FPLC standard operating procedure – AKTA system (GE)[1]

Before any run should commence, please fill the "Column Form"

Introduction

The Fast Performance Liquid Chromatography (FPLC) is essentially a computerized-control pump with a high degree of column selection. With the use of the FPLC, one can separate and characterize protein mixture as well as pure proteins. See appendix A for overview scheme of the AKTA purifier customized system in the Zarivach laboratory. The AKTA prime utilizes a single pump.

Materials & Equipment

- EtOH 20% (HPLC grade) – minimum 1L
- MQ – minimum 1L
- Appropriate column
- Appropriate buffers

Pre-run checklist

1. Check that the main waste bottle is empty.
2. Check that the pump waste bottle is empty.
3. Check that the carousel is positioned correctly and that there are enough clean tubes.
    - Check there is sufficient volumes of buffers, MQ and EtOH 20% for the coming run(s).
    - Check pump and tubing:

Note: Tube A1 is the default buffer tubing

- Tube A1 should be placed in MQ bottle; Tube A1 should be connected to position #1 on buffer selection valve IV-908 (module #6); In case there is need to transfer **ANY** tubing from one bottle to another, make sure you wash the tube with MQ water before placing it in it's new position.

[1] Reference: ÄKTAprime plus User Manual 11-0026-44 Edition AA, GE

Preparing for a Run

1. Switch on the AKTA prime via main switch (see scheme, appendix A).
    a. Several self tests (~30-40 second length) are performed in this process – if there is any error with the system a notification should be displayed on the LCD monitor.
2. The display should show "Template".
3. Initiate "Primer View" software at the PC station 

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Wash system's pump:

4. Place the selected "A" tube (1-8) and "B" tube in MQ bottle (a minimum of 1L is required before commencing any run)
5. Verify the waste capillaries are placed within the waste bottles (one for the injection loop outlet and another for the main waste bucket).
6. Within the panel:
    a. Go to "Templates" (press "OK") ⇒ "Application templates" ⇒ "System Wash Template" ⇒ Choose[2] the inlet to be washed (A1 & B are automatically washed). ⇒ scroll to the OK position and press the "OK" button.
    b. Press "OK" to start the wash template.
    c. Replace the first collection tube which contains small amounts of MQ.
        - In case air bubbles were identified within the tubing, follow the instructions on purging air from the pump and tubing (see below).

Connecting & conditioning the column:

7. Place the A1 tube in the running buffer bottle.
8. Connect the column's inlet port to the inlet (green) tubing through the 1/16'' male connector[3]; usually, this tubing is connected directly to the to the UV cell detector (see scheme, appendix A).

[2] Select the inlets to be washed with the arrows and then pressing "OK"

9. Connect the column's outlet port to the UV detector connection with an orange PEEK tube.
10. See that the delivery arm is positioned accordingly (see appendix B).
11. Wash the column with 2CV[4] of MQ by performing the following steps:
    a. Go to "Manual Run" ⇒ "Set Method Base" choose ml ⇒ "Set gradient" check "off" ⇒ "Set flow rate" ⇒ set according to column specs ⇒ "set pressure limit" set according to column specs ⇒ "Set Injection valve pos" set to "Load" position ⇒ "Set buffer valve" set the appropriate buffer valve which is in the MQ bottle ⇒ "Start Run".
    b. To stop the wash step you can either:
        - Press the "Pause/cont" button
        - Go to "Manual Run" ⇒ "Set flow rate" and set it to 0.00.
12. Perform system's wash with the inlet tubing placed within the running buffer (steps 4-6).
13. Wash the column with the running buffer (repeat step 11).
14. The system is read for a run.

<u>Loading sample via loop</u>

Before starting procedure, remove aggregates from protein solution by either centrifuging sample at >13Krpm for a minimum of 10' at 4°C or utilizing a 0.22µM filter (which can be connected directly to the injection port).

1. Connect loop via ports 2 and 6 on injection valve.
2. In the system's menu go to "Manual Run" ⇒ "Set Injection valve pos" ⇒ set to "Load" position.
3. Wash loop with MQ, at least x2 loop volume (LV).
4. Connect round-tip needle to the syringe and wash with 1ml of MQ.
5. Repeat step 2 and 3 with protein's buffer.
6. Slowly draw protein sample into the syringe and remove any bubble formed.

