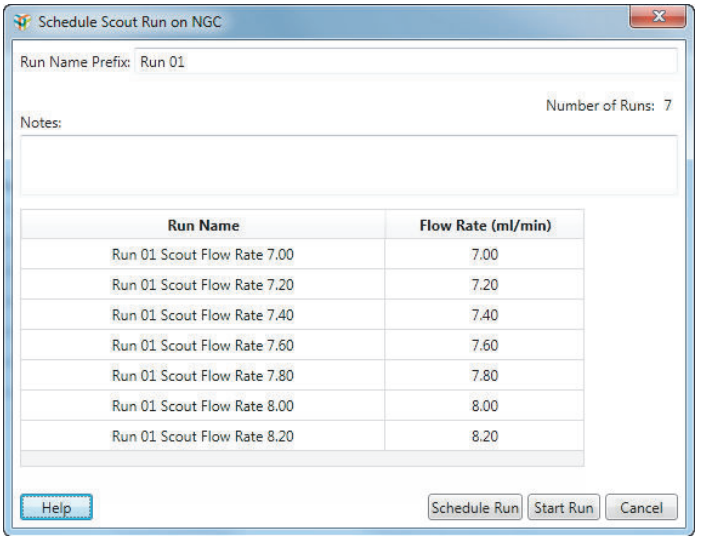
When you collect fractions during scouting, the fractions get appended to the fractions collected in the previous run. The collection skips a tube between each scout run and goes to the next available tube.

Tip: See Running a Method and Collecting Fractions on page 177 for more information about running methods.

To run a scout method

1. Open the method to run.
2. On the toolbar, click Start Run.

   The Schedule Scout Run dialog box displays the number and names of runs that are scheduled, and their scout parameter.



3. (Optional) In the dialog box you can change the run name prefix. The run name prefix for each run changes dynamically as you type the new prefix. The run prefix can consist of up to 35 characters.

4. Do one of the following:
   - Click Start Run to begin the run immediately.
   - Click Schedule Run to put the methods in the run queue and run them later.

   Tip: To remove a run from the run queue, click  .

7 Evaluating Results

In the Evaluation window, you can process and analyze chromatography data. The results can be viewed in a variety of ways. A single run appears when you open a run. Multiple runs appear in separate tabs when you open multiple runs individually. Multiple runs can also be compared in a single tab view. The runs in this view can be either stacked or overlaid for comparison.

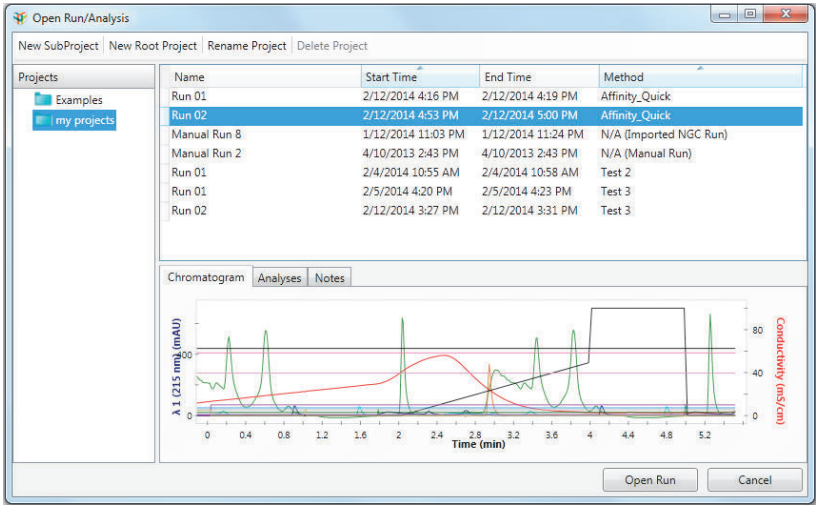
There are two types of results files: runs and analyses. A run is the set of saved data that results from running an experiment manually or using a method automatically. An analysis file is data from a run on which peak integration has been performed. A trace comparison, which consists of multiple runs displayed as an overlay or stack, is also considered an analysis. You can perform peak integration on a single run or on multiple runs in a single space.

You can import Unicorn and BioLogic DuoFlow™ software data files into an analysis project as well as NGC™ files exported from ChromLab™ software running on another computer. See Chapter 8, Importing and Exporting Data for more information.

Managing Analysis Projects

You can open a run or analysis in the Open Run/Analysis dialog box and organize runs and analyses by creating projects and subprojects. You can also create a root project in which to store your projects and subprojects.

Saved projects and subprojects are listed in the left pane. Runs in the selected project or subproject are listed in the right pane with start and end times and method type.



For the selected run, a read-only image of the chromatogram, a list of saved analyses, and run notes appear in tabs in the lower pane.

To create a root project, project, or subproject

1. Select File > Open Run/Analysis to open the dialog box.
2. At the top of the dialog box, click the button for the type of project to create.
3. Type a name for the project in the box that appears above the Projects pane and then click Save.

   The project you created appears in the Projects pane.

To group the runs list by method

▶ Right-click the runs list and choose Group by Method in the menu that appears.

To display run data for a single run

▶ Select a run and click Open Run.

The Evaluation window displays the run data.

To display data for multiple runs

1. Hold down Ctrl or Shift and select multiple runs from the list in the right pane.

   The Open Runs button becomes a dropdown list.

2. In the dropdown list, do one of the following:

   - Select Open in Multiple Tabs to view each run in a separate tab.
   - Select Open as a Trace Comparison to view the selected runs in one new Analysis tab.

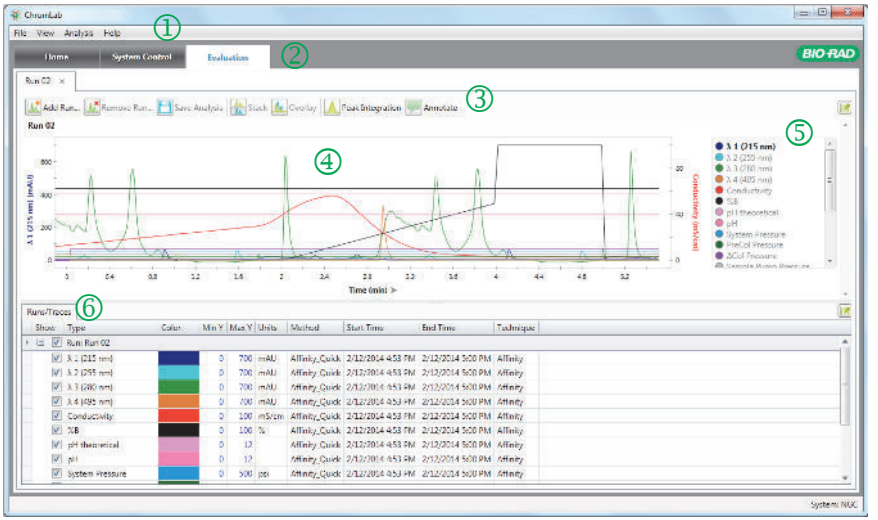
   Tip: To see the runs in each method group, expand the list by clicking the plus sign.

To open a saved analysis

1. Select a run in the runs list.
2. In the Analysis tab, select an analysis.
3. Click Open.

Evaluation Window

In the Evaluation window you can display run data for a single run or multiple runs. Multiple runs can be displayed in separate tabs or in a single Analysis tab as a trace comparison. Data appear as individual traces in the chromatogram. Pausing the pointer over a trace in a chromatogram displays a tooltip with coordinate information. The trace table that appears below the chromatogram documents the details of each trace.



LEGEND

1	Menu bar provides quick access to File, View, Analysis, and Help menu commands.
2	Tabs access main functional areas.
3	Tab toolbar buttons provide quick access to commands.
4	Chromatogram displays analysis results as traces.
5	Chromatogram legend defines trace colors and display options. It also displays the wavelength value in nanometers for UV traces.
6	Runs/Traces, Peaks, and Fractions tabs display analysis data in tabular form.

File Menu Commands

Open Run/Analysis — opens the Open Run/Analysis dialog box in which you can select runs to display in the Evaluation window.

Add Run — opens the Add Runs dialog box in which you can select additional runs to display in the same tab for multiple runs trace comparison.

Remove Run from Analysis — opens a dialog box in which you can choose runs to remove from a multiple runs trace comparison.

Show Method — opens the method for the displayed run in the Method Editor window. When the displayed run is a scout run, this command opens the method associated with the run. The method is identified by an asterisk after its name (for example, Method Name: Scout Flow Rate 2.00*).

Show Scout Method — (available only when the displayed run is a scout run) opens the original method from which the scout method was created. The Method Editor includes the Scout Parameters tab from which you can view all scouting runs associated with the scout method and their settings.

Close Run/Analysis — closes the displayed run or analysis.

Close All Runs/Analyses — closes all open runs and analyses.

Save Analysis — saves the displayed analysis.

Copy Analysis — creates a copy of the displayed analysis.

Export — exports a single run as a .csv file, which can be imported into spreadsheet applications such as Excel, or as an .ngcRun file, which can be imported by ChromLab software on another computer. See Exporting Data on page 268 for more information.

Import NGC File — imports .ngcRuns files exported from ChromLab on another computer. You can select the destination project and name for the imported project. See Importing an NGC Method or Run on page 263 for more information.

Import Unicorn Data — opens a dialog box in which you can import a data file into the NGC database. See Importing Unicorn Data Files on page 265 for more information.

Import DuoFlow Data — opens a dialog box in which you can import a data file into the NGC database. See Importing BioLogic DuoFlow Data Files on page 267 for more information.

Rename Run/Analysis — opens a dialog box in which you can rename the displayed run or analysis.

Delete Run/Analysis — deletes the displayed run or analysis.

Analysis Notes — enables you to view and edit notes for the displayed analysis.

Run Report — compiles and displays a report of the displayed run. You can choose sections to appear in the report, including the chromatogram, column performance statistics, system information, the run/event log, and annotations. The report can be saved in .pdf, .doc, and .ppt file formats.

Analysis Report — compiles and displays a report of the displayed analysis, including participating runs, chromatograms, peak parameters, fractions, and peaks table data, based on columns that you choose to include in the report. The report can be saved in .pdf, .doc, and .ppt file formats.

Method Report — compiles and displays a report of the displayed method, including the relevant method settings and steps.

Preferences — opens dialog boxes in which you can do the following:

- Select pressure units for all system and software pressure values. This is a global setting.
- Set up an SMTP server to send email messages about system notifications from the ChromLab computer.

Exit — closes ChromLab.

View Menu Commands

Overlay View — displays multiple runs in a single chromatogram window.

Stack View — displays multiple runs in a stack, one above the other, sorted alphabetically by name.

Show Pre-Injection — displays data collected before the injection point.

Show Baseline — following peak integration, shows the baseline curves in the chromatogram.

Show Peak Area —following peak integration, shows the peak areas in the chromatogram.

Show Peak Labels —following peak integration, shows the start, end, and apex labels of peaks in the chromatogram.

Show Peaks for All Traces — following peak integration, shows baseline, peak area, and peak labels for all integrated traces.

Show Chromatogram — displays one or more chromatograms. Clear this command to hide chromatograms.

Show Table — displays one or more tables. Clear this command to hide tables.

Lock UV Scales — when selected, locks the y-axes of all UV traces to the same y-scale range. When not selected, each multi-wave axis can use a different y-scale range.

Analysis Menu Commands

Set Injection Point — opens a dialog box in which you can specify the injection point (where x = 0) of displayed runs.

Peak Integration — calculates and displays peaks and their values. If integration has already been performed, opens the Peak Integration pane.

Manual Integration — following peak integration, opens the Manual Integration pane.

Delete Peak List — deletes previously calculated peaks. All peaks are removed from traces you selected in the traces list in the peak integration pane.

Analyze Column Performance — following a column performance test run, detects the largest peak within the column performance phase and opens the Column Performance pane.

Tools Menu Command

Flow Rate Converter— opens the Flow Rate Converter tool, which enables you to determine the flow rate to use for each column in the method based on the column size and the initial rate entered. A rate entered in ml/min is converted to cm/h and L/h; a rate entered in cm/hr is converted to ml/min and L/h. You can copy the result in the converter and paste it into your method.

Help Menu Commands

Help — displays screen-level help topics and links to installed manuals.

About — displays version and copyright information about ChromLab software.

Tab Toolbar Commands

Add Run — opens the Add Runs dialog box in which you can select a run to display in the same tab.

Remove Run — opens a dialog box in which you can choose runs to remove from a multiple-run trace comparison.

Save Analysis — saves the displayed analysis and display settings you selected.

Stack — displays multiple runs in a stack, one above the other, sorted alphabetically by name.

Overlay — displays all multiple runs in a single chromatogram window.

Peak Integration — calculates and displays peaks and their values. If peak integration has already been performed, opens the Peak Integration pane.

Annotate — adds a note to the chromatogram at the specified point on the x-axis. Multiple notes can be added to a chromatogram.

Charts in View — visible when multiple runs are displayed in stacked view, this setting enables you to select the number of runs in the view.

Customizing the Chromatogram

You can change the color of the traces, show or hide selected traces, control the range of the y-scale for each trace, and annotate the chromatogram at specific points on the x-scale. ChromLab saves trace display and run view settings and uses them when displaying subsequent runs.

Displaying Traces

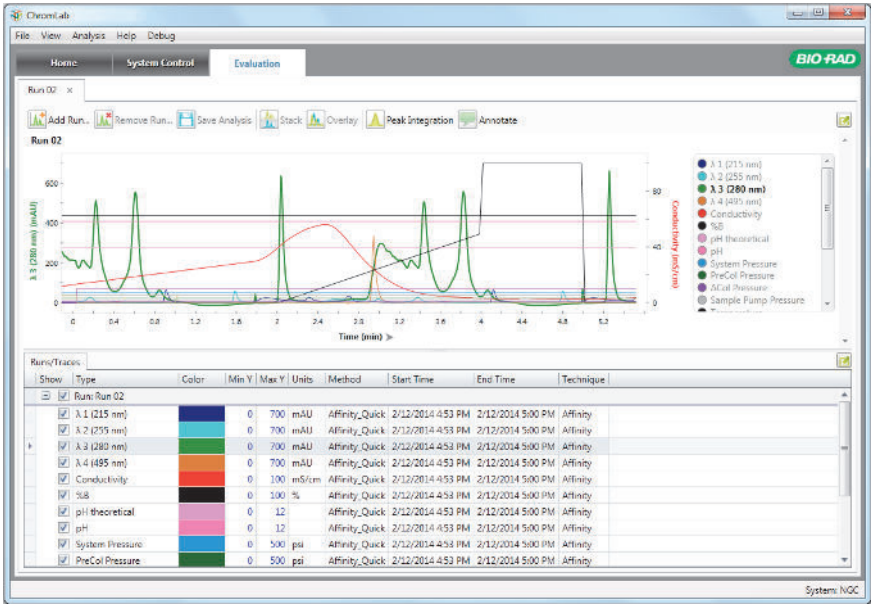
The available traces in each configuration appear in the following order in the System Control and Evaluation windows chromatogram legends. Not all traces appear in all configurations.

- UV Traces (in numerical order, λ1, λ2, λ3, λ4)
- Conductivity
- %B
- pH Theoretical
- pH (measured)
- System Pressure
- PreCol Pressure (in numerical order for each inline column switching valve)
- ΔCol Pressure (in numerical order for each inline column switching valve)
- Sample Pump Pressure
- Temperature
- Flow Rate (system pump)
- Sample Pump Flow Rate
- SIM 1 (provided trace name is displayed)
- SIM 2 (provided trace name is displayed)

Selecting the Active Trace

When a trace is selected, it appears bolded, the row corresponding to the trace is selected in the trace table, and the primary y-axis (on the left of the chromatogram) changes to the units and scale of the trace. For example, when UV is selected, the units and scale of the y-axis display as mAU.

