

Protein Science - PEL-00031 General Operation of BioRad NGC Chromatography Instrument

Purpose

These are the procedures for initiating a liquid chromatography experiment to purify protein.

Equipment

- NGC Chromatography System
- ChromLab software
- Chromatographic column with stationary phase

Procedures

A. System Preparation

All users should read the NGC Chromatography Systems and ChromLab Software Instrument Guide Version 3.3 and receive proper training before attempting to use this instrument.

1. Power up the NGC Chromatography System, and then open ChromLab software, making sure the software properly links with the system.
2. In the ChromLab software, open the controller windows for both the system pump and the sample pump and check that the pressure limits are set to 0.8 MPa (116 psi). **Caution:** Do not change the pressure limit on the system unless a plexiglass shield is in place for operation of high-pressure columns.
3. Move the system pump and sample pump input lines into the appropriate buffers and purge the necessary input lines with the appropriate buffers.
4. Proceed to attach the chromatographic column with stationary phase.

B. Experiment

1. Open “Method” in the ChromLab software and check the settings for the operation.
2. Move the sample pump input lines into the sample materials.
3. Start the “Method” run.

C. Experiment with High-Pressure Column

1. Open “Method” in the ChromLab software and check the settings for the operation.
2. Move the sample pump input lines into the sample materials.
3. Place a shield in front of the high-pressure column.
4. Start the “Method” run.

D. System Cleaning

1. Remove the chromatographic column with stationary phase from the NGC Chromatography system.
2. Place all the system pump input lines into water. Place all the sample pump input lines into 1N NaOH.
3. In the ChromLab software, open the controller windows for both the system pump and the sample pump and check that the pressure limits are set to 0.8 MPa (116 psi).
4. Purge all the sample input lines with 10 mL of 1N NaOH, making sure the injection valve position is in “Sample Pump Direct Inject/ System Pump Waste.” Allow the system to sit for 20 minutes. Then purge all the sample input lines with 10 mL of water, followed by 10 mL of 20% Ethanol.
5. Return the injection valve to “Manual Load Loop” and system pump to “Column” and purge all system input lines with 20 mL of water.