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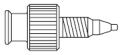
3 

[4] Column Volume; there are situations that require more than 2CV wash length

Note: Before injection, make sure flow rate is correctly set, flow path is set to column position, bufferValve choice is set to the correct tubing.

7. If using filter, connect filter to syringe and connect the filter nozzle to the injection port.
8. There are two loop filling techniques: one is aimed at minimizing sample loss ("Partial"), the other is aimed at reproducible sample loading ("Complete").
    a. Partial filling technique ⇒ Inject your sample at half the loop's volume.
    b. Complete filling technique ⇒ inject your sample at twice the loop's volume.
9. Do not disconnect the syringe until the end of the run.

Perform the run

15. Start run by performing the following steps:
    a. Go to "Manual Run" ⇒ "Set Method Base" check "ml" ⇒ "Set gradient" check "off" ⇒ "Set flow rate" ⇒ set according to column specs ⇒ "set pressure limit" set check it is correct ⇒ "Set Injection valve pos" check "Load"/"inject" position according to run step ⇒ "Set buffer valve" set the appropriate buffer valve which is in the buffer bottle ⇒ "Start Run".
    b. The following parameters can be changed along the run process:
        - Flow path
        - Flow rate
        - Fraction size (initiate collection of fractions once is set to other than "0.0")
        - Buffer valve position (by default choose "A1")
        - Injection valve position
        - Gradient ⇒ only when a gradient is in process. If gradient initiation is needed, switch to Pause/Hold (via buttons "Pause/Cont" or "Hold/Cont") and then initiate the gradient.
        - % Concentration of B
        - Autozero
        - EvenMark

Loading sample via buffer valve (sample volume >10ml)

10. Filter sample via 0.22μM filter
11. Connect an inlet tubing to port#8 and the other end place within the 50ml falcon tube containing the sample (make sure the tubing reach the bottom of the tube and is fastened).
12. Go to the system's menu ⇒ "Manual Run" ⇒ "Set Flow Rate" set to appropriate loading flow rate ⇒ "Start Run" ⇒ Press "OK".

Perform gradient run

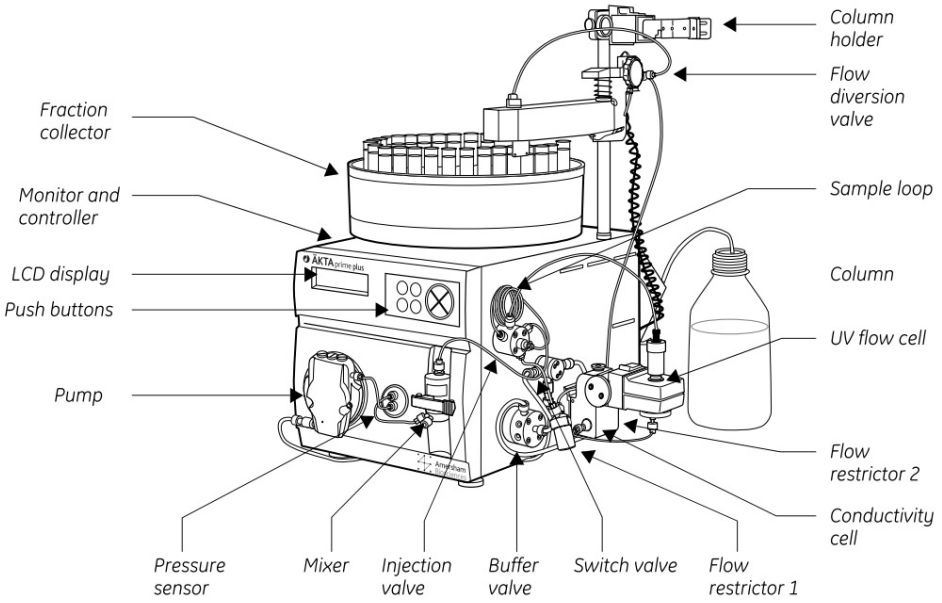
13. If in the middle of a run, press "Pause/Cont".
14. Place tubing "B" into the buffer "B" solution bottle.
15. Linear Gradient ⇒ Go to the system's menu ⇒ "Manual Run" ⇒ "Set Gradient" set on ⇒ "Set Length" