Integrating the ALIAS Autosampler with the NGC Chromatography System

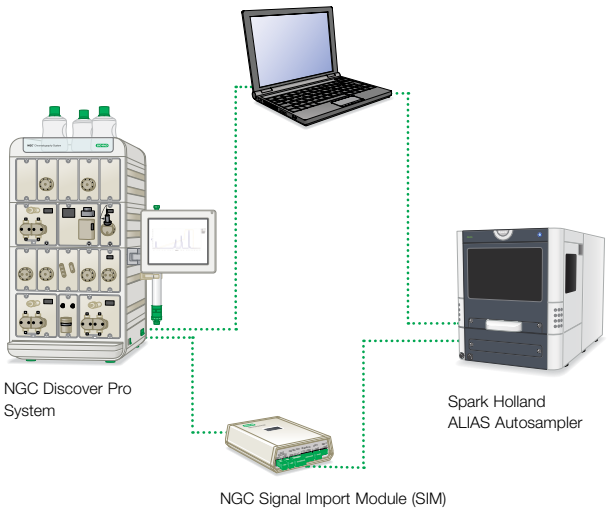
Protocol

Protein Purification

Bulletin 7031

Introduction

In protein purification, it is often necessary to analyze small protein aliquots acquired from large-scale purification. The ALIAS Autosampler, along with the NGC Chromatography System, allows multiple small-scale injections for analysis. Here we describe how to integrate the ALIAS Autosampler with the NGC Discover Pro System to automate sample injections, enabling high precision and accuracy with every run.



Materials

Bio-Rad Laboratories

- NGC Chromatography System with ChromLab Software Version 4.0 or higher
- 2.0 mm flat-head screwdriver (provided with NGC Fittings Kit, catalog #7884017)
- NGC Signal Import Module (SIM, catalog #7884016)

Additional Tools

- ALIAS Autosampler (catalog #SP841.002)
- ALIAS Cool Autosampler (catalog #SP841.003)
- ALIAS Service Manager Software Version 5.2.0
- SparkLink Software Version 5.3

Procedure

Connecting External Devices to the NGC SIM

WARNING! These procedures require you to work with exposed wires. Ensure that your systems are powered off and unplugged before performing this task.

1. Hardware connections

1.1. Connect the ALIAS cable to your PC (either serial or USB) to establish a connection between your computer and the autosampler.

1.2. Connect the ALIAS Autosampler DIGITAL IN (input) (4-wired) to the NGC SIM DIGITAL OUT (output).

1.2.1. Loosen screws on DIGITAL OUT (output) positions on the NGC SIM.

1.2.2. Connect orange and brown wires to ground (GND) position.

1.2.3. Connect red wire to position 1.

1.2.4. Connect black wire to position 2.

1.3. Connect the ALIAS Autosampler DIGITAL OUT (3-wired) to NGC SIM DIGITAL IN.

1.3.1. Loosen screws on DIGITAL IN positions on the NGC SIM.

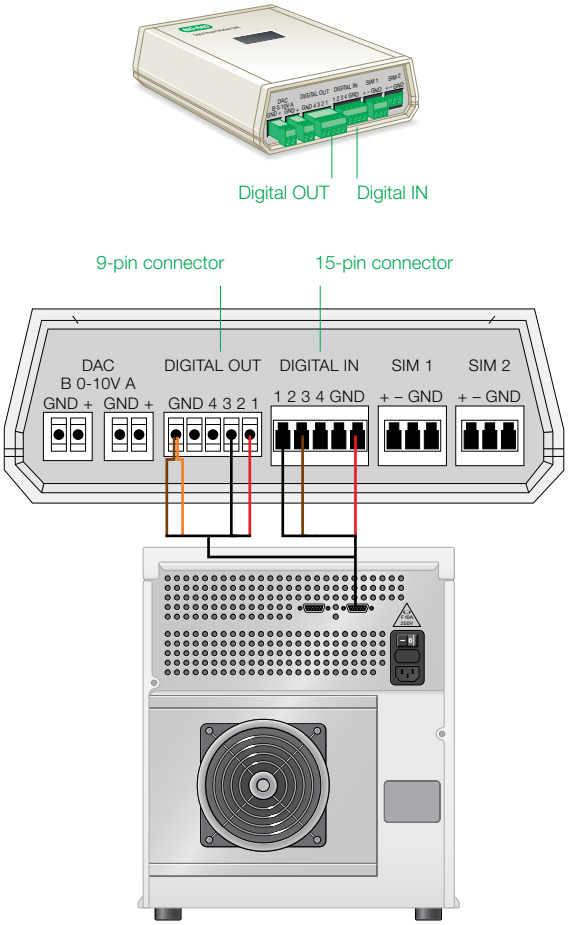
1.3.2. Connect red wire to GND position.

1.3.3. Connect black wire to position 1.

1.3.4. Connect brown wire to position 2.

1.4. Plug the SIM into the peripheral port on the base of your NGC Instrument.

1.5. Power cycle the NGC Instrument.



Connections between the NGC SIM and ALIAS Autosampler.

2. Software connections

You will need to adjust the software settings on the ALIAS Service Manager (ASM), SparkLink Configuration, and SparkLink Software as well as NGC ChromLab Software. If additional details are needed during ALIAS Software setup, please refer to the ALIAS Autosampler instruction manual (191.0010; Edition 9, 2016).

2.1. Set up ASM Software.

2.1.1. Open ASM Software and navigate to Communication.

2.1.2. Adjust communication settings as follows:

– Set Comport to appropriate communication port

– Set Protocol to RS232

2.1.3. Navigate to instrument settings.

2.1.4. Adjust the instrument settings as follows:

– Set Tray and Flow Path settings to match the configurations of the ALIAS Instrument you purchased

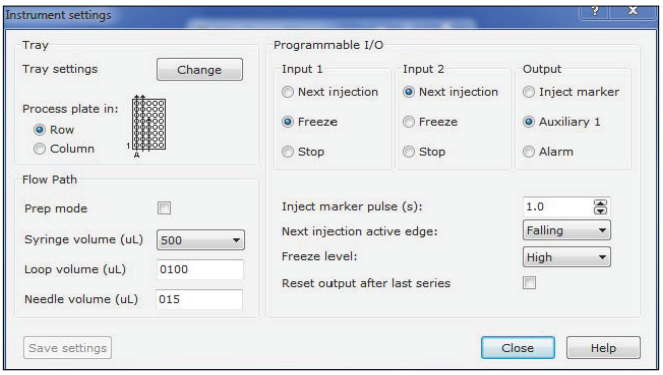
– Set Input 1 to Freeze

– Set Input 2 to Next injection

– Set Output to Auxiliary 1

– Set Next injection active edge to Falling

– Set Freeze level to High



2.2. Set up SparkLink Configuration Software.

2.2.1. Open SparkLink Configuration Software.

2.2.2. Create a new configuration that corresponds to those of the ALIAS Instrument.

2.2.3. Save configuration and then select activate.

2.2.4. Close SparkLink Configuration Software.

2.3. Set up SparkLink Software.

SparkLink settings appear in the ALIAS Autosampler as follows.

2.3.1. Open SparkLink Software.

2.3.2. Select New under the Project tab to create a new project.

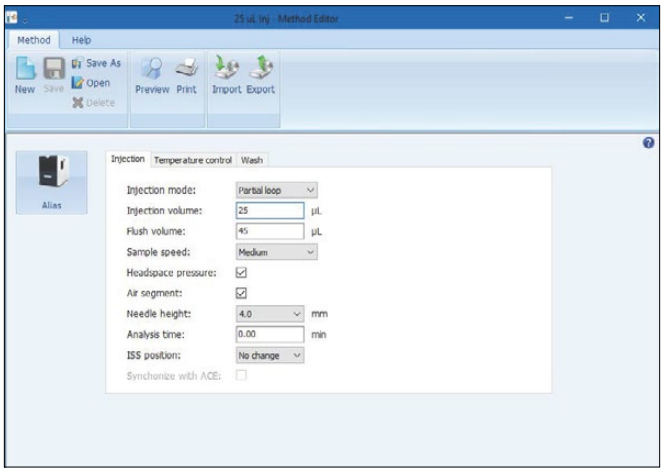
2.3.3. Name the project and then select Create.

2.3.4. In the Project tab, click Select to begin working in the new project.

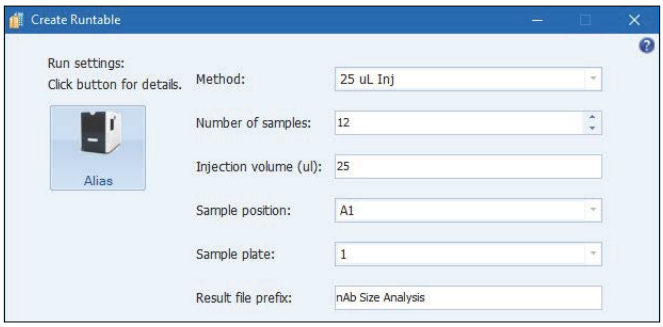
2.3.5. In the Methods tab, select Method Editor to create a new method.

2.3.6. In the Method Editor, set the appropriate injection mode, injection volume, and sample speed for the experimental samples. Additional information on the flush volume, headspace pressure, air segment, and needle height can be found in the ALIAS instruction manual.

2.3.7. Set Analysis time to 0.00 min. This will instruct the autosampler to pick up the next sample immediately after the wash. *If using tray cooling or injection volumes greater than 100 µl, please see additional instructions in the Appendix.*

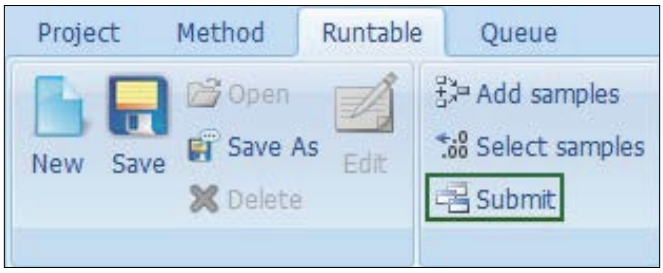


2.3.8. In the Runtable tab, select New to create a new runtable based on the created method for the respective samples/vials.



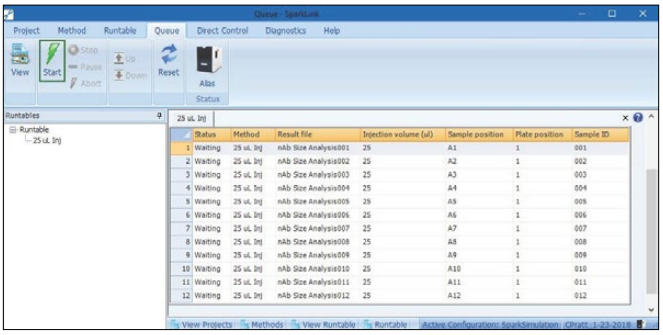
2.3.9. Input relevant information related to your unique experimental conditions and then click Create.

2.3.10. Select Submit in the Runtable tab to send the runtable to the queue.



2.3.11. Select OK in the Sample Positions dialog box.

2.3.12. Navigate to the Queue tab and select start to power the ALIAS Instrument.



2.3.13. After configuring ChromLab Software as described in the next section, the ALIAS Autosampler will be ready to carry out sample instructions.

Note: Always start SparkLink first, before the NGC System.

For more details on setting up SparkLink Software, refer to the ALIAS Autosampler instruction manual (191.0010; Edition 9, 2016).

2.4. Set up ChromLab Software.

The NGC System configuration within ChromLab Software will need to be modified to both send and receive SIM signals. To do this, the fluidic scheme must be modified to include a SIM (see the Fluidic Scheme Configurations section referencing the SIM in Chapter 3 of the NGC Chromatography Systems and ChromLab Software User Guide, #10000049092). Do not include the autosampler on the sample inject valve within the fluidic scheme. Once the SIM has been added to the fluidics scheme, navigate to the Method Editor window to create Custom Equilibration and ALIAS Sample Application phases in your method. These custom phases will provide reliable communication from the NGC System to the ALIAS Autosampler.

2.4.1. To add a SIM.

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).

2.4.2. To create the Custom Equilibration phase.

In the Method Outline tab:

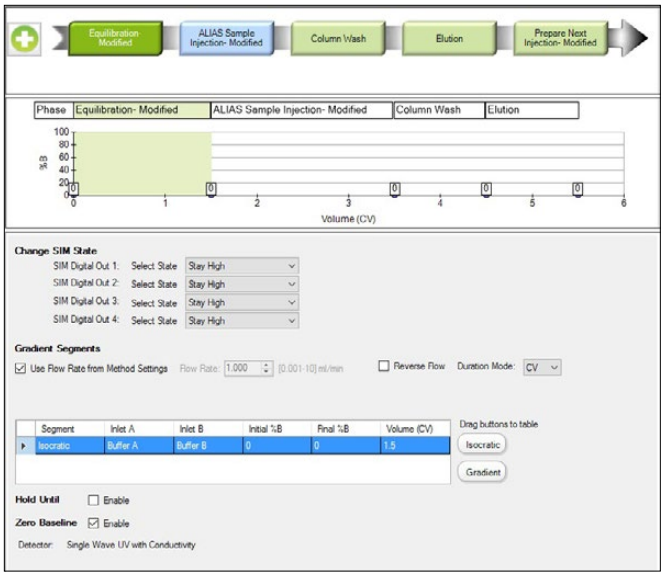
– Add an Equilibration phase from the Standard Phase Library to the Method Outline

