

Integrating the CETAC ASX-560 Autosampler with the NGC™ Chromatography System

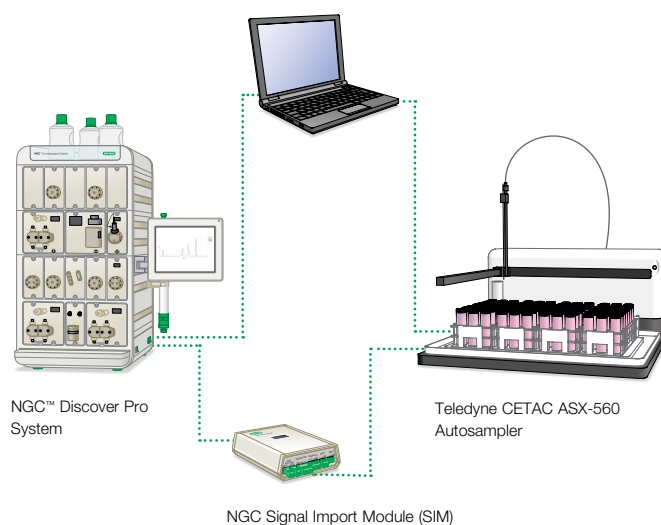
Protocol

Protein Purification

Bulletin 6896

Introduction

Protein purification is a common laboratory technique that is currently only semi-automated, requiring user intervention when purifying multiple large-volume samples. Here we describe how to integrate the CETAC ASX-560 Autosampler with the NGC Discover™ Pro Chromatography System to automate sequential protein purification without sample cross contamination, enabling rapid and reproducible results.



Materials

Bio-Rad Laboratories

- NGC Chromatography System with ChromLab™ Software Version 4.0 or higher
- NGC Sample Pump Module (catalog #7884004)
- NGC Inlet Valve Module (catalog #7884006)
- NGC Signal Import Module (SIM) (catalog #7884016)
- NGC Air Sensor Module (catalog #7885017)

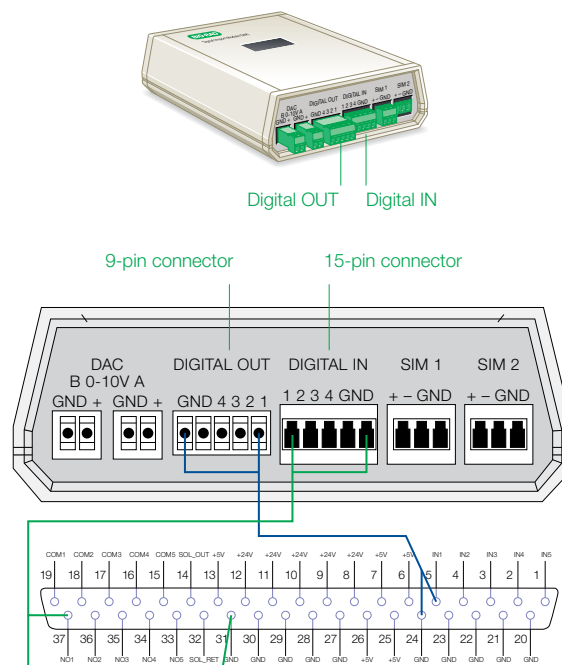
Additional Tools

- Teledyne CETAC ASX-560 (A56-99-0027) or ASX 280 (A28-99-0027). Instrument ships with cable connecting I/O box with NGC SIM.

Procedure

1. Connecting the ASX-560 Autosampler to the NGC SIM

- 1.1. Connect the green ends of the provided cable to the NGC SIM DIGITAL IN and DIGITAL OUT.
- 1.2. Connect auxiliary I/O end of the provided cable to the connector on the CETAC ASX-560 Autosampler.
- 1.3. Power up both instruments.



INx, Input; GND, Ground; SOL, Solenoid; NOx, Output; COMx, Common; 9-pin connector wiring (—); 15-pin connector wiring (—)

2. Plumbing connections between the NGC System and the autosampler sipper

Note: The ASX-560 Autosampler sipper should be plumbed with a large sample probe/sipper (1.0 mm ID). This will be designated by two blue bands at the top of the installed probe. A smaller sample probe/sipper will cause cavitation, resulting in less than the entire sample being loaded.

- 2.1. Plumb the sample probe/sipper to position 1 on the NGC Inlet Valve Module. Place an air sensor between the inlet valve module and the sample pump, allowing the maximum amount of sample to be loaded.
- 2.2. Plumb position 8 of the NGC Inlet Valve Module to the post-sample injection wash buffer or equilibration buffer.

3. Programming the CETAC ASX-560 Autosampler

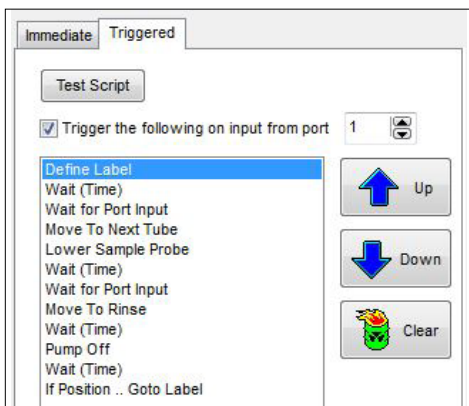
The ASX-560 Autosampler is controlled by AScript, a step-based software program with line by line commands. The following is the AScript script that allows sample loading onto the NGC System. Ensure that the script is written in the Triggered tab and "Trigger the following on input from port 1" is selected.

- Step 1: Define Label (START)
- Step 2: Wait (Time, 30 sec)
- Step 3: Wait for Port Input (Port 1, Open)
- Step 4: Move to Next Tube
- Step 5: Lower Sample Probe (140 mm)
- Step 6: Wait (Time, 60 sec)
- Step 7: Wait for Port Input (Port 1, Closed)
- Step 8: Move to Rinse (140 mm)
- Step 9: Wait (Time, 360 sec)
- Step 10: Pump Off
- Step 11: If Position... Go to Label (< (# Samples),
Go to Label START)

The first Wait for Port Input (Step 3) is the opening signal from the NGC System during equilibration, telling the autosampler to go to the sample and lower the sample probe.

The second Wait for Port Input (Step 7) is the closing signal from the NGC System during sample application, telling the autosampler that the sample has finished loading and to wash the probe.

The last step, If Position... Go to Label, makes a loop out of the script so that multiple samples can be loaded automatically. This script can be used for as many samples as the ASX-560 Autosampler can hold without cross-contamination from sample to sample.



4. Programming the NGC System

The NGC configuration within ChromLab Software will need to be modified to both send and receive signal import module (SIM) signals. To do this, the fluidic scheme must be modified to include a SIM module (see the Fluidic Scheme Configurations section referencing the SIM in Chapter 3 of the NGC Chromatography Systems and ChromLab Software User Guide, #10000049092). Once the SIM module has been added to the fluidics scheme, navigate to the Method Editor window and add initialization, equilibration, and ASX-560 Sample Application phases to your method. Customize them to provide reliable communication from the NGC System to the ASX-560 Autosampler.

4.1. Adding a SIM Module

In the Method Settings tab:

- Add a SIM Module to your fluidics scheme (see Chapter 3 System Control, Fluidic Scheme Configurations, in the NGC Chromatography Systems and ChromLab Software User Guide, #10000049092)

4.2. Creating the Initialization Phase

In the Method Outline tab:

- Select Create New Phase from the Standard Phase Library
- Type Initialization in the dialog box that pops up to name the new phase

In the Method Steps tab:

- Drag a SIM Control step from the Step Library to the top of the Method Steps table. The phase is automatically renamed Initialization Modified

Note: A Gradient Segments step is not required for the initialization phase. Remove if desired.

4.3. Creating the Equilibration Phase

In the Method Outline tab:

- Add an Equilibration phase from the Standard Phase Library to the Method Outline

In the Method Steps tab:

- Drag a SIM Control step from the Step Library to the top of the Equilibration Phase in the Method Steps table. The phase is automatically renamed Equilibration Modified

In the Method Outline Tab:

- Use the Change SIM State step to modify SIM Digital Out 1 to Stay High. This will signal the CETAC sipper to move to the appropriate sample and lower the probe

4.4. Creating the CETAC ASX-560 Sample Application Phase

In the Method Outline tab:

- Add a Sample Application phase from the Standard Phase Library to the method outline

In the Method Steps tab:

- Drag a SIM Control step from the Step Library to the bottom of the Sample Application Phase in the Method Steps table. The phase is automatically renamed Sample Application Modified
- Drag a Load Inject Sample step from the Step Library to the bottom of the Sample Application phase in the Method Steps table

In the Method Outline tab:

- Select Inject Sample on Column with Sample Pump on both Sample Loading steps **A1** **A2**
- Select Post-Injection Sample Pump Wash **B1**, set the
 - Buffer Position to S1 Port 8 **B2**
 - Flow Rate to 5 ml/min **B3**
 - Volume to 20 ml **B4**
- Set the Sample Position to S1 Port 1 **C1**
- Select Interrupt Injection If Air is Detected **C2**
- Set the Volume to a value larger than your sample volume **C3**

- Set all SIM states to Stay Low in the Change SIM State step **D1** **D2** **D3** **D4**
- In the second Sample Loading Step, select Pre-Injection Sample Pump Wash **E1** and set the Buffer Position to S1 Port 1 **E2**

This reprimes the line from the sipper of the ASX-560 Autosampler to the sample inlet valve of the NGC System using buffer from the rinsing reservoir. Set Flow Rate and Volume to match your system setup. Do not let the Flow Rate exceed 7 ml/min.

- In the second Sample Loading Step, deselect Interrupt Injection If Air is Detected **F1**
- In the second Sample Loading step, set Volume in the Direct Inject with Sample Pump step to 0.02 ml **F2**
- Deselect SIM Output 2 under Injection Point in both Sample Loading steps **G1** **G2**

Note: SIM Output 2 must be deselected in the Sample Application phase each time the method is opened.

The screenshot displays the method configuration interface for a sample application phase. At the top, a process flow diagram shows: Initialization → Equilibration → CETAC Sample Application → Elution. Below this, the configuration is divided into two sample loading steps (A1 and A2) and a 'Change SIM State' section.

Step A1 (Sample Loading):

- Sample Loading:** Inject Sample on Column with Sample Pump
- Pre-Injection Sample Pump Wash:** Pre-Injection Sample Pump Wash **B1**, Post-Injection Sample Pump Wash
- Direct Inject with Sample Pump:** Sample Position: S1 Port 1 **C1**, Interrupt Injection If Air is Detected **C2**, Flow Rate: 1.000 ml/min, Volume: 100.00 ml **C3**
- Post-Injection Sample Pump Wash with Buffer:** Buffer Position: S1 Port 8 **B2**, Flow Rate: 5.000 ml/min **B3**, Volume: 20.00 ml **B4**
- Change SIM State:** SIM Digital Out 1-4: Select State Stay Low **D1** **D2** **D3** **D4**
- Interrupt Injection:** Interrupt Injection Above UV, λ (280 nm), 2000 mAU
- Injection Point:** SIM Output 2 **G1**

Step A2 (Sample Loading):

- Sample Loading:** Inject Sample on Column with Sample Pump
- Pre-Injection Sample Pump Wash:** Pre-Injection Sample Pump Wash **E1**, Post-Injection Sample Pump Wash
- Pre-Injection Sample Pump Wash with Buffer:** Buffer Position: S1 Port 1 **E2**, Flow Rate: 7.00 ml/min, Volume: 20.00 ml
- Direct Inject with Sample Pump:** Sample Position: S1 Port 1, Interrupt Injection If Air is Detected **F1**, Flow Rate: 1.000 ml/min, Volume: 0.02 ml **F2**
- Interrupt Injection:** Interrupt Injection Above UV, λ (280 nm), 1 mAU
- Injection Point:** SIM Output 2 **G2**

4.5. Saving your custom phases

After adding these custom commands, rename and save these phases to the Custom Phase Library.

For more information about using the Method Steps tab, see Chapter 5 Method Editor, Method Steps View, in the NGC Chromatography Systems and ChromLab Software User Guide, #10000049092.

5. Starting a method using the CETAC ASX-560 Autosampler and NGC System

- 5.1. Execute the AScript (created in Step 4 of this protocol) for the number of samples to be run.
- 5.2. Start the NGC method and match the number of multiple sequential runs to the number of samples in the autosampler.

Screenshot of the "Schedule Run on NGC" dialog box. The "System Name" is set to "NGC" and the "Run Name" is "CETAC Samples". The "Multiple Runs" checkbox is checked, and the "Number of Runs" is set to 34. The "Append Fractions" radio button is selected. There are checkboxes for "Email When Run Completes" and "Create Run Report", both of which are unchecked. At the bottom, there are buttons for "Help", "Schedule Run", "Start Run", and "Cancel".

The CETAC ASX-560 Autosampler and the NGC Chromatography System are now communicating and able to perform sequential large-scale purifications.

Visit bio-rad.com/web/CETAC for more information.

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