Note: The secondary y-axis (on the right side of the chromatogram) does not change when you select the active trace. See Changing the Axes on page 210 for information about changing its axes.



To select a trace

▶ Do one of the following:

- Click the trace in the chromatogram.
- Click the trace item in the legend.
- Select a row in the trace table.

Showing or Hiding a Trace

To show or hide a trace

▶ In the trace table, select or clear the Show checkbox in the trace row.

Changing the Axes

The chromatogram has two y-axes. The left axis is the primary axis. Its default trace is UV absorbance. The default trace of the right axis is Conductivity. You can change the units of either y-axis by clicking its label, which is a toggle, to show the next trace's scale and units.

Alternatively, you can change the units of the primary axis by choosing the active trace in the chromatogram, as described in Selecting the Active Trace on page 209.

To change x-axis units

▶ Click the x-axis title to switch between the available options.

For runs that were performed from saved methods, the x-axis unit can be changed to Time (min), Volume (ml), or Column Volume (CV). For runs that were performed manually, the x-axis unit can be changed to Time (min) or Volume (ml).

Controlling the Range of the Y-Scale

For each trace, you can change the maximum and minimum y values in the trace table to set the chromatogram scale. By default, traces that share the same UV units have the same y-scale range. When you change the y-scale range (Min Y or Max Y) of a trace, the new range applies to all traces in the same run that have the same units. In a display in which traces from different runs are overlaid in a single chromatogram, changing the y-scale range of one trace changes the y-scale range of all overlaid traces that have the same units. When traces from each run are stacked in the display, the scale for each of the stacked chromatograms can be different.

To change the Max Y and Min Y values in the trace table

▶ Enter maximum and minimum values in the appropriate trace table row, ensuring that the maximum value always exceeds the minimum value.

To rescale UV units individually for multiple or overlaid traces

▶ Right-click a trace in the trace table or chromatogram and clear Lock UV scales in the menu that appears.

All traces are unlocked and can be rescaled individually.

To lock UV units for multiple or overlaid traces

▶ Right-click a trace in the trace table or chromatogram and select Lock UV scales in the menu that appears.

To restore the default y-scale range

▶ Right-click a trace in the trace table and select Restore Default Y Scale Range in the menu that appears.

Changing Trace Colors

You can change trace color to increase print quality or to more clearly distinguish one trace from another.

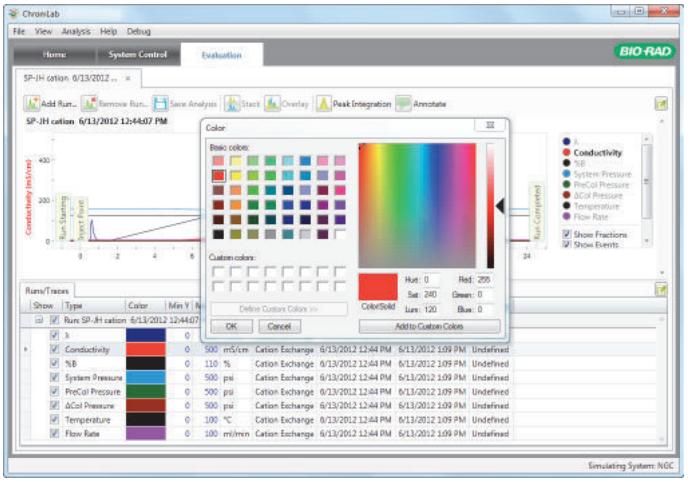
The default trace colors in the System Control window are identical to the default colors shown in the Evaluation window. Each trace color denotes a type of default trace. The trace colors are defined in the chromatogram legend.

Color changes you make in the System Control window to a run in progress or before initiating a run are saved and appear when the run is opened in the Evaluation window.

When you change the color of a trace in the Evaluation window, the color change applies to the trace in the table, legend, and chromatogram. Color changes are saved with the specific run in evaluation settings. They do not affect System Control window settings or other runs.

To change the trace color

1. In the Runs/Traces tab, double-click a color cell in the table to open the Color chooser.
2. Select a color in the Color chooser and click OK to apply it to the trace.



To revert to the default trace color

▶ Right-click the trace color in the table and choose Restore Default Color on the menu that appears.

Zooming In and Out

To zoom in on a section of the chromatogram

▶ While clicking in the chromatogram, drag the pointer to mark the zoom region of interest.

To zoom out from a section of the chromatogram

▶ Do one of the following:

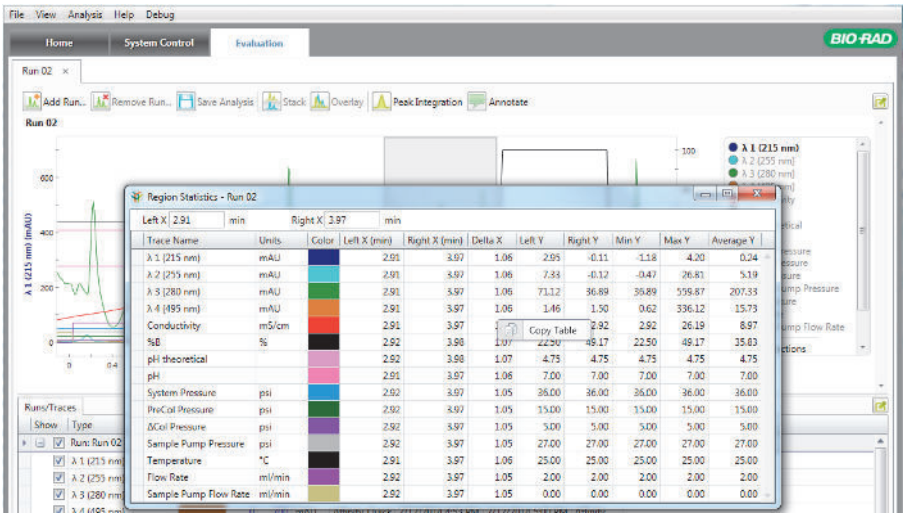
- To zoom out to the previous zoom level, double-click the chromatogram or right-click the chromatogram and choose Undo Zoom on the menu that appears.
- To zoom out to the full scale view, right-click a chromatogram and choose Reset Zoom on the menu that appears.

Obtaining Statistics of a Selected Region on the Chromatogram

For each trace, you can view specific statistics for a selected region on the chromatogram. Selecting a region on the chromatogram creates the Region Statistics table, which displays the following data:

- **Trace name** — the type of trace. Only traces selected in the Runs/Traces table appear in the Region Statistics table.
- **Units** — the trace's units.
- **Color**
- **Left X (min)** — the value of the left x-axis point of the selected region, specified in the selected x-axis units (min/ml/CV).
- **Right X (min)** — the value of the right x-axis point of the selected region, specified in the selected x-axis units (min/ml/CV).
- **Delta X** — the range of the x-axis (right x-axis – left x-axis) of the selected region, specified in the selected x-axis units (min/ml/CV).
- **Left Y** — the value of the trace on the left y-axis point of the selected region.
- **Right Y** — the value of the trace on the right y-axis point of the selected region.
- **Min Y** — the minimum y-axis value of the specific trace within the region.
- **Max Y** — the maximum y-axis value of the specific trace within the region.
- **Average Y** — the average y-axis of the specific trace within the region.

You can modify the selected region on the chromatogram or in the Region Statistics table. Resizing the selected region updates the values in the Region Statistics table. You can also copy the statistics in the table and paste those data into a spreadsheet. Closing the Region Statistics table clears the selected region on the chromatogram.



To select a region on the chromatogram

▶ Right-click the start point in the chromatogram and drag the pointer to the end point.

A grey box appears over the selected area and the Region Statistics table opens.

To resize the selected region

▶ Do one of the following:

- On the chromatogram, drag an edge of the grey box to the new point.
- In the Region Statistics dialog box, type new values in the Left X or Right X fields located above the table.

To copy the contents of the table

▶ Right-click in the Region Statistics table and select Copy Table.

You can paste the contents into a .doc or .txt file or a spreadsheet.

To clear the selected region on the chromatogram

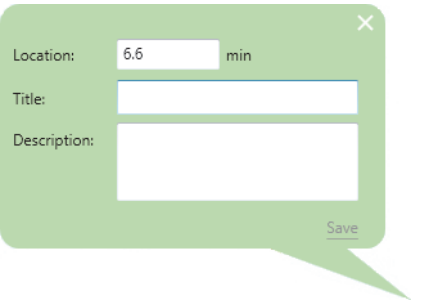
▶ Close the Region Statistics table.

Annotating the Chromatogram

In the Evaluation window, you can add notes to the chromatogram to associate observations with data points. The annotation dialog box contains three fields: Location (in time, volume, or CV), Title, and Description. When an annotation is saved, the title appears at the specified location on the x-axis. The description appears in the run report.

To add annotations

1. Click Annotate on the toolbar and drag the icon to the target location on the chromatogram. The green annotation dialog box opens with the Location field filled in.



   Tip: You can edit the Location field.

2. Type a title for the annotation.
3. (Optional) Type a description for the annotation.
4. Click Save to save the annotation.

   Note: Clicking ☒ closes the dialog box without saving the annotation.

To edit an annotation

1. Double-click the annotation on the chromatogram to open its dialog box.
2. Edit the annotation and click Save to save the changes.

To delete an annotation

▶ Double-click the annotation to open its dialog box and click Delete.

Copying the Chromatogram

A copied chromatogram image can be pasted into a document or presentation file or saved in a variety of image formats optimized for either screen or print display.

To copy a chromatogram

▶ Right-click a chromatogram and select Copy Chromatogram on the menu that appears.

The chromatogram is copied to the clipboard, from which you can paste it into another application.

To save a chromatogram to an image file

1. Right-click a chromatogram and select Save Chromatogram As.
2. In the menu that appears, choose a file format and click Save.

Tip: For display on a computer, save your chromatogram in .png, .jpeg, or .gif format. For best print results, save your chromatogram in .tiff or .bmp format, which produce higher resolution files.

Customizing the Trace Table Display

The details of each trace in a run appear in a row in the trace table, one row for each trace. By default, trace information is grouped by run. Use the table to select the visible traces, to change their colors, and to change the axes' scale.

You can customize the table display further by changing the table grouping, sorting the columns, and changing the column order. You can also copy the table.

Changing Table Grouping

Traces can be grouped by run or by type. Traces can also be ungrouped. Groups can be expanded to display their contents or collapsed so only the group name appears in the table.

To change how traces are grouped

- Right-click in the table and select Group By Run or Group By Type on the menu that appears.

To ungroup traces

- Right-click in the table and select Group By Ungroup on the menu that appears.

To expand or collapse groups

- Do one of the following:
  - Click the plus or minus sign next to the group name.
  - Right-click in the table and select Expand All Groups or Collapse All Groups on the menu that appears.

Sorting Table Columns

When the trace table is not grouped, traces are sorted in default order.

To sort table columns

▶ Do one of the following:

- Select a column, right-click the column heading, and then select a Sort option on the menu that appears.
- Click a column heading to toggle between ascending and descending table data order.

  Tip: An up or down arrow near the column title indicates that the table was sorted using this column.

Ordering and Selecting Columns

Changes you make to the selection and order of columns in the Runs/Traces table apply to all your subsequent runs. These settings are specific to the user.

To change column display order

▶ Drag columns to new locations in the trace table.

Showing or Hiding Columns

Note: You can hide trace table columns without losing the data the columns contain.

To hide trace table columns

▶ Click the heading of the column to hide and drag it out of the table.

To display a hidden column

1. Right-click a column heading and select Show Column Chooser on the menu that appears.
2. Drag the heading of the column you want to display from the Column Chooser to the location in the table where you want it to appear.

   The column heading and data reappear in the table.

Copying a Table

You can copy a table to the clipboard and then paste it into a spreadsheet or another type of application. The copied table appears in the application with the following adjustments:

- The color value is copied in hexadecimal format.
- The show/hide state is copied as a Boolean value.

To copy the table to the clipboard

▶ Right-click the trace table and select Copy Table on the menu that appears.

The table is copied to the clipboard. You can now paste it into another application.

Showing or Hiding Pre-Injection Data

In the Evaluation window, the injection point is defined as the x = 0 of the run. For method runs, the injection point is taken from the method and set to the time/volume in which the sample was injected, usually in the Sample Application phase. For manual runs, the injection point is set to time/volume = 0. By default, data collected before the injection point are not shown.

To show pre-injection data

▶ Select View > Show Pre-injection.

To hide pre-injection data

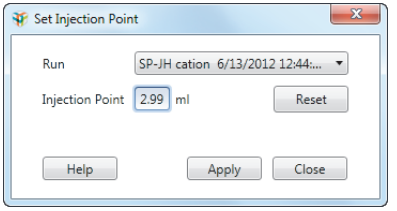
▶ On the View menu, clear the Show Pre-injection option.

Manually Setting the Injection Point (x Alignment)

You can manually set the injection point for any run, including a run displayed in a trace comparison. The injection point is saved with the run (the x = 0 point). A change to the injection point in one view affects other views that include the run.

To set the injection point for a run manually

1. Select Analysis > Set Injection Point.
2. In the Set Injection Point dialog box, type the new injection point.



3. If several runs are open in the same window, select a run on the Run menu.
4. Click Apply to align the data according to the setting you typed or click Reset to restore the original setting.

Managing Runs

You can rename or delete a single run displayed in the Evaluation window. You can also choose how to display multiple runs.

Note: You cannot delete a run that is part of another analysis.

When you make the following changes in single run view, they are saved automatically: trace colors, y-scale range, x-axis units, and show/hide state. In addition, changes to the selection and order of table columns are globally saved and are applied to any run displayed in single run view after the changes are made.

Renaming a Single Run

You can rename a single run displayed in the Evaluation window. A run name can consist of up to 100 characters.

To rename a single run

1. With the run displayed, select File > Rename Run.

   The Rename Run dialog box appears, with the current run name in the Name box.

2. Replace the current name with a new name.
3. Click Save.

Deleting a Single Run

You can delete a single run displayed in the Evaluation window. When you delete a single run, its tab closes automatically.

To delete a single run

1. With the run displayed, select File > Delete Run.

   A dialog box appears, asking you to verify that you want to delete the run.

2. Click Yes to delete the run.

   If the run is part of an analysis, ChromLab cancels the deletion and notifies you.

Viewing Multiple Runs in Separate Tabs

ChromLab software can display multiple runs in the Evaluation window. Each run opens in a separate tab so you can view each one individually. When you select a run that is already open, the system highlights the tab for that run.

To open multiple runs in separate tabs

1. Do one of the following:
   - Click Open/Run Analysis in the Home window Evaluation pane.
   - If the Evaluation window is already open, select File > Open Run/Analysis.
2. Hold down Ctrl or Shift and select multiple runs from the list in the right pane.

   The Open Runs button becomes a dropdown list.
3. On the dropdown list, select Open in Multiple Tabs to view each run in a separate tab.

To close a tab

▶ Select File > Close Run/Analysis or click the small x to the right of the tab name.

To close all tabs

▶ Select File > Close All Runs/Analyses.

Comparing Traces

ChromLab can display multiple runs selected in the Open Run/Analysis dialog box in a single tab view. This view enables you to compare the traces from different runs. Runs displayed in the tab can appear either stacked for individual display or overlaid for comparison.

To open multiple runs in one tab

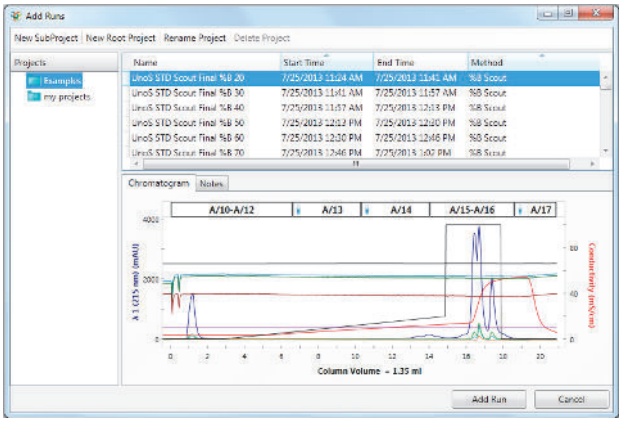
1. In the Home window, click Open Run/Analysis in the Evaluation pane.
2. In the Open Run/Analysis window, hold down Ctrl or Shift, select the runs to add, and click Open Run.

You can add more runs to an open run or analysis by clicking Add Run on the Evaluation window toolbar.

To add more runs to an open analysis

1. Click Add Run on the Evaluation window toolbar.

   The Add Run dialog box opens. A list of runs appears in the right pane.



2. Select one or more runs and click Add Run.

   The runs are added to the analysis and appear stacked in the Evaluation window.

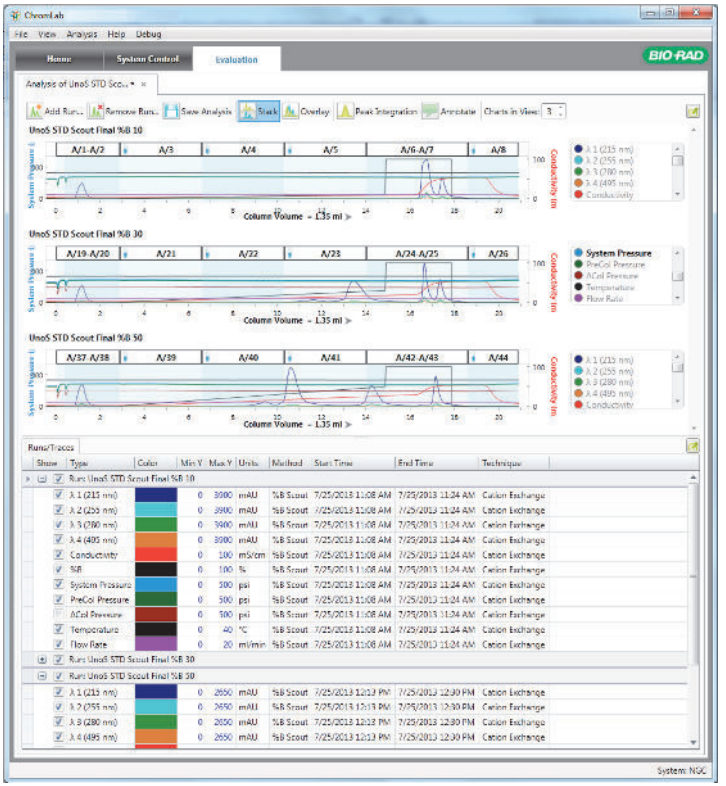
Viewing Multiple Runs in One Tab

In this view, a single table displays information for all traces in all the displayed runs. You can select a trace, zoom in or out, and change trace colors. You can also show or hide specific traces and spread the different runs across the y-scale, as described in Offsetting Traces — Overlay Mode on page 228.

Stacked View

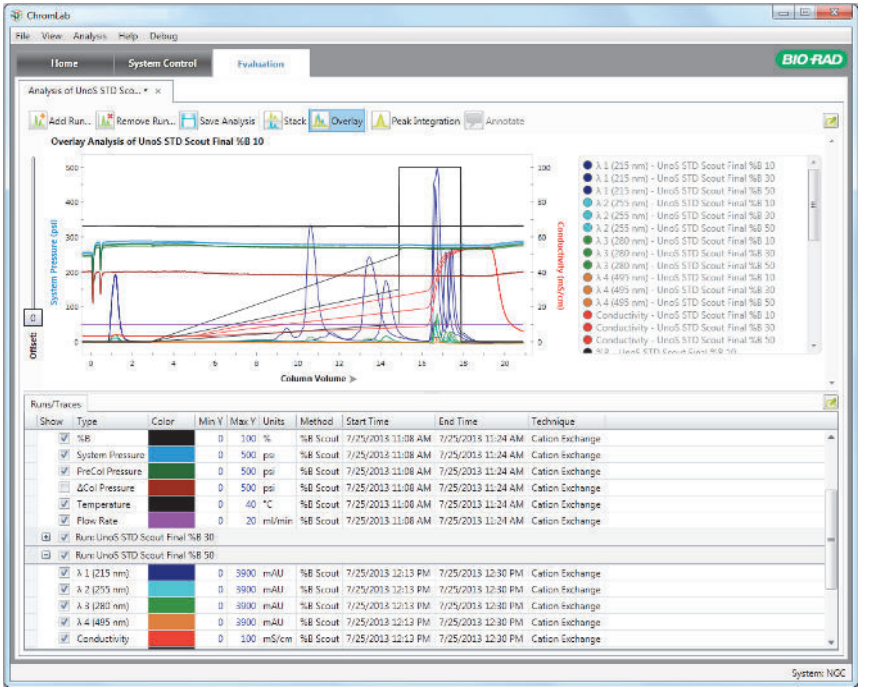
Each run appears in a separate chromatogram. On the toolbar, the Charts in View box indicates the number of runs in the view. You can scroll in this box to view chromatograms for additional runs. A single table displays information for all traces in all the displayed runs.

When runs are stacked, the x-scale and left y-scale units are identical in all chromatograms. Changing the scale units in one chromatogram changes the scale units in all the chromatograms in the stack. Similarly, when you zoom in on one chromatogram in a stack, the zoom applies to all the other chromatograms in the stack.


ChromLab
File View Analysis Help Debug
Home
System Control
Evaluation
BIO-RAD
Add Runs
Remove Runs
Save Analysis
Stack
Overlay
Peak Integration
Annotate
Charts in View:
Uno6 STD Scout Final %B 10
A/1-A/2
A/3
A/4
A/5
A/6-A/7
A/8
Column Volume - 1.35 ml
Uno6 STD Scout Final %B 30
A/19-A/20
A/21
A/22
A/23
A/24-A/25
A/26
Uno6 STD Scout Final %B 50
A/37-A/38
A/39
A/40
A/41
A/42-A/43
A/44
System Pressure
PreCol Pressure
ACol Pressure
Temperature
Flow Rate
Conductivity
Runs/Traces
Show
Type
Color
Min Y
Max Y
Units
Method
Start Time
End Time
Technique
Run: Uno6 STD Scout Final %B 10
λ 1 (215 nm)
λ 2 (255 nm)
λ 3 (280 nm)
λ 4 (495 nm)
Conductivity
%B
System Pressure
PreCol Pressure
ACol Pressure
Temperature
Flow Rate
Run: Uno6 STD Scout Final %B 30
Run: Uno6 STD Scout Final %B 50
Cation Exchange
System: NGC

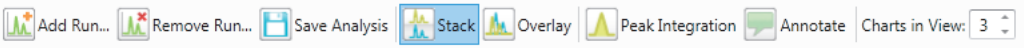
Overlay View

All visible traces from all selected runs appear in a single chromatogram.



To switch between stacked and overlay views

▶ Click Stack or Overlay on the Run tab toolbar or on the View menu.



Customizing the Trace Comparison Table

Multiple runs in a single tab can be grouped by run or by type. Multiple runs can also be ungrouped. In the Evaluation window, the default view shows multiple runs with traces grouped by run. Runs are sorted alphabetically. When the trace table is grouped by type, the trace types are sorted in the default order, as described in Displaying Traces on page 208.

Expanding or Collapsing Groups

When groups are expanded, all their data are visible. When groups are collapsed, the group rows are visible but not the content inside each group.

To collapse or expand groups of data

- Click +/- near the group name.

Hiding All Traces in a Group

To hide all traces in a group

- Clear the Show checkbox in the group row.

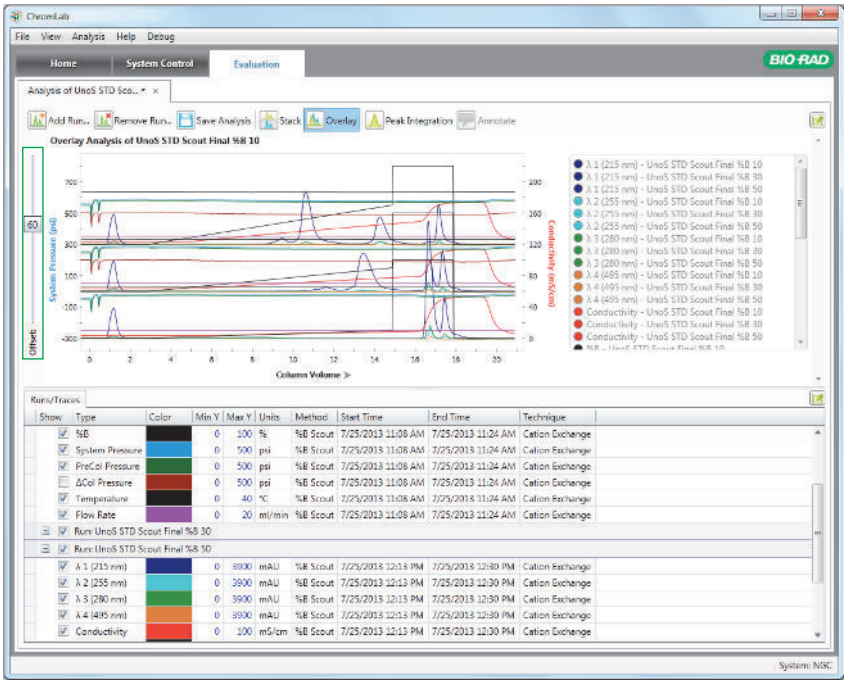
Showing All Traces in a Group

To show all the traces in a group

- Select the Show checkbox in the group row.

Offsetting Traces — Overlay Mode

When two or more traces from different runs are too close together to distinguish, Offset can be used to shift traces so you can view them separately. Traces from the first run remain in their original locations. Traces from other runs are shifted. If more than two runs are involved, each additional run has its own offset. The last run in the list is shifted the most.



Note: Offsetting the y-scale of a trace does not change trace values.

To offset a trace

▶ With two or more runs displayed in the Evaluation window, use the Offset slider at the left side of the chromatogram to select an offset number from 0–100, with 0 meaning no offset and 100 meaning maximum offset.

Offset changes appear in the chromatogram as you move the slider.

Managing Analyses

Removing Runs from a Trace Comparison

When you view multiple runs, you can remove one or more runs from the analysis. You cannot remove all runs from the view.

To remove a run from a multiple run analysis

1. Do one of the following:
   - On the Run tab toolbar, click Remove Run.
   - Select File > Remove Run from Analysis.

   The Remove Run from Analysis dialog box opens with the multiple runs listed and the current run selected.
2. (Optional) Select additional runs to remove from the analysis.
3. Click Remove to remove the run or runs from the analysis.

Saving a Trace Comparison Analysis

The Run tab displays an asterisk to indicate that the displayed trace comparison has been modified and has not yet been saved. Saving an analysis saves the following attributes:

- List of runs
- Table data grouping
- x-axis units (time, vol, CV)
- View selected when saved (overlay or stacked)

When you save a new analysis, the Save Analysis dialog box opens with the default analysis name filled in. Text you add in the Notes box is saved with the analysis. This text can be of any length, and it can be copied and pasted.

Tip: When a saved analysis is open, you can view its notes text by choosing Analysis Notes on the File menu.

To save an analysis

1. Do one of the following:
   - Click Save Analysis on the Run tab toolbar.
   - Select File > Save Analysis.
2. Type a name for the analysis in the Save New Analysis dialog box.
3. Click Save.

Once an analysis has been saved, the following actions are automatically saved when you apply them:

- Showing or hiding a trace or group of traces
- Changes to the minimum or maximum y-axis values
- Changes to a trace color

Copying an Analysis

To create a copy of an analysis

1. With the analysis displayed and saved, select File > Copy Analysis.
2. Type a name for the new analysis in the Copy Analysis dialog box.
3. Click Save.

Renaming an Analysis

To rename an analysis

1. With the analysis displayed, select File > Rename Analysis.

   The Rename Analysis dialog box appears, with the current run name in the Name field.
2. Replace the current name with a new name up to 50 characters long.
3. Click Save.

Deleting an Analysis

To delete an analysis

1. With the analysis displayed, select File > Delete Analysis.

   A dialog box appears, asking you to verify that you want to delete the analysis.

2. Click Yes to delete the analysis.

Reports

ChromLab supports three kinds of reports, including run and analysis reports. For more information, see Chapter 9, Reports.

Exporting Run Data

You can export run data as an NGC file, which can be loaded onto a different NGC system or another computer running ChromLab, or as a .csv file, suitable for importing into other applications. You can also export run data with the method used to generate it or export only the run or method itself.

For more information, see Chapter 8, Importing and Exporting Data.

Peak Integration

ChromLab software uses an algorithm to find peaks in UV traces and calculate the necessary data for analysis, such as retention volume and peak area. The results appear in the Peaks table at the bottom of the Evaluation window and in the chromatogram.

You can perform peak integration on a single run or on multiple runs in a single view.

When you click Peak Integration on the Run tab toolbar, peak integration is performed automatically using default settings, which appear in the Peak Integration pane to the right of the chromatogram. You can change the parameter settings or the traces selected for integration and run peak integration again with the new settings.

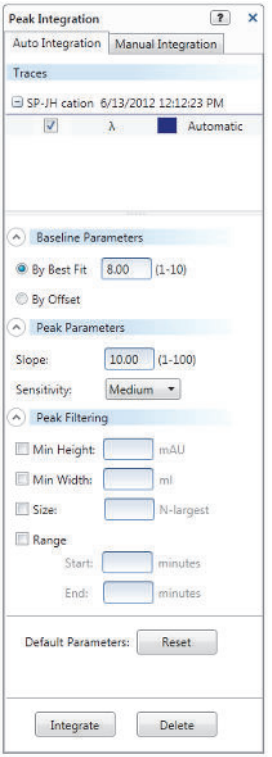
You can also adjust peaks manually. Doing so adjusts only the selected trace and displays only this trace's peaks in the chromatogram. See Adjusting Peaks Manually on page 243 for details.

Tip: SIM traces for external detectors connected to the NGC instruments are also used for peak detection and integration. In this case, the y-axis units defined in System Settings for the external detectors are used for peak calculations. See Device Input Tab on page 78 for more information.

Automatic Peak Integration Settings

In the Peak Integration pane, the Auto Integration tab displays settings that affect baseline calculation and peak detection. Changing these settings enables you to optimize the way peaks are detected and recognized, specific to your data.

Note: By default most tab sections are collapsed. Expanding the sections displays the options shown in the following illustration.



Traces

A list of shown UV traces grouped by run. By default all shown UV traces are selected for peak integration.

You can exclude traces from integration by clearing their checkboxes.

Baseline Parameters

You can select from two baseline calculation methods.

By Best Fit — the baseline is calculated from the data. The baseline curve connects the low points of the data. This setting describes the radius of a disk rolling along the curve from underneath.

By default, the best fit radius parameter is set to 8. This value can to be set from 1 to 10. The smaller the radius, the more low points on the curve it touches.

By Offset — the baseline is a straight horizontal line that has a constant offset (y) value. The default value is zero, but you can specify a different offset value.