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 all SIM Digital Out states to Stay High



2.4.3. To create the custom ALIAS Sample Application Phase.

In the Method Outline tab:

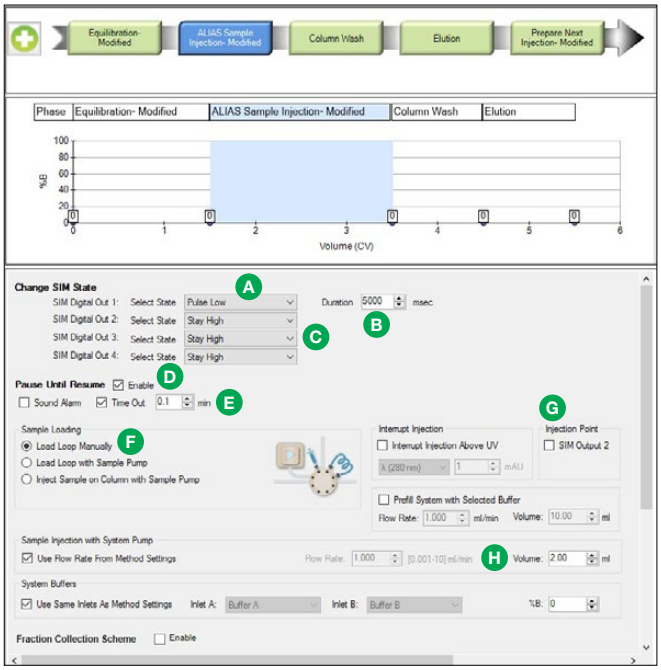
- Open Phase Library
- Select Create a New Phase and name it ALIAS Sample Injection

In the Method Steps tab:

- Drag Fraction Collection from the Step Library to the top of the ALIAS Sample Injection phase. This should be the first step in the phase
- Right click and delete gradient segments
- Drag SIM Control from the Step Library and place directly after the Fraction Collection step
- Drag Pause from the Step Library and place directly after the SIM control step
- Drag Load Inject Sample from the Step Library and place directly after the Pause step

In the Method Outline tab:

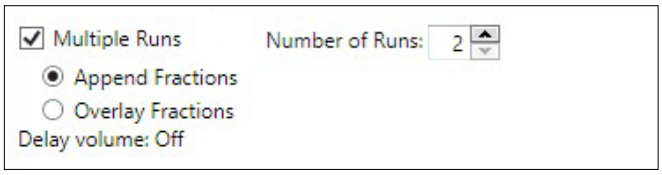
- Under Change SIM State, change the state for SIM Digital Out 1 to Pulse Low A
- Set the duration to 5,000 msec B
- All other states for SIM digital outputs should be set to Stay High C
- Under Pause Until Resume, click the checkbox to enable pause D
- Check Time Out and set to 0.1 min E
- Under Sample Loading, select Load Loop Manually F
- Under Injection Point, deselect SIM Output 2 G
- Under Sample Injection with System Pump, input a volume that is sufficient to properly load the sample onto the column. This volume should account for sample volume and the volume required to move the sample to the NGC System H



3. Starting a method using the ALIAS Autosampler and NGC System

3.1. Execute the Runtable (created in Step 2.3 of this protocol) for the number of samples to be run.

3.2. Start the NGC method and select Multiple Runs. Match the number of multiple sequential runs to the number of samples in the ALIAS autosampler.



The ALIAS Autosampler and the NGC Chromatography System are now able to communicate and perform sequential analytical protein purification.

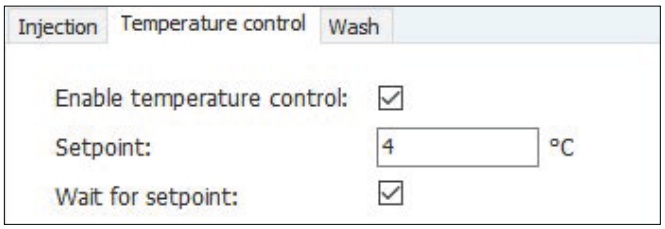
Appendix

The following instructions apply if tray cooling of the ALIAS is being used or if the sample injection volume is over 100 µl.

To prevent the sample from sitting too long at room temperature while the method for the previous sample is being executed or to allow for adequate loading time of larger samples, the following adjustments need to be made in both SparkLink and NGC ChromLab Software.

To make changes to SparkLink Software

- Open SparkLink Software and navigate to the Methods tab (see section 2.3 of this guide for instructions on how to navigate to this section of the software)
- In the Method Editor, set the Analysis time to 0.25 min
- In the Temperature Control tab, program the required temperature for the samples



To create an additional Prepare Next Injection phase in ChromLab Software

Note: This phase should be placed at the end of your method.

In the Method Outline tab:

– Open Phase Library

– Select Create a New Phase and name it Prepare Next Injection

In the Method Steps tab:

– Drag a SIM Control step from the Step Library to the Prepare Next Injection phase

– Right click and delete gradient segments

– Drag a Hold Until step from the Step Library and place directly after the SIM Control step

– Drag another SIM Control step from the Step Library and place directly after the Hold Until step

In the Method Outline tab:

– Under the first Change SIM State, set SIM Digital Out 1 to Stay Low A

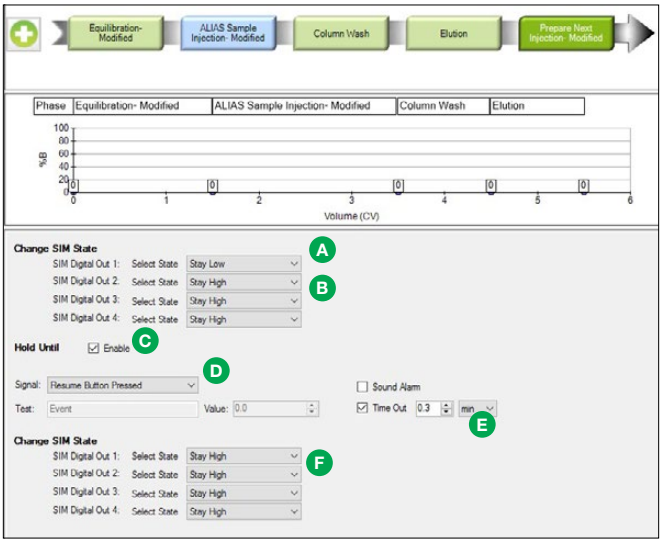
– Set all other SIM Digital Out states to Stay High B

– Enable the Hold Until setting C

– Set Signal to Resume Button Pressed D

– Set Time Out to 0.3 min E

– Under the second Change SIM State section, set all SIM Digital Out states (1–4) to Stay High F



Visit bio-rad.com/NGCautosampler for more information.

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *bio-rad.com* ***USA*** *1 800 424 6723* ***Australia*** *61 2 9914 2800* ***Austria*** *43 01 877 89019* ***Belgium*** *32 03 710 53 00* ***Brazil*** *55 11 3065 7550* ***Canada*** *1 905 364 3435* ***China*** *86 21 6169 8500* ***Czech Republic*** *36 01 459 6192* ***Denmark*** *45 04 452 10 00* ***Finland*** *35 08 980 422 00* ***France*** *33 01 479 593 00* ***Germany*** *49 089 3188 4393* ***Hong Kong*** *852 2789 3300* ***Hungary*** *36 01 459 6190* ***India*** *91 124 4029300* ***Israel*** *972 03 963 6050* ***Italy*** *39 02 49486600* ***Japan*** *81 3 6361 7000* ***Korea*** *82 2 3473 4460* ***Mexico*** *52 555 488 7670* ***The Netherlands*** *31 0 318 540 666* ***New Zealand*** *64 9 415 2280* ***Norway*** *47 0 233 841 30* ***Poland*** *36 01 459 6191* ***Portugal*** *351 21 4727717* ***Russia*** *7 495 721 14 04* ***Singapore*** *65 6415 3188* ***South Africa*** *36 01 459 6193* ***Spain*** *34 091 49 06 580* ***Sweden*** *46 08 555 127 00* ***Switzerland*** *41 0617 17 9555* ***Taiwan*** *886 2 2578 7189* ***Thailand*** *66 2 651 8311* ***United Arab Emirates*** *971 4 8187300* ***United Kingdom*** *44 01923 47 1301*

Bulletin 7031 Ver B US/EG *18-0851 1118 Sig 0118*