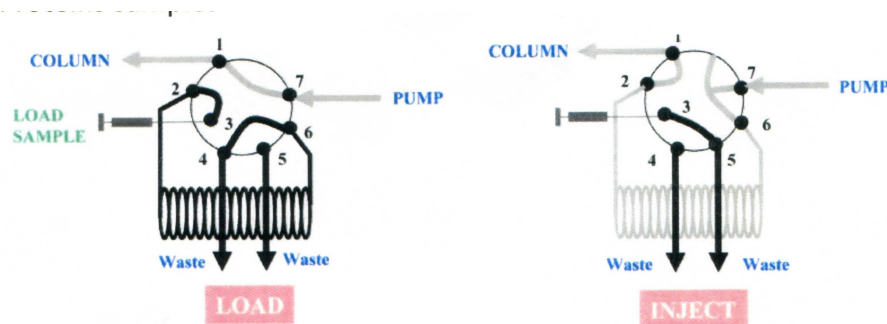


## AKTA FPLC System standard operating procedure

Note: First-time users must coordinate an introduction with Zhe before planning their first FPLC run

### Materials & Equipment

- Appropriate column
- Appropriate buffers chilled in AKTA refrigerator
- Protein samples



### Pre-run checklist

Start Unicorn Software if it's not on already. Unicorn software consists of four panels: *Unicorn Manager, Method Editor, System Control, and Evaluation*

- 1) On the AKTA, check that the fraction collector base unit status light on the right is lit green and that the rack is positioned securely in place.
- 2) On the Unicorn, check the bottom of the system control panel to make sure the path of the run is colored green (means system is connected).
- 3) Check that the main waste bottle is empty (by the cold box).

\*If the connection between Unicorn and AKTA is lost, try following:

1. Restart Unicorn. (The fraction collector will swing during the search for connection.)
2. Restart the PC and AKTA FPLC itself.
3. Check the CU950 box behind the laptop. All three green LEDs on the CU950 box should be lit (Power, PC, and SYSTEM). Flashing LED indicates that particular component is not connected.

### Creating User Account in the Unicorn:

- 1) From an existing user account, go to Unicorn Manager. Click on Administration-> User Setup. In the User Setup, click on New, fill out user's name and position and assign user's folder.
- 2) In the User Setup, click on Edit and click on System 1 to grant the access to the new user
- 3) Now, log on with the new user's account

### Creating Methods:

- 1) To create new methods, go to Method Editor and click on Method Wizard. Go through the step-by-step instructions to create a new method.
- 2) Otherwise, copy the Method files from an existing user (David's). Method and Results files are located in C: Unicorn/Local/File/UserName
- 3) Modify the Method by opening the file in Method Editor and click on Variables. Make sure the type of column is selected correctly. Modify the column position, injection sample volume, fraction size and any other variables. Make sure the column position is selected correctly, and injection sample volume is consistent with the size of the sample loop connected on the AKTA.
- 4) For each size exclusion chromatography column, create two Methods. For column wash, generate a Method with Empty Sample Loop and Fraction Collection sizes both at 0 ml. For the real run, choose appropriate Empty Sample Loop volume and Fraction collection sizes. Save both Methods.

### Column Equilibration before your run

- 1) For gel filtration runs, make sure there is at least 150mM NaCl in the buffer.
- 2) There are two pumps (A and B) with each AKTA. Put pump A into your buffer. On the Unicorn, go to System Control Panel -> Pump -> Pump Wash-> Pump A-> ON and then click on Execute. Pump wash replaces the solution in the pump space (~30mL) to your buffer. Pump wash continues until the indicator light on the System Control turns from red (Pause) to green (Ready).
- 3) Click on END at the top of the System Control panel to finish the Pump Wash.
- 4) Start column equilibration by click File->Run and select a column wash Method. Columns should be equilibrated after about 1.2 column volume (CV).

### Prepare Your Run

Protein sample loading:

- 1) Concentrate your samples to ~0.5mL for 10/300 columns and ~3mL for 16/60 columns.
- 2) IMPORTANT!** Filter or centrifuge your sample to remove any insoluble or precipitation.
- 3) Change the sample loop to the size of your choice. Make sure to screw the loop in tightly. (Make sure the size of your sample loop is larger than your sample volume)
- 4) Wash the sample loop by injecting at least 2 sample loop volume through the loop and collect waste from the short waste loop at position 4 with a beaker. Make sure there is no leakage and leave the syringe on the Injection Valve.
- 5) Load your sample by putting the short waste loop into the bottom of your sample tube and pull the syringe gently until the sample tube is empty. (Make sure no air bubbles are trapped in the loop).
- 6) Add small amount of buffer to the empty sample tube and pull the syringe again to push the sample into the sample loop.

- 7) Keep pulling the syringe until the solution front is about 1cm away from the inlet of the short waste loop.
- 8) Make sure sample + buffer added to the sample loop is < the size of the sample loop.

Fraction collection:

- 1) Bend the caps of the 2mL or 1.7 mL tubes downwards and place the tubes into the glass tubes on the Fraction Collector F950. Make sure the tubes are all centered.
- 2) Place the collector rack back and make sure it's positioned securely in place.

The Run

- 1) Start the run by clicking on File->Run in the System Control Panel. Select a run method (with sample injection and fraction collection).

### **Evaluation**

- 1) Go to the Evaluation Panel and open a result.
- 2) Right click on the result and select Properties to select the traces to be shown.
- 3) Right click on the result and select Peak Integrate to display peak positions.

### **After your run**

- 1) Collect your fractions and clean the fraction collector
- 2) Pick up the pump, rinse it with water, and place it back into ddH<sub>2</sub>O.
- 3) Start the pump wash from system control panel.
- 4) Wash (equilibrate) the used column with water.
- 5) Place the pump into storage solution (20% ethanol)
- 6) Start the pump wash from system control panel.
- 7) Wash (equilibrate) the column with storage solution.
- 8) Fill the syringe with water and rinse the injection loop.