Peak Parameters

You can specify initial values for two parameters that participate in the peak detection process: slope and filter sensitivity.

Slope — slope defines the threshold for peak slope. The bigger the slope, the steeper the peaks that are filtered out. Slope values range from 1–100.

Sensitivity — sensitivity determines the strength of the filter used for smoothing the curve before finding peaks. The Low sensitivity setting filters out higher frequency components in the signal, eliminating small, narrow peaks. The higher the sensitivity setting, the more higher frequency components get through the filter. This allows smaller and narrower peaks to be detected.

Peak Filtering

You can specify the following thresholds for valid peaks.

Min Height — this setting specifies the minimum height for a valid peak in mAU units.

Min Width — this setting specifies the minimum baseline width for a valid peak in ml units.

N-Largest — this setting specifies area-wise filtering. It picks N largest peaks in descending order.

Range — these settings define the range within which peaks will be detected. The range units are determined from the chromatogram x-axis units.

Default Parameters

Reset — restores the baseline parameters, peak detection parameters, and peak filtering setting to their defaults.

Note: You must click Integrate to recalculate peaks with the default settings.

Integrate and Delete

Integrate — detects peaks, using current parameter values, for the selected traces and updates the Peaks table with detected values.

Delete — deletes previously calculated peaks. All peaks are removed only from traces selected in the traces list.

Starting Peak Integration

When you select Peak Integration, a single run becomes an analysis, and the system prompts you for an analysis name when you first save it.

To start peak integration for the first time

▶ Click Peak Integration on the Run tab toolbar.

The Peak Integration pane opens, and peak integration is performed automatically using default settings.

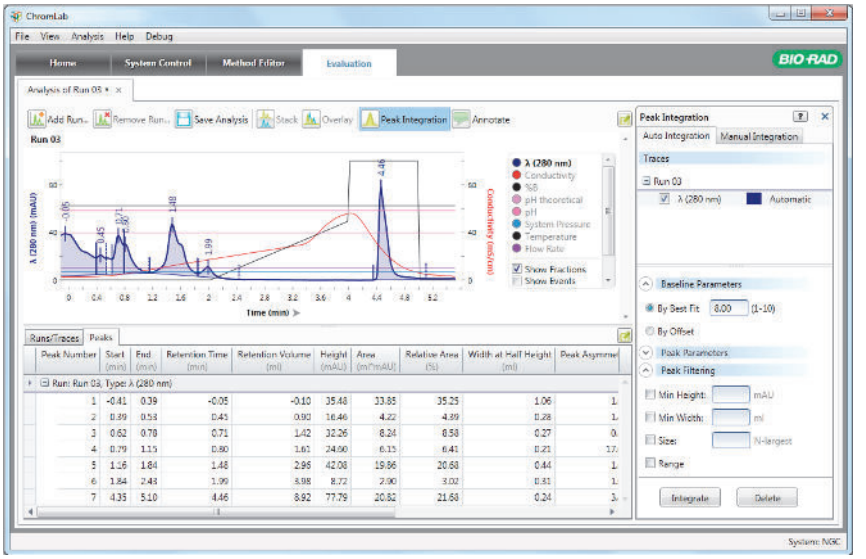
To perform peak integration with changed settings

▶ Select the traces of interest, change the settings in the Peak Integration pane, and then click Integrate at the bottom of the pane.

Updated peak integration results appear in the chromatogram and in the Peaks table.

Tip: You can apply different settings for individual traces by integrating each trace separately with its own settings. Clear the checkbox for the other traces in the list and click Integrate.

Displaying Peak Results — the Chromatogram



In the chromatogram, a peak's start, end, and apex are indicated by small vertical lines at the relevant x points. The color of each line matches the corresponding trace.

The retention time, volume, or column volume appears near the line of the peak apex.

Tip: The example screen displays the retention time, but you can display the column volume instead by clicking the Time title near the bottom of the chromatogram.

Start Peak appears as a continuous vertical line located at the relevant x point.

End Peak appears as a dashed vertical line located at the relevant x point.

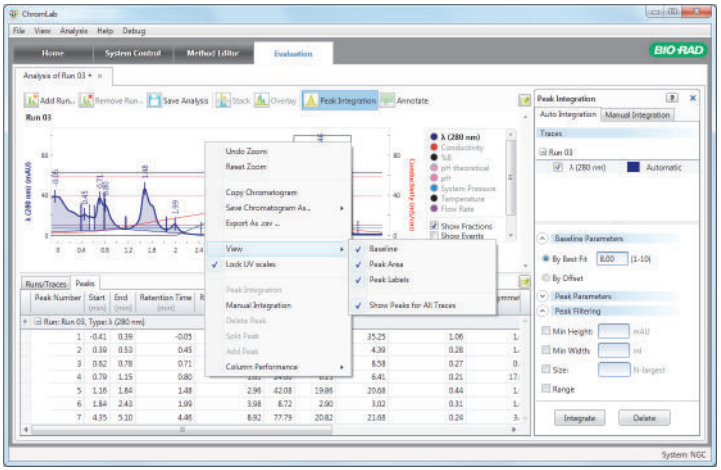
Pausing the pointer on a peak start, end, area, or apex displays a tooltip that shows details of the item.

The calculated baseline appears on the chromatogram when peaks are detected. The baseline is drawn according to the selected baseline calculation in the same color as the associated trace. Pausing the pointer on a baseline displays a tooltip that provides details about the baseline and the trace.

Changing Peak View Options

You can choose to hide or show the baseline, peak area, or peak labels, or all of these options.

Both the chromatogram and Peaks table highlight information about the item you select in either one. Items you select in the chromatogram are also highlighted in the Peaks table and vice versa. Similarly, changes you make in one display are reflected in the other.



When you click a peak item (apex, start, end, or peak area) in the chromatogram or a peak row in the Peaks table:

- The peak row in the table is selected.
- The peak area is highlighted.
- Peak start, end, and apex appear in boldface.
- The relevant trace is selected.

To hide or show peak view options

▶ Right-click in the chromatogram, choose View, and select the option or options you want in the dropdown menu. The same options also appear on the View menu.

Displaying Peak Results — the Peaks Table

Each peak is described in a Peaks table row. The following fields are displayed for each peak:

- **Peak number** — sequential.
- **Run name**
- **Trace type** — UV traces display wavelength value in nanometers, for example (280 nm). SIM traces display the peak's height and area in the relevant units.
- **Start** — the beginning of the peak, displayed in the units of the currently displayed x-axis.
- **End** — the end of the peak, displayed in the units of the currently displayed x-axis.
- **Retention volume** — the elution volume at maximum peak height.
- **Retention time** — the time at maximum peak height.
- **Height (mAU)** — the peak height at the retention volume/time, measured from the UV trace to the calculated baseline.
- **Area (ml*mAU)** — the area under the curve between the UV trace and the baseline.
- **Relative area (%)** — the relative peak area of all peak areas in a trace.
- **Width at half height (ml)** — the width (in ml) of the peak at 50% of the maximum peak height.

- **Peak asymmetry** — the asymmetry factor, defined as the distance from the center line of the peak to the back slope divided by the distance from the center line of the peak to the front slope. All measurements are made at 10% of the maximum peak height.

  Note: The asymmetry factor of a peak is usually similar to the tailing factor of the same peak, but the two values cannot be directly converted.

- **Fractions** — all fractions that reside between start and end. Multiple fractions are displayed first to last.

- **Peak type** — the integration type for a peak.

  - **Automatic** — the peak was determined automatically and has not been manually adjusted.

  - **Manual** — the peak has been manually adjusted or added.

- **Path length (cm)** — the path length of the UV detector's flow cell. The default length is 0.5 cm. The NGC system has three optional flow cells, which are used to calculate the protein concentration: 0.2, 0.5, and 1.0 cm. This field can be modified to display the value of the flow cell in use. When the value in one row is changed the cell flow path length for all peaks in the same run is updated accordingly.

- **Extinction coefficient ($(mg/ml)^{-1}$ cm^{-1})** — the extinction coefficient of the protein, used to calculate the protein concentration. By default this field is empty. You can enter a value up to three significant digits. Coefficient values entered in the Peaks table automatically populate the extinction coefficient values for relevant fractions in the Fractions table. Coefficient values entered in the Fractions table are *not* automatically populated to the Peaks table.

- **Concentration (mg/ml)** — the calculated concentration of the protein for the specified peak. This value is based on the following calculation:

  (peak area/peak volume) / (extinction coefficient x path length x 1,000)

- **Molecular mass (kDa)** — the molecular mass of the protein. By default this field is empty. Molecular mass values that you enter in the Peaks table are automatically populated to the Fractions table for relevant fractions. Molecular mass values that you enter in the Fractions table are *not* automatically populated to the Peaks table.
- **Molarity (µM)** — the calculated molar concentration of the protein for the specified peak. This value is computed from the calculated concentration and molecular mass.
- **280/260** (or 280/255) — the ratio of absorbance at 280 nm and 260 nm (or 255 nm) used to determine purity of protein for each peak in the 280 trace. The ratio appears in the 280 nm trace section of the table. The ratio is calculated using baseline-subtracted values of the UV trace at the indicated retention time or volume of the 280 nm peak.

  Note: This column appears in the Peaks table after peak integration is performed when both the 280 nm and 260 nm (or 255 nm) traces are detected. Depending on which trace is present, the column name can be either 280/260 or 280/255.

Table Display Order and Column Selection

You can change the order of the table columns. Column selection and order settings are specific to the user and apply to subsequent peak integration results tables.

To change column display order

▶ Drag columns to new locations in the Peaks table.

Showing or Hiding Columns

As in the Runs/Traces table, you can show or hide columns in the Peaks table by choosing Show Column Chooser in the context menu.

Note: You can show or hide columns in the table without affecting the data the columns contain.

To hide columns in the displayed Peaks table

▶ Click the column heading to hide and drag it out of the table.

To display a hidden column

1. Right-click a column heading and select Show Column Chooser on the menu that appears.
2. Drag the column heading you want to show from the Column Chooser to the location in the table where the column should appear.

   The column heading and data reappear in the table.

Copying the Peaks Table

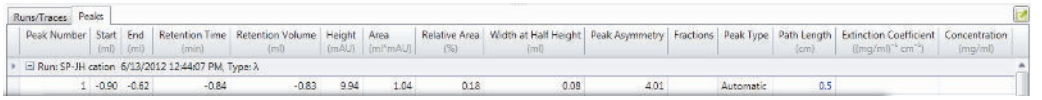
To copy the table

▶ Right-click the table and select Copy Table on the menu that appears.

The copied table can be pasted into a spreadsheet or another kind of document.

Grouping or Ungrouping the Peaks Table

When groups are expanded, all their data are visible. When groups are collapsed, the group rows are visible but not the content inside each group. By default, peaks are grouped by run and type. Each combination of run and trace type is shown as a group. Relevant peaks reside in the group. In the Peaks table, the grouping settings for each run appear in the run title row directly below the column headings.



To group peaks

▶ Right-click the table and select Group By Run or Group By Type on the menu that appears.

Note: In Peaks tables of multiple runs in a single view, you can also select Group By Run and Type.

To ungroup peaks

▶ In the table, right-click a group of peaks and select Ungroup on the menu that appears.

To expand or collapse groups of peaks

▶ Right-click the table and select Expand All Groups or Collapse All Groups on the menu that appears.

Clearing Extinction Coefficient Values in the Peaks Table

Note: Clearing a coefficient in the Peaks table also clears the values in the Extinction Coefficient and Concentration columns in the Fractions table for the fractions that contain that peak.

To clear the extinction coefficient for a specific peak

▶ Select the value and click Delete.

The cell in the extinction coefficient column clears as well as the value in the associated Concentration column.

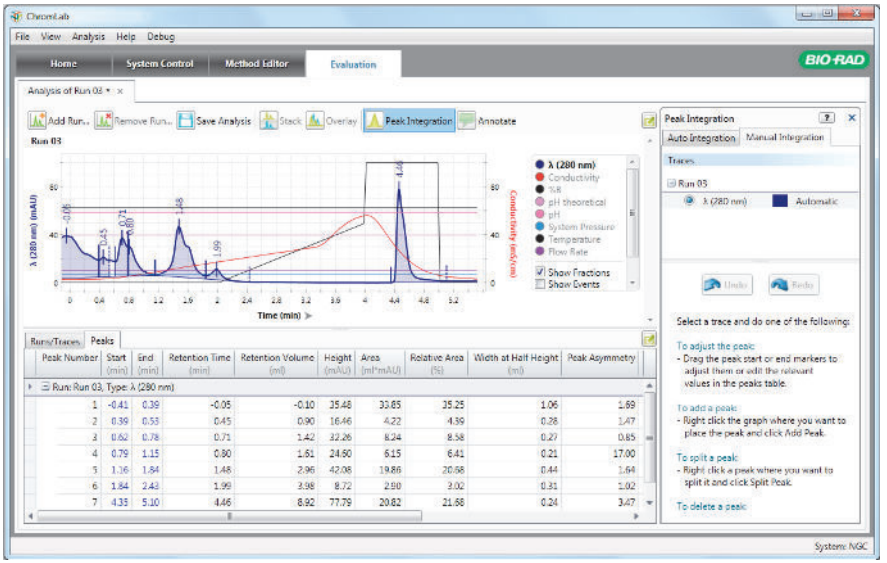
To clear all values in the extinction coefficient column

▶ Right-click the table and select Clear Extinction Coefficient on the menu that appears.

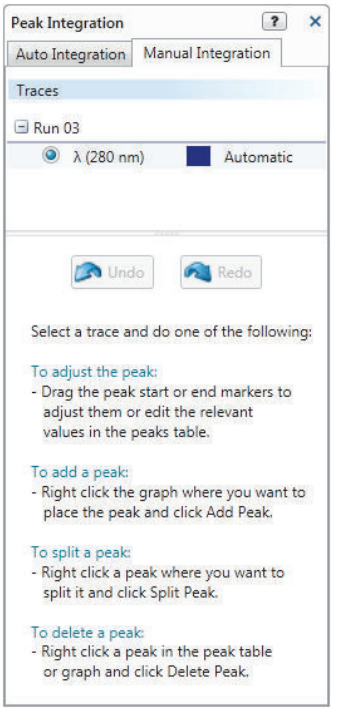
All values entered in this column clear as well as all values in the Concentration column.

Adjusting Peaks Manually

You can manually adjust the peaks found in automatic peak integration. You can also add a peak to a chromatogram location in which no peak was detected. When you select the Manual Integration tab in the Peak Integration pane, grid lines appear in the chromatogram.



The Manual Integration tab displays a list of runs in the current analysis and their integrated UV traces. You can select and adjust one peak at a time. Only the selected trace can be adjusted and only its peaks appear in the chromatogram. This tab also displays instructions for manually adjusting peaks. Undo and Redo buttons make it easy to experiment with different settings for a given trace.



You can adjust peaks by selecting commands on the menu that appears when you right-click in the chromatogram or Peaks table.

To add a peak

▶ Right-click a chromatogram location that does not contain a detected peak and then click Add Peak on the menu that appears.

A new peak is added to the trace with a default width that you can later adjust. The system recalculates and updates Peaks table statistics.

To change the start and end points of a peak in a chromatogram

1. Pause the pointer over a peak start or end line.

The cursor changes to a pair of arrows, indicating that you can move the line.

2. Click the line to select it, and drag the line to another position.

As you move the line, your changes are also applied to the Peaks table.

To change peak start or end in the Peaks table

1. In the chromatogram or in the Peaks table, select the peak to edit.
2. In the Peaks table, change the Start or End value or both in the highlighted row.

To split a peak

- In the chromatogram or in the Peaks table, right-click the peak to split, and then click Split Peak on the menu that appears.

  The peak splits into two peaks. The split X point appears where the pointer was located when you selected Split Peak. The Peaks table is updated to contain two peaks instead of one. Table statistics are also recalculated and updated.

To delete a peak

- Right-click a peak in the chromatogram or in the Peaks table, and then click Delete Peak on the menu that appears.

  The peak is deleted from the chromatogram and the Peaks table and the table statistics are recalculated and updated to exclude the deleted peak.

To undo or redo a manual peak adjustment action

- Select a trace in the chromatogram, and click Undo or Redo in the Manual Integration tab to cancel or reapply the most recent action performed on the trace.

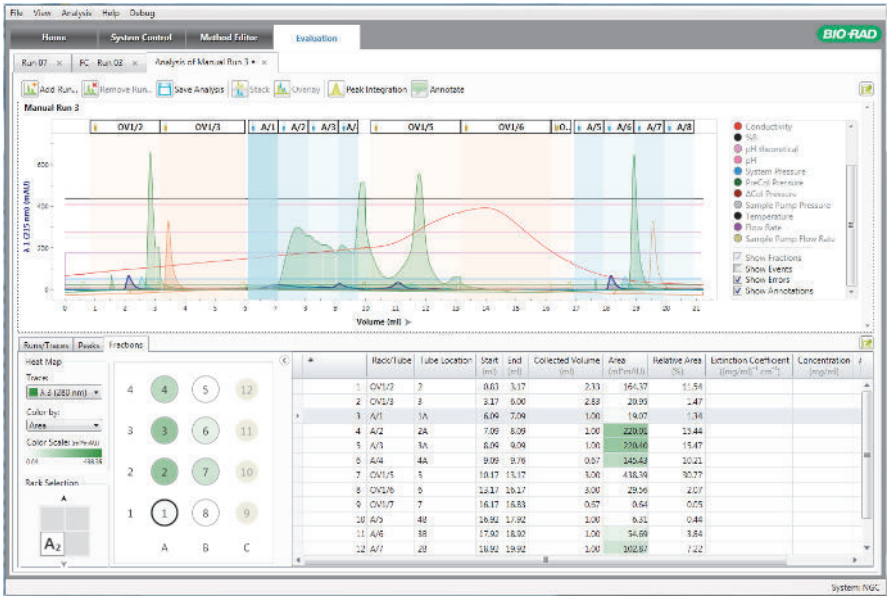
Saving Peak Integration Data

When you perform peak integration on a single run, the results are saved in an analysis. Peak integration settings and results are saved when you save the analysis and are loaded when you subsequently open the analysis.

Evaluating Fractions

When fraction collection is enabled the details of each fraction in the run appear in a row in the fraction table below the chromatogram, one row for each collected fraction. The location of the fraction appears in the rack image to the left of the fraction table. Use the fraction table, rack display, and the chromatogram to find fractions of interest. If multiple racks were used, use the rack selection area to choose the racks of interest.

Displaying Fraction Collection Results



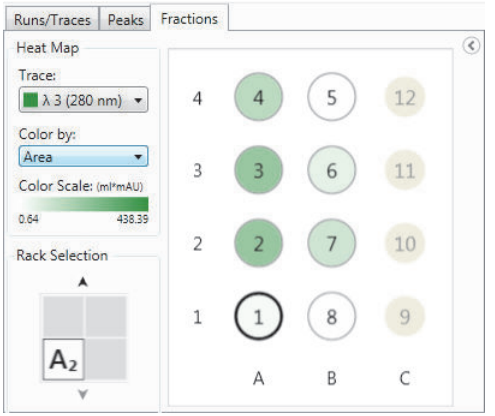
The chromatogram displays the fractions collected for each run as blocks of alternating shades of color:

- **Blue** — for fractions collected via the BioFrac™ fraction collector. The rack and tube number appear at the top of each block.

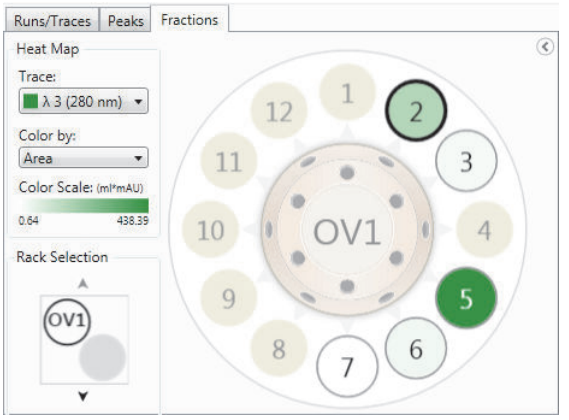
- **Orange** — for fractions collected via an outlet valve. The outlet valve and port number appear at the top of each block.

A heat map, indicating the relative amounts or purity within each fraction tube, appears after peak integration is performed. Items you select in the chromatogram are highlighted in the rack or outlet valve display and fractions table and vice versa.

Rack Display



Outlet Valve Display



Displaying Fraction Collection Results — the Fractions Table

Like the Traces table, the Fractions table is available when the run is opened in the Evaluation window. The Fractions table is available only when a single run is open in the Evaluation window. It is not displayed for multiple run analyses or for trace comparisons.

The following fields are displayed for each fraction collected:

- **#** — sequential row number.
- **Rack/Tube** — the rack and tube number of the fraction.
- **Tube location** — the location of the tube in the plate or rack based on its numbering scheme.
- **Start** — the beginning of the collection, displayed in the units of the currently displayed x-axis.
- **End** — the end of the collection, displayed in the units of the currently displayed x-axis.
- **Collected volume (ml)** — the total fraction volume collected in that tube.
- **Area (ml*mAU)** — the area of the fraction between the UV trace selected in the heat map and its baseline. This column appears after peak integration is performed.
- **Amount (mg) —** the amount of protein in the fraction based on the trace selected in the heat map. This column appears after peak integration is performed and the extinction coefficient value is entered into the table.
- **Concentration (mg/ml)** — the calculated concentration of the protein for the specified fraction based on the trace selected in the heat map. This column appears after peak integration is performed and the extinction coefficient value is entered into the table.
- **Relative area (%)** — the relative fraction area of all fraction areas in a trace. This column appears after peak integration is performed.

- **Extinction coefficient ((mg/ml)$^{-1}$ cm^{-1})** — the extinction coefficient of the protein, used to calculate the protein concentration. ChromLab automatically populates this field when the extinction coefficient is entered or changed in the Peaks table.

  Note: Changing a single coefficient in the Peaks table affects only coefficients for the fractions that contain that peak.

- **Molecular mass (kDa)** — the molecular mass of the protein. By default this field is empty. Molecular mass values that you enter in the Peaks table are automatically populated to the Fractions table for relevant fractions. Molecular mass values that you enter in the Fractions table are *not* automatically populated to the Peaks table.

- **Molarity (µM)** — the calculated molar concentration of the protein for the specified peak. This value is computed from the calculated concentration and molecular mass.

- **280/260** (or 280/255) — the ratio of average absorbance at 280 nm and 260 nm (or 255 nm) used to determine the purity of protein for each fraction. The ratio appears in the 280 nm trace section of the table. The ratio is calculated using baseline subtracted values of the UV traces area within the fraction.

  Note: The column appears after Peak Integration is performed when both the 280 nm and 260 nm (or 255 nm) traces are detected. Depending on which trace is present, the column name can be either 280/260 or 280/255.

Calculating Protein Concentration for Fractions

You can calculate and view the protein concentration for individual and pooled fractions after you perform peak integration. Peak integration is performed using default settings. If necessary, you can adjust the baseline by changing the Best Fit or Offset parameters and reintegrating. The baseline is used to calculate the area under the curve of the UV trace in each fraction. See Baseline Parameters on page 233 for more information.

For pooled fractions, the protein concentration is calculated by a weighted average of the fractions included in the pool.

To calculate protein concentration for individual or pooled fractions

▶ Click Peak Integration on the Run tab toolbar.

After you perform peak integration, the following columns appear in the Fractions table:

- Area
- Relative Area
- Extinction Coefficient
- Concentration
- Amount

The extinction coefficient and concentration fields are automatically populated when the extinction coefficient is entered in the Peaks table. If a fraction spans multiple peaks that have different extinction coefficient values, these values are not imported and the extinction coefficient field in the Fractions table displays the word Multiple. In this case, you can manually enter the extinction coefficient field in the Fractions table.

You can manually change the extinction coefficient of a fraction in the Fractions table. Doing so will not change the coefficient of the peak in the Peaks table.

Viewing Fraction Details

To view details of a single fraction

▶ Do one of the following:

- Select the location of the tube or outlet valve port in the chromatogram.
- Select the row in the table that corresponds to the fraction of interest.
- Select the fraction of interest in the fraction display to the left of the table.

  Tip: If the fraction of interest is in another container, change the display by clicking the fraction's number in the rack selection panel.

To select multiple fractions

▶ Do one of the following:

- To select a range of fractions, Shift-click the first fraction in the range and then click the last fraction in the range.
- To select a group of discrete fractions, hold down the Ctrl key and click each fraction of interest individually.

Pooling Fractions

You can select multiple fractions in the chromatogram or the Fractions table to group (or pool) together in order to see calculated concentrations. Creating a pool also selects groups of fractions in the rack display.

Note: The pool must consist of adjacent tubes in the chromatogram or rows in the table. You can pool multiple fractions from the same outlet valve port by right-clicking in the Fractions table and selecting Pool Outlet Valve/Port. You cannot pool disconnected fraction collector fractions.

In the Fractions table, the pool of fractions collapses to a single line. The pool is identified in the Fractions table by the following:

- **A plus (+) sign** — appears in the first cell of the collapsed row.
- **Fraction number** — the range of fractions in the pool (for example, Fractions 1–4).

- **Tube location** — the location of the group of pooled tubes in the rack.
- **Start** —the beginning of the collection of the first fraction in the pool.
- **End** — the end of the collection of the last fraction in the pool.
- **Collected volume (ml)** — the total fraction volume collected in all tubes in the pool.
- **Area** (**ml*mAU)** — the sum of all areas in the pool. This column appears after peak integration is performed.
- **Relative area** (**%)** — the relative pool area of all areas in a trace. This column appears after peak integration is performed.
- **Amount (mg)** — the sum of all fraction amounts in the pool. This column appears after peak integration is performed.
- **Concentration (mg/ml)** — the calculated protein concentration for the specified pool. This value is the weighted average by volume of the fraction concentrations in the pool.
- **Molarity (µM)** — the calculated molar concentration of the protein for the pool. This value is the weighted average by volume of the molar concentrations for the fractions in the pool.

To create a fraction pool

1. In the Fractions table, hold down Ctrl or Shift and select multiple adjacent rows of fractions.
2. Right-click the group of adjacent fractions and select Pool.

To expand the pool in the Fractions table

▶ Do one of the following:

- In the Fractions table, click the plus sign in the first cell.
- Right-click the Fractions table and select Expand all pools.

To clear a pool

▶ In the Fractions table, right-click the pool row and select Unpool.

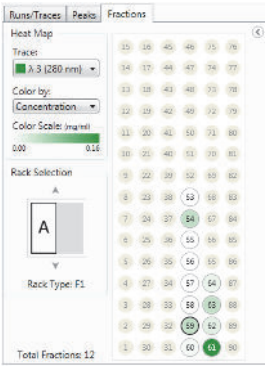
Viewing Fraction Collection Results — the Heat Map

A configuration of the fraction collector (the rack display or the outlet valve) appears to the left of the Fractions table. The image displays the configuration of the selected fraction collector when the run was executed. After peak integration, a heat map of the different fraction properties appears. If a multi-wavelength UV detector was used for the run, you can select a trace for the heat map (the default trace is 280 nm). The color of the heat map is based on the color of the chosen trace in the chromatogram. Tubes or outlet valve ports that do not contain fractions appear shaded.

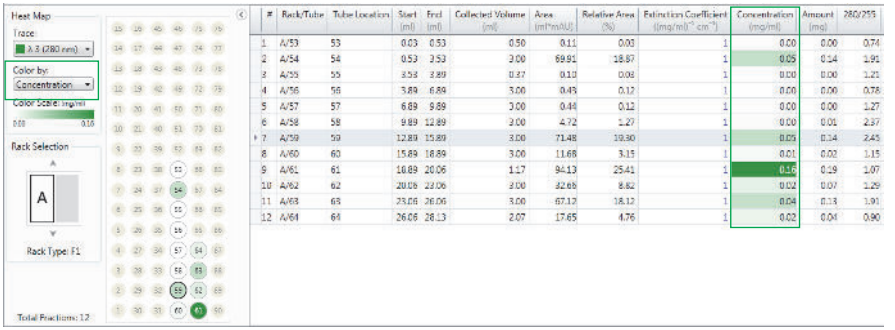
Note: Because OV1 Port 1 directs flow either to the diverter valve of the BioFrac fraction collector or to waste, it always appears shaded in the outlet valve display. When two outlet valves are used to collect fractions, OV1Port 12 also appears shaded.

You can base the heat map on any of the following columns in the Fractions table by selecting from the Color by dropdown list:

- Area
- Concentration
- Amount
- Molarity
- 280/260 (or 255)



The color scale is based on the column chosen and is relative among all available fractions of the run. A darker color represents a greater value and tubes or valve positions that do not contain fractions appear dimmed, for example



Viewing Heat Map Details

To change the displayed trace

▶ Select another UV trace from the dropdown list (available only if the multi-wavelength UV detector was used in the method).

To change the values to display

▶ Select Area, Concentration, Amount, Molarity, or 280/260 (or 255) from the Color by dropdown list.

To hide the rack display

▶ Click the collapse arrow to the right of the fraction display.

To select a rack or plate to view

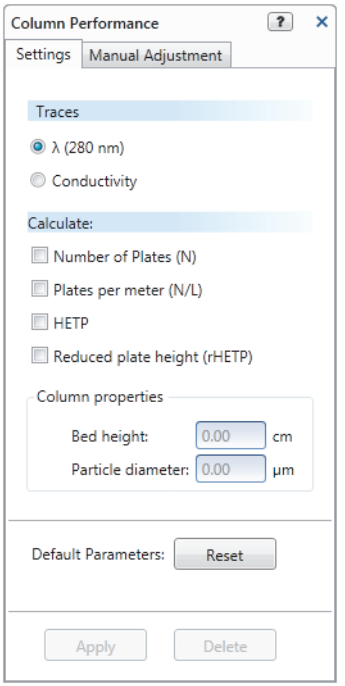
▶ Click the relevant display icon in the rack selection panel. Use the up and down arrows next to the panel to scroll to the relevant rack, plate, or outlet valve if necessary.

Column Performance Analysis

Method runs that include the Column Performance Test phase can be analyzed for column performance. ChromLab software analyzes the performance of the column in use and provides quality statistics including the number of theoretical plates (N), the number of plates per meter (N/L), height equivalent to a theoretical plate (HETP), and the reduced plate height (rHETP). The results appear in the Column Performance tab in the table at the bottom of the Evaluation window and in the chromatogram.

Column Performance — Settings

When you click Analyze Column Performance on the Analysis menu, the Column Performance pane appears to the right of the chromatogram. The default settings in the Settings tab are used to automatically calculate various column performance attributes. You can change the default settings and choose which quality measurements to calculate and display in the Column Performance table.



Traces

You can choose to analyze the column's performance based on either a single UV trace or the conductivity trace.

Calculate

You can calculate and display the following measurements:

Number of plates (N) — displays the number of theoretical plates, an indication of column efficiency. Theoretical plate numbers are an indirect measure of peak width for a peak at a specific time retention. Columns with high plate numbers are considered to be more efficient. The formula used to calculate number of plates is:

$N = 5.54 * (\text{peak retention time}/\text{peak width at half height})^2$

Plates per meter (N/L) — the number of theoretical plates per meter, a value used to compare theoretical plate numbers between columns. This measurement requires the medium bed height (in cm) for the column in use. The formula used to calculate plates per meter is:

plates/meter = N/L

where L = medium bed height

Height equivalent to theoretical plate (HETP) — the height equivalent to a theoretical plate, a value used to determine the number of theoretical plates contained in any length of column. Shorter plate heights indicate more efficient columns. This measurement requires the medium bed height. The formula used to calculate HETP is:

HETP = L/N

Reduced HETP (rHETP) — the reduced plate height, a value used to compare the efficiency of multiple columns packed with different particle sizes. Columns with rHETP ≤ 2 are considered well packed and more efficient. This measurement requires the medium bed height and the average particle diameter (in μm) for the column in use. The formula used to calculate rHETP is:

rHETP = HETP/Dp

where Dp = average particle diameter

Analyzing Column Performance

When analyzing the performance of predefined columns, ChromLab obtains the values for medium bed height and average particle diameter from the column's properties table and automatically calculates all measurements.

When analyzing the performance of custom columns, ChromLab automatically calculates only the number of plates. After you manually enter the values for medium bed height and average particle diameter, ChromLab calculates the measurements you select.

All initial values are based on the UV trace. You can change the trace to calculate measurements based on conductivity.

To analyze column performance

1. Select Analysis > Analyze Column Performance.

   The Column Performance pane appears beside the chromatogram. The Column Performance table appears in the bottom pane populated with the known values for the column.

2. (Optional) In the Traces section in the Column Performance pane, change the trace to use.

3. In the Calculate section, select or clear the checkboxes of the measurements to calculate.

4. In the section, enter the values for bed height and particle diameter to calculate N/L, HETP, or rHETP.

   Tip: For predefined columns, you can change the prepopulated values for bed height and particle diameter in this section without changing the values in the Method Editor.

5. Click Apply to calculate the selected column performance statistics and display them in the table.

   Tip: You can include the column performance statistics in the Run report. See Run Reports on page 281 for information about creating Run reports.

Resetting the Column Performance Properties

To reset the properties to their default values

▶ Click Reset to reset the properties checkboxes to their default values.

Deleting Column Performance Data

To delete column performance data for the displayed trace

▶ Click Delete.

Note: This also removes the Column Performance table.

Column Performance — Manual Adjustment

In the Manual Adjustment tab of the Column Settings pane you can manually adjust the start and end points of the peak within the column performance phase range. Adjustments to the start and end points appear in the relevant column of the Column Performance table in the bottom pane. You can also adjust the start and end points on the Column Performance table. These changes appear at the appropriate points on the chromatogram.

To change the peak start or end points on the chromatogram

▶ Drag the peak's start and/or end marker to the adjusted time value.

To change the peak start or end points on the Column Performance table

▶ Type the adjusted time value in the relevant column.

To undo the adjustment

▶ Click Undo in the Manual Adjustment tab.

To redo the adjustment

▶ Click Redo in the Manual Adjustment tab.

Displaying Column Performance Results — the Chromatogram

The chromatogram displays the column performance analysis peak as hatched and shaded. The peak's start, end, and apex are indicated by small vertical lines at the relevant x points.

The retention time or column volume appears near the line of the peak apex.

Start Peak appears as a continuous vertical line located at the relevant x point.

End Peak appears as a dashed vertical line located at the relevant x point.

Pausing the pointer on a peak start, end, area, or apex displays a tooltip that shows details of the item.

Displaying Column Performance Results — the Column Performance Table

The results of the column performance analysis are detailed in the Column Performance table. The table displays the following fields for the column performance peak:

- **N** — displays the calculated number of theoretical plates for the column.
- **N/L (cm^{-1})** — displays the calculated number of theoretical plates per meter for the column.
- **HETP (cm)** — displays the calculated height equivalent to a theoretical plate for the column.
- **rHETP** — displays the calculated reduced plate height for the column.
- **Bed height (cm)** — displays the value for medium bed height as specified in the Settings pane.
- **Particle diameter (µm)** — displays the value for average particle diameter as specified in the Settings pane.
- **Run start time**
- **Column type** — displays the type of column. The column type is set in the Method Settings pane in the Method Settings view.
- **Start** — the beginning of the peak, displayed in the units of the currently chosen x-axis.
- **End** — the end of the peak, displayed in the units of the currently chosen x-axis.
- **Retention time** — displays the elution time at maximum peak height.
- **Retention volume** — displays the elution volume at maximum peak height.
- **Height (mAU)** — displays the peak height at the retention volume/time, measured from the UV trace to the calculated baseline.

- **Width at half height (ml)** — displays the width (in ml) of the peak at 50% of the maximum peak height.
- **Peak asymmetry** — displays the asymmetry factor, defined as the distance from the center line of the peak to the back slope divided by the distance from the center line of the peak to the front slope. All measurements are made at 10% of the maximum peak height.
- **Peak type** — displays the integration type for a peak.
  - **Automatic** — the peak was determined automatically and has not been manually adjusted.
  - **Manual** — the peak has been manually adjusted or added.

8 Importing and Exporting Data

Data files can be imported in the Home, Method Editor, or Evaluation window when you choose Import on the File menu. You can import the following data files:

- NGC™ data files, including regular and scouting methods, methods with runs, and run files exported from ChromLab™ software running on another computer
- Unicorn (v. 5 and v. 6) data files
- BioLogic DuoFlow™ data files

Importing an NGC Method or Run

You can import a method with or without runs or import a run in the Home, Method Editor, or Evaluation window. Method and run files are saved with the following extensions:

Method	.ngcMethod
Method with runs	.ngcMethodRuns
Run	.ngcRun

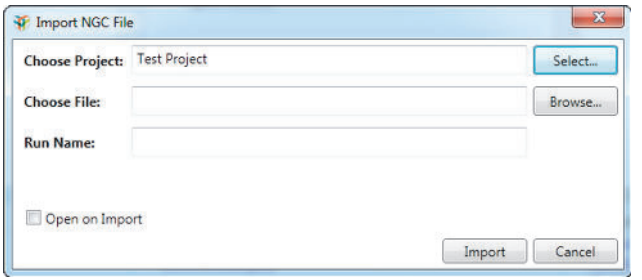
You can import a method with its associated runs even if the target folder already contains a method with the same name. In these cases:

- All new runs are appended to the existing method
- Existing runs are not imported twice

To import a method or run

1. In the Home, Method Editor, or Evaluation window, select File > Import and choose NGC File.

   The Import NGC File dialog box appears.



2. Click Select. In the Select Project dialog box, do one of the following:

   - Choose a destination project for the method or run and click Select Project.
   - Rename a project using the appropriate project button at the top of the dialog box and select the project to rename. Click Select Project.
   - Create a new sub- or root project and click Select Project.

3. In the Import NGC File dialog box, click Browse to display the Open dialog box.
4. Select the method or run file to import and click Open.

   Tip: To import multiple methods or runs at once, hold down Ctrl or Shift and select each file.

5. (Optional) In the Name box, type another name for the method or run.

   Note: This option is not available if you selected multiple methods or runs to import.

6. (Optional) Select Open on Import to open in the Evaluation window upon import.

   Note: This option is not available if you selected multiple methods or runs to import.

7. Click Import. During the import a status dialog box appears. When all methods and runs have successfully imported, the status displays Completed.

8. Click OK to close the dialog box.

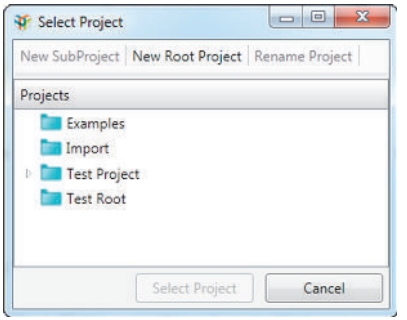
   The files are imported into the project you selected. Imported data files appear listed in the Open Run/Analysis dialog box available on the File menu in the Evaluation and Home windows.

Importing Unicorn Data Files

Imported Unicorn data are added to the NGC database. You can import a Unicorn run file in .asc or .csv format. The imported run can be compared with other runs in the database.

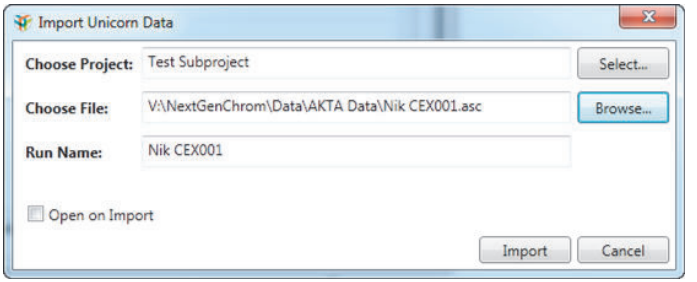
To import a Unicorn data file

1. In the Home or Evaluation window, select File > Import > Unicorn Data.
2. In the Import Unicorn Data dialog box, do one of the following:
   - Click Select and choose a destination project for the imported run.
   - Create or rename a project using the appropriate project button at the top of the dialog box and select the project. You can also create a new sub- or root project.



3. Click Select Project.
4. In the Import Unicorn Data dialog box, click Browse to display the Select Unicorn File dialog box.
5. Select a run file and click Open.

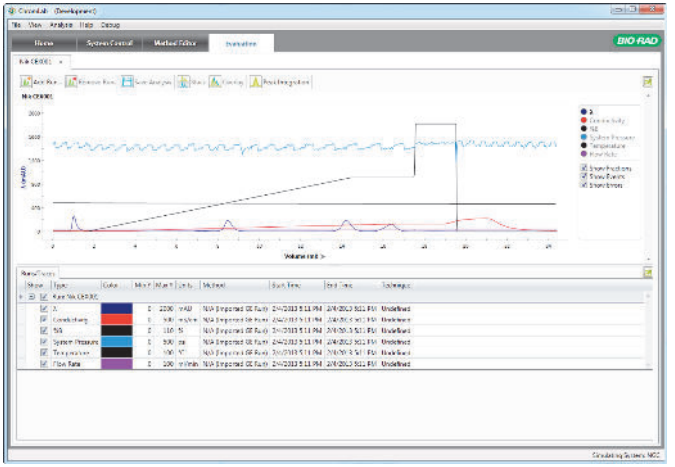
   The name of the run file and the project you selected appear in the Import Unicorn Data dialog box.



6. (Optional) In the Run Name box, type another name for the run you want to import.
7. (Optional) To have the run file open in the Evaluation window upon import, select Open on Import.
8. Click Import. Once the run has been imported, click OK.

   The data file is imported into the project you selected. Imported data files appear listed in the Open Run/Analysis dialog box available on the File menu in the Evaluation and Home windows.

9. If it is not already open, select the run to display its chromatogram and trace table in the Evaluation window.

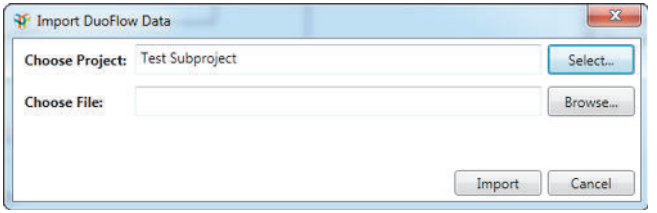


Importing BioLogic DuoFlow Data Files

BioLogic DuoFlow data are imported in a BIODB.txt file. Imported BioLogic DuoFlow data are added to the NGC database.

To import a BioLogic DuoFlow data file

1. In the Home or Evaluation window, select File > Import > DuoFlow Data.
2. In the Import DuoFlow Data dialog box, click Select and choose a project from the list of projects that appears.



3. In the Select DuoFlow File dialog box, select a data file to import.

4. Click Import.

   ChromLab imports all runs in the data file.

To open an imported run

1. Select File > Open Run/Analysis and select the project into which you imported the data file.
2. Select a run in the Open Run/Analysis dialog box and click Open Run.

   The run you selected appears in the Evaluation window.

Exporting Data

You can export run data with the method used to generate it or export only the run or method itself.

Note: You can export run data without the associated method only in the Evaluation window.

You can export run data as an NGC file, which can be loaded onto a different NGC system or another computer running ChromLab, or as a .csv file, suitable for importing into other applications. See Exporting Run Data as a .csv File on page 273 for more information.

Exported data files are saved with the following extensions:

Method (only)	.ngcMethod
Method with runs	.ngcMethodRuns
Run	.ngcRun
Run	.csv

Note: You cannot export scouting methods that have associated runs or scouting runs themselves. You can export only scouting methods.

Exporting Data as an NGC File

You can export method and run information from the Home, Method Editor, or Evaluation windows.

- In the Home window, you can export single or multiple runs, methods alone, and methods with their associated runs at the same time. You do not need to open the runs or methods to export them.
- In the Method Editor window, you open the method and export all of its runs with it.
- In the Evaluation window, you open the run and export the run alone, the method alone, or the run with its method.

By default, exporting a run does not export its method information — its column volume, for example. As a result, any report you subsequently generate based on an imported run will not include method information.

Exporting a method with its associated runs ensures that method information appears in run reports you subsequently generate.

You can also export only the method associated with a run.

To export data from a single run with its method

1. With a run displayed in the Evaluation window, select Export > Run with Method.

   The Save As dialog box appears, prepopulated with the method and file type.

2. Browse to the folder where you want to save the exported run data and method and click Save. Once the run has been imported, click OK.

To export a method with all of its runs

1. With the method displayed in the Method Editor window, select File > Export Method with Runs.

   The Save As dialog box appears, prepopulated with the method and file type.

2. Browse to the folder where you want to save the exported method and its runs and click Save. Once the method and its runs have been imported, click OK.

To export only the method

1. With a run displayed in the Evaluation window, do one of the following:

   - Select File > Export > Method only.
   - Select File > Show Method to display the method in the Method Editor window and then choose Export Method.

   The Save As dialog box appears, prepopulated with the method and file type.

2. Browse to the folder where you want to save the exported method and click Save. Once the method has been imported, click OK.
3. Click OK.

To export only the run data

1. With a run displayed in the Evaluation window, select File > Export > Run only (*.ngcRun).

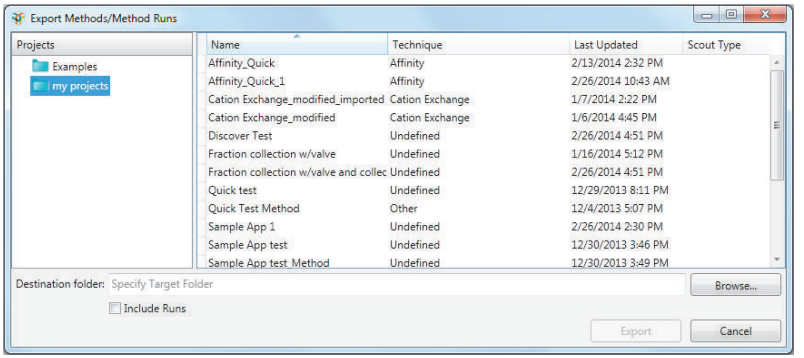
   The Save As dialog box appears, prepopulated with the run name and file type.

2. Browse to the folder where you want to save the exported run data and method and click Save. Once the run data have been imported, click OK.

To export multiple methods and associated runs

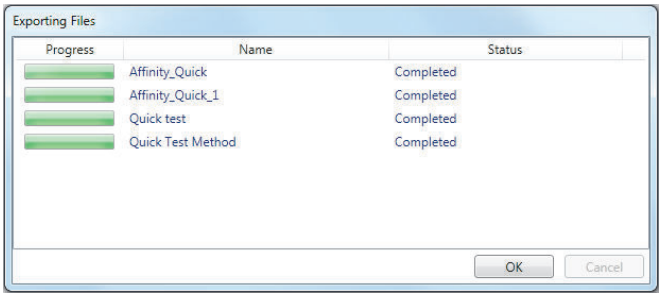
1. In the Home window, select File > Export > Method/Method Runs.

   The Export Method/Method Runs dialog box appears.



2. Select the project folder that contains the methods that you want to export in the left pane.
3. Hold down Ctrl or Shift and select multiple methods from the list in the right pane.
4. Click Browse to specify a target folder into which to save the method data.
5. (Optional) Select Include Runs to export the associated run data.
6. Click Export.

   During the export a status dialog box appears. When all method and run data have successfully exported, the status displays Completed.



7. Click OK to close the dialog box.

To export multiple runs

1. In the Home window, select File > Export > Runs.

   The Export Runs dialog box appears.

2. Select the project folder that contains the runs that you want to export in the left pane.
3. Hold down Ctrl or Shift and select multiple runs from the list in the right pane.
4. Click Browse to specify a target folder into which to save the run data.
5. (Optional) Select Include Methods to export the associated methods.
6. Click Export. After all run data have been successfully exported, click OK.

Exporting Run Data as a .csv File

Exporting run data to a .csv file enables you to employ various standard file formats, such as spreadsheet formats, so you can include the data in reports and custom process it. When you export run data, the following rules apply:

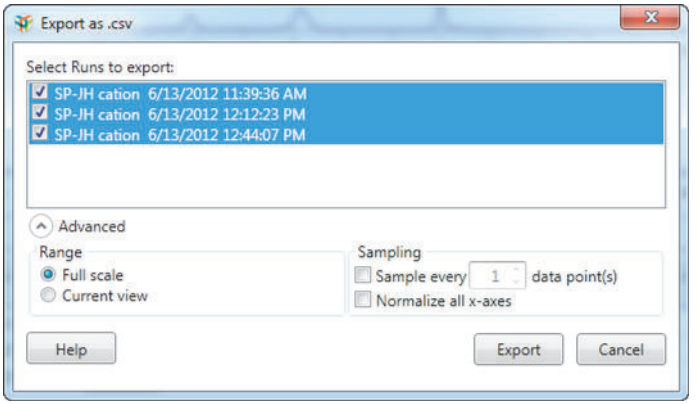
- Only traces marked Show in the Run table are exported.
- By default, all data points (y values) are exported, even if they are not currently in scale.
- The x-axis scale (units) is determined by the currently displayed axis.
- If an injection point is set, it determines the x-axis zero point.
- By default, data are exported so that each trace has two columns: X data and Y data. Because the sampling rates of trace types vary, different traces may have different numbers of values.

To export chromatogram data to a .csv file

1. With a run displayed, do one of the following:
   - Select File > Export > As .csv.
   - Right-click in the chromatogram and choose Export As .csv on the menu that appears.

     The Export as .csv dialog box appears.

   Runs currently displayed in ChromLab are listed and preselected.



2. Clear the checkbox for any run data you do not want to export.
3. To change the default settings, click Advanced to expose additional settings.
4. Under Range, select Full Scale to export the entire range of data or select Current view to export only the visible range.
5. Under Sampling, select Sample every n data points to reduce the number of data points by a factor you enter. This reduces the amount of data exported.
6. Select Normalize all x-axes to force all traces to use the same axis as the UV or the most frequent trace (presented in a single column) and multiple Y columns, one for each exported trace.

   Note: Having a single x-axis makes it easier to draw results charts when the exported data are opened in spreadsheet applications.

7. Click Export.

   The Save .csv file dialog box opens in which you can edit the file name and select a destination folder for the .csv file.

8. Click Save.

Exporting Diagnostic Logs

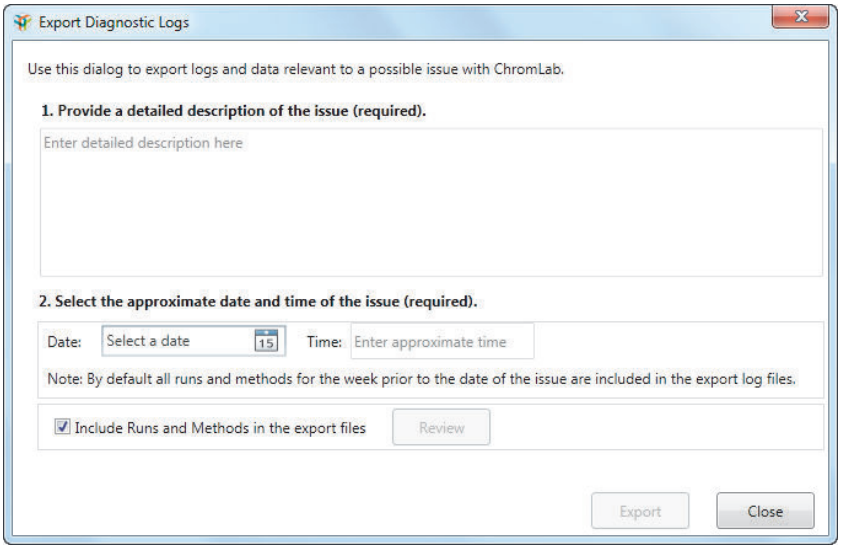
In ChromLab, you can export all critical information that Bio-Rad Technical Support requires to diagnose issues. The information includes system information, current log files, the associated runs and methods for a specified date range when the issue was noted, and a description of the issue or issues.

Note: Lamp information is not included in the exported diagnostic logs. To obtain lamp status, in the System Control window select File > System Information and click Get Lamp Status in the Detector tab.

By default, the log files and data are exported to a zip file and saved to the ChromLab computer's desktop. You can choose to save the file to another location.

To export diagnostic logs

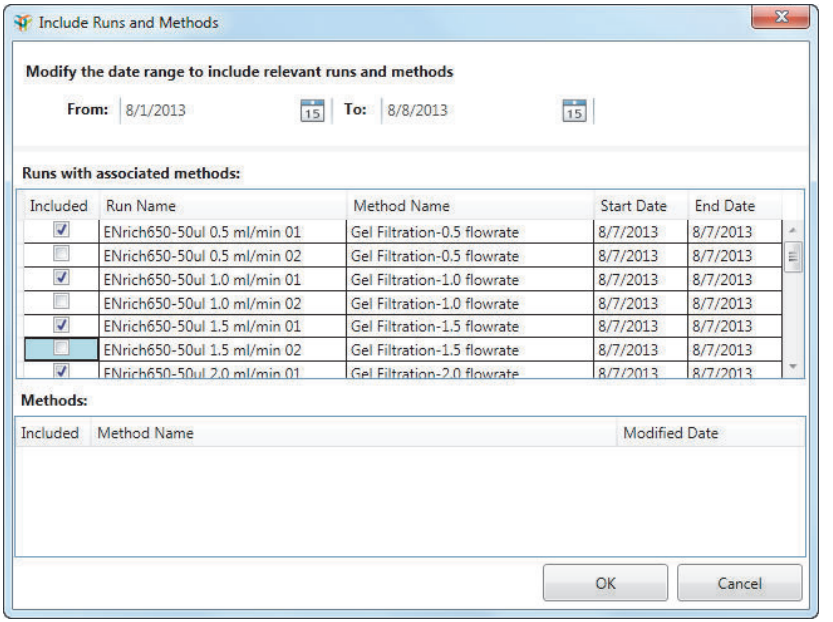
1. In the Home, System Control, or Method Editor window, select Help > Export Diagnostic Logs. The Export Diagnostic Logs dialog box appears.



2. Complete the required steps in the dialog box.

3. (Optional) Clear the Include Runs and Methods in the export file checkbox to exclude all method and run data from the export file.

4. (Optional) Click Review to display the Include Runs and Methods dialog box. This dialog box lists the runs with methods and separately lists the methods performed during the selected period. For example:



5. In the Include Runs and Methods dialog box you can

   - Change the date range.
   - Clear the checkboxes for entries not relevant to the issue. The data for these entries will not be included in the logs.

- Do one of the following:
  - Click OK to accept any changes and return to the Export Diagnostic Logs dialog box.
  - Click Cancel to revert any changes and return to the Export Diagnostic Logs checkbox.

6. In the Export Diagnostic Logs dialog box, click Export.

   By default, the ChromLabLog.zip file is saved to the ChromLab desktop. If necessary, you can navigate to another folder in which to save the file.

7. Click Save to save the ChromLabLog.zip file.

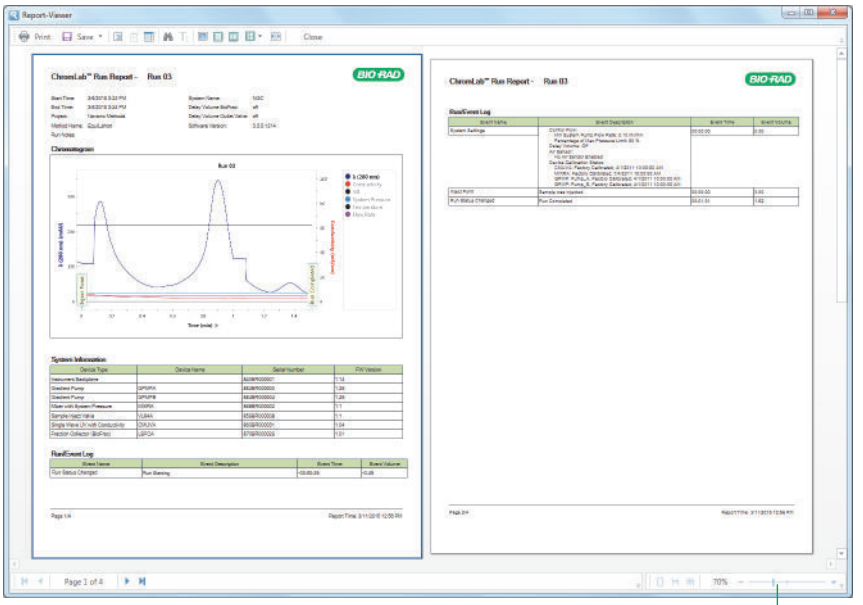
9 Reports

The three report formats available in ChromLab™ software make it easy to publish method, run, and analysis data in attractive, detailed reports. The single run report includes all information about a single run without peak integration analyses. The method report includes all information about the method. The analysis report includes all information in the single run report as well as a list of included runs and information about trace comparison and peak detected runs.

You can print reports without leaving ChromLab, and you can save reports in .pdf, .ppt, or .doc format.

Producing a Report

After you generate the data you want to include, you can easily create a formatted report with ChromLab. You can refine the report with Report Viewer dialog box commands and options. You can save or print the report, specify page size, and choose from several viewing options. You can also change the view by moving the scroll bar in the bottom right corner of the dialog box.



Method Reports

Note: The Scout column and Scouting Parameters table appear in the Method report only if the method is a scouting method.

To generate a Method report

▶ With a run, method, or analysis displayed, select File > Method Report.

ChromLab automatically generates a formatted report of all information in the method and displays the report in the Report Viewer dialog box.

Note: You cannot generate a method report if multiple runs appear in an analysis.

Run Reports

To generate a Run report

1. In the Evaluation window, select File > Run Report.

   The Run Report Options dialog box displays a list of options to include in the report (for example, the chromatogram, column performance statistics, system information, the run/event log, and annotations).

2. By default, ChromLab generates all options in the Run report. Clear the checkboxes of the options that you do not want to include in the Run report.

3. Click OK.

   ChromLab automatically generates a formatted report of selected options and displays the report in the Report Viewer dialog box.

Analysis Reports

Note: You must first perform peak integration analysis in order to generate an Analysis report.

To generate an Analysis report

1. In the Evaluation window, select File > Analysis Report.

   The Analysis Report Options dialog box displays a list of the columns that appear in the displayed Peaks table.

2. Select the checkboxes of the columns to include in the Analysis report.

   Note: You are limited to 12 columns.

3. Click OK.

   ChromLab automatically generates a formatted report of the selected columns as well as the peak parameters and the chromatogram. The analysis report appears in the Report Viewer dialog box.

   If fractions were collected, the Fractions table and all of its displayed columns appear as a separate table in the report. Pooled fractions appear in the report as a row in the Fractions table. The fractions in the pool appear as separate entries below the pooled row.

   Tip: Trace comparisons (if performed) in the peak integration analysis also appear in the Analysis report.

Printing a Report

To print a report

▶ With the report displayed in the Report Viewer dialog box, click Print in the toolbar, select a printer, and click Print.

Saving a Report

To save a report

- With the report displayed in the Report Viewer dialog box, click Save and select a file format from the dropdown list that appears.

  The Export Settings dialog box appears. You can expand this dialog box to display detailed settings. Available settings depend on the print format you selected.

A Database Management

ChromLab™ software stores all data (for example, methods, templates, and runs) in a database using Microsoft SQL Server 2008 R2. Bio-Rad highly recommends backing up this database regularly. Backing up the database on a different drive from the one on which ChromLab is running is also recommended.

If preventing data loss is critical to your operation, consider installing ChromLab on a RAID 1 drive. With RAID level 1, data are written identically to two hard drives. If one hard disk crashes, data are available on the redundant drive.

The backup procedure saves the NGC™ database into a zip (.bak) file. The size of the .bak file is approximately the same size as the NGC database itself. You need free disk space that is at least equal to the size of the NGC database to store the .bak file. Restoring the database requires the same amount of free disk space as the size of the .bak file.

Use ChromLab Administration to back up and restore the NGC database. ChromLab Administration backs up all NGC data, including all methods, runs, and analyses.

Important: ChromLab Administration backs up only the current NGC database. You cannot use this tool to back up a 1.0 or 2.0 database. As well, ChromLab Administration restores only the current NGC data. You cannot use this tool to restore an NGC database that was backed up using third-party tools such as SQLBackupAndFTP or SQL Backup Pro.

Backing Up the NGC Database

You cannot start ChromLab Administration while ChromLab is running on the client computer. Before you back up the NGC database, ensure that ChromLab is not running on the client computer and that the NGC instrument is idle, that is, no manual or method operation is running.

Note: You must have Windows administration privileges on the ChromLab computer to run ChromLab Administration.

To back up the ChromLab database

1. If you have not done so, determine the size of the NGC database.

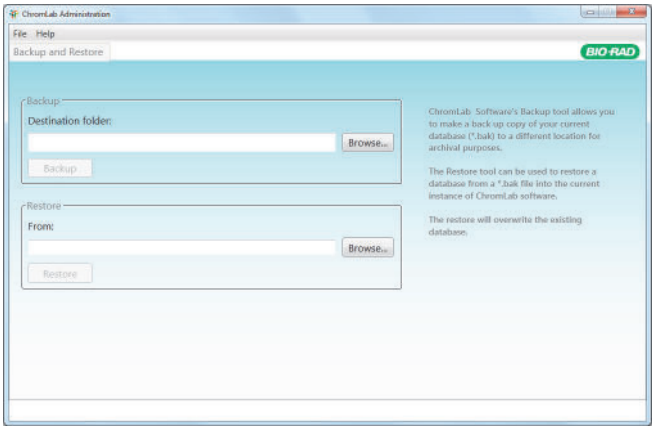
   a. Navigate to C:\ProgramData\Bio-Rad\NGC\Database.

   b. Right-click on the Database folder and select Properties.

   c. On the General tab, note the Size value.

   Ensure that the disk on which you plan to save the backup zip file has free disk space that is at least equal to the size of the NGC database.

2. On the Start menu, select ChromLab > ChromLab Administration.



3. In the Backup section, click Browse to browse to a location into which to save the NGC backup (.bak) file.

4. Click Backup.

   A status bar appears displaying the backup progress. Depending on the size of your database, the backup can take some time.

5. When the backup completes, close ChromLab Administration.

6. Restart ChromLab on the client computer.

   Tip: You cannot start ChromLab on the client computer while ChromLab Administration is running.

Restoring the NGC Database

Important: Before you restore the NGC database, close ChromLab on the computer and shut down the NGC instrument.

Note: You must have Windows administration privileges on the ChromLab computer to run ChromLab Administration.

To restore the ChromLab database

1. If you have not done so, determine the size of the NGC backup zip file.

   a. Navigate to the disk on which you saved the NGC backup zip file.

   b. Right-click on the backup file and select Properties.

   c. On the General tab, note the Size value.

   Ensure that the disk on which you plan to restore the NGC database has free disk space that is at least equal to the size of the backup file.

2. On the Start menu, select ChromLab > ChromLab Administration.

3. In the Restore section, click Browse to browse to the location where you saved the NGC backup (.bak) file.

4. Click Restore.

   A status bar appears, displaying the restore progress. Depending on the size of your database, the restore can take some time.

5. When the restore completes, close ChromLab Administration.
6. Restart the NGC instrument.
7. Restart ChromLab on the client computer.

   Tip: You cannot start ChromLab on the client computer while ChromLab Administration is running.

B Multicolumn Purifications

Protein purification can involve challenging separations of complex mixtures that might not provide sufficient resolution of target proteins from their impurities in a single chromatographic step. Such cases may require multiple discrete experiments involving multiple columns and different column chemistries.

Multicolumn purifications combine a series of columns, often with different or orthogonal chemistries in sequence or in tandem, in a single separation protocol. Most common protein purification techniques can be incorporated into a single protocol utilizing multiple columns, providing convenience through automation for proteins purified on a routine basis. A typical example of a multicolumn chromatographic scheme consists of an affinity purification step, via a histidine (His) or glutathione-S-transferase (GST) tag, followed by one or more additional experiments involving size exclusion columns for desalting or aggregate removal, or ion exchange columns for separating complex mixtures.

To streamline the combination of such disparate chromatographic methods, an automated chromatography system with a flexible design and versatile control software is essential. With their flexibility and scalability NGC™ chromatography systems can be easily customized to meet your multicolumn purification requirements.

Multicolumn Purification Method Templates

ChromLab™ software includes several multicolumn purification templates. The templates comprise preprogrammed methods for binding and eluting samples from multiple columns in an automated sequence. They also offer multidimensional chromatography strategies whereby the target fraction eluted from one column is loaded onto another for a second dimension of purification.

You can customize the preprogrammed templates by defining, among others, the following variables:

- Column type and size for your sample
- Method base unit
- Sample volume
- Step duration and length

Multicolumn Sequential Purification Templates

Sequential binding and elution is used when multiple samples must be purified on multiple columns. The samples are injected sequentially either by using a sample pump with sample inlet valve or through sample loops. Each sample is loaded onto a column and washed to remove contaminants that can cause sample degradation. The columns are then eluted using either step or linear gradient protocols in a sequence. The fractions are collected with the BioFrac™ fraction collector or an outlet valve. The sequential purification templates primarily utilize affinity techniques but can be modified to include other techniques.

Table 7 on page 291 defines the multicolumn sequential purification templates.

Table 7. Multicolumn sequential templates

Template Name	Template Description
Affinity (1 ml) — Linear Gradient	This method is used to purify many samples quickly. Using a sample inlet valve and the sample pump, the method loads sample onto separate affinity columns and washes unbound material off in quick succession. This process can be repeated for up to five columns. This method is designed to reduce the target protein's exposure to proteases. The target proteins are then eluted from each column sequentially using a linear gradient.
Affinity (1 ml) — Step (Manual Load)	This method is used to purify many samples quickly. Using a loop valve (a column switching valve plumbed to the injection valve in the loop position with multiple sample loops), the method loads sample onto separate affinity columns and washes unbound off material in quick succession. This process can be repeated for up to five columns. This method is designed to reduce the target protein's exposure to proteases. The target proteins are then eluted from each column sequentially using a step gradient.
Affinity (1 ml) — Step Gradient	This method is used to purify many samples quickly. Using a sample inlet valve and the sample pump, the method loads sample onto separate affinity columns and washes unbound material off in quick succession. This process can be repeated for up to five columns. This method is designed to reduce the target protein's exposure to proteases. The target proteins are then eluted from each column sequentially using a step gradient.
Affinity (5 ml) — Linear Gradient	This method is used to purify many samples quickly. Using a sample inlet valve and the sample pump, the method loads sample onto separate affinity columns and washes unbound material off in quick succession. This process can be repeated for up to five columns. This method is designed to reduce the target protein's exposure to proteases. The target proteins are then eluted from each column sequentially using a linear gradient.

Table 7. Multicolumn sequential templates, continued

Template Name	Template Description
Affinity (5 ml) — Step Gradient	This method is used to purify many samples quickly. Using a sample inlet valve and the sample pump, the method loads sample onto separate affinity columns and washes unbound material off in quick succession. This process can be repeated for up to five columns. This method is designed to reduce the target protein's exposure to proteases. The target proteins are then eluted from each column sequentially using a step gradient.

Multicolumn Tandem Purification Templates

Tandem purifications are used when two different chromatography techniques are combined into one method. First, samples are injected, bound to a column and washed to remove any major contaminants. Next, the target fractions are eluted and either applied directly onto another column in tandem or stored temporarily in a sample loop or container. If stored, they are later reinjected onto a second column. In either case, the second column is then eluted and the purified fractions are collected with the BioFrac fraction collector or an outlet valve. ChromLab software's multicolumn tandem purification templates utilize affinity, desalting, and size exclusion chromatography (SEC) techniques but can be modified to include other techniques.

Table 8 defines the multicolumn tandem purification templates.

Table 8. Multicolumn tandem templates

Template Name	Description
2-D Affinity (1 ml) > SEC (24 ml)	This method is used to purify up to four samples. Using a sample inlet valve and the sample pump, the method loads different samples onto separate affinity columns and washes unbound material off in quick succession. This process may be repeated for up to four columns. This method is designed to reduce the target protein's exposure to proteases. The target proteins are then eluted from each column sequentially using a step gradient. The eluted fraction is stored in a loop valve. The fraction is then injected onto a size exclusion column for a final analytical or preparative purification step.

Table 8. Multicolumn tandem templates, continued

Template Name	Description
2-D Affinity (5 ml) > Desalting (50 ml)	This method is used to purify up to four samples. Using a sample inlet valve and the sample pump, the method loads different samples onto separate affinity columns and washes unbound material off in quick succession. This process may be repeated for up to four columns. This method is designed to reduce the target protein's exposure to proteases. The target proteins are eluted from each column sequentially using a step gradient. The eluted fraction is stored in a loop valve (a column switching valve plumbed to the loop ports on the sample inject valve with multiple sample loops). The fraction is then injected onto a desalting column to perform a buffer exchange or to remove salt.
Affinity (1 ml) > Desalting (10 ml)	This method is used to purify up to five samples. Using a sample inlet valve and the sample pump, the method loads different samples onto separate affinity columns and washes unbound material off in quick succession. This process may be repeated for up to five columns. This method is designed to reduce the target protein's exposure to proteases. The target proteins are eluted from each column sequentially using a step gradient. Each eluted fraction is then passed immediately through a desalting column to perform a buffer exchange or to remove salt.
Affinity (5 ml) > Desalting (50 ml)	This method is used to purify up to five samples. Using a sample inlet valve and the sample pump, the method loads different samples onto separate affinity columns and washes unbound material off in quick succession. This process may be repeated for up to five columns. This method is designed to reduce the target protein's exposure to proteases. The target proteins are eluted from each column sequentially using a step gradient. Each eluted fraction is then passed immediately through a desalting column to perform a buffer exchange or to remove salt.

Performing Multicolumn Purification Chromatography

To perform multicolumn purification chromatography on an NGC system you must

- Plumb the NGC system
- Prime the NGC system
- Select and save a Method template
- Run the method

Plumbing the NGC System

How you set up and plumb the NGC system depends on the type of method you plan to run. This section provides information about plumbing the system to use the multicolumn purification templates.

Plumbing NGC Discover Pro Systems

This table lists the tasks for plumbing NGC Discover™ Pro systems. See Chapter 5, Plumbing and Priming the NGC Chromatography Systems, in the NGC Chromatography Software Installation Guide for detailed instructions.

Plumbing NGC Discover Pro Systems

	Task
1.	Tube #6 between the outlet port on the sample inlet valve and the inlet port on the sample pump **Note:** Remove the inlet tubing from the sample pump inlet manifold if it is installed.
2.	Connect one of the following: ■ Tube #4 from the Common inlet port on the BioFrac fraction collector diverter valve to port 1 on the outlet valve ■ Waste tubing to port 1 on the outlet valve
3.	Inlet tubing to the remaining ports on the sample inlet valve
4.	Outlet tubing to the remaining ports on the outlet valve

Plumbing the NGC Discover Pro System to Use Multicolumn Purification Templates

The tables in this section list the additional tasks for plumbing NGC Discover Pro systems to perform multicolumn purification. Ensure that you complete the plumbing tasks in the section Plumbing NGC Discover Pro Systems on page 295 before continuing.

Tip: These templates require either the NGC Discover Pro or the NGC Discover Pro +1CSV (column switching valve) fluidic scheme.

Multicolumn Sequential Affinity — Step and Linear Gradient Templates

	Task
▶	Affinity columns at ports 1–5 on column switching valve 1 (C1)

Multicolumn Sequential Affinity — Step (Manual Load) Template

	Tasks
1.	Insert a second column switching valve, (referred to as the loop valve) near the sample inject valve on the NGC instrument and map as C2
2.	Short length of tubing from port F on the sample inject valve to the left inlet port on the loop valve (C2)
3.	Short length of tubing from port E on the sample inject valve to the right inlet port on the loop valve (C2)
4.	One 5 ml loop tubing for each affinity column to the top and bottom ports on the loop valve (C2) For example, if you have two affinity columns ■ Attach loop tubing to Column Top port 1 and Column Bottom port 1 ■ Attach loop tubing to Column Top port 2 and Column Bottom port 2
5.	Tubing from port 2 on the outlet valve to the sample pump port on the sample inject valve
6.	Affinity columns at ports 1–5 on C1

Multicolumn Tandem 2-D Affinity Templates

	Tasks
1.	Insert a second column switching valve (referred to as the loop valve) near the sample inject valve on the NGC instrument and map as C2
2.	Short length of tubing from port F on the sample inject valve to the left inlet port on the loop valve (C2)
3.	Short length of tubing from port E on the sample inject valve to the right inlet port on the loop valve (C2)
4.	One 5 ml loop tubing for each affinity column to the top and bottom ports on the loop valve (C2) For example, if you have two affinity columns ■ Attach loop tubing to Column Top port 1 and Column Bottom port 1 ■ Attach loop tubing to Column Top port 2 and Column Bottom port 2
5.	Tubing from port 2 on the outlet valve to the syringe port on the sample inject valve
6.	Affinity columns at ports 1–4 on C1
7.	Desalting or SEC column at port 5 on C1

Multicolumn Tandem Affinity Templates

	Tasks
1.	Insert a second column switching valve (C2) into the NGC instrument
2.	Short length of tubing from outlet port on C1 to the inlet port on C2
3.	Short length of tubing from outlet port on C2 to the UV detector
4.	Affinity columns at ports 1–5 on C1
5.	Desalting column at port 5 on C2

Priming the NGC System

Important: Whenever you add or remove a module or change or upgrade your NGC system hardware configuration, you must replumb and reprime the system.

To prime the system

1. Power on the NGC system and its connected computer.
2. To enter manual mode, do one of the following:
   - On the computer, click Manual Run in the ChromLab Home window or click the System Control tab and then click in the fluidic scheme.
   - On the touch screen, click Enter Manual Mode if the application is not already in manual mode.
3. (Optional) If your hardware configuration has changed, select a fluidic scheme that matches your current system configuration.
   a. Select Tools > Change Fluidic Scheme.
   b. Select the appropriate fluidic scheme in the Fluidic Scheme Selector pane.
   c. Click Select to choose the manual mode fluidic scheme.
4. Insert appropriate inlet lines into the buffer, storage solution, and/or cleaning solutions to be primed and flushed through the system.
5. Prime the system.

   See Chapter 5, Plumbing and Priming the NGC Chromatography Systems, in the NGC Chromatography Systems and ChromLab Software Installation Guide for detailed instructions.

Selecting and Saving a Template

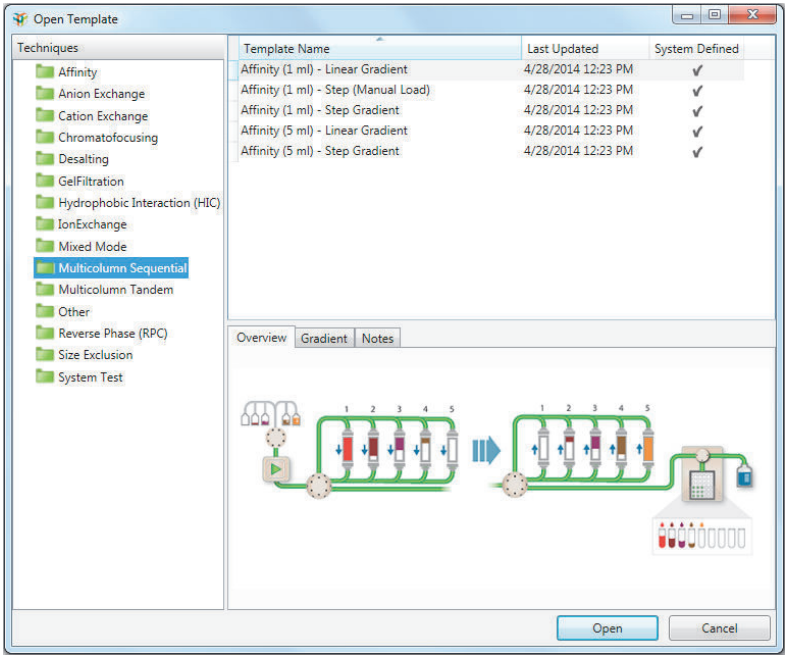
Tip: You must save the template as a method before you can use it.

To select a Method template and save it as a method

1. In ChromLab on the computer, do one of the following:

   - In the Home window, click Open Method Template.
   - In the Method Editor window, click Open Template on the toolbar.

   The Open Template dialog box appears. All supplied Method templates are organized in folders by technique.



2. In the left pane, click either the Multicolumn Sequential folder or the Multicolumn Tandem folder and select a template in the list that appears in the upper right pane.

In the lower-right pane

- The Overview tab displays a graphic representation of the purification steps for the selected template.
- The Gradient tab displays the gradient graph of the selected template.
- The Notes tab displays explanatory text that you can edit in the Method Settings view.

3. Double-click a template name to open the template in the Method Editor window.
4. In the Method Settings view, edit general settings like column type, method base unit, and wavelength in the Phase Parameters pane.
5. Select File > Save As to open the Save Method As dialog box.
6. Select a project folder or create a new subproject or root project in which to save the method.
7. Type a name for the new method, and then click Save.

Running Multicolumn Purification Methods

You run multicolumn purification methods the same way you run regular methods. See Running a Method and Collecting Fractions on page 177 for detailed instructions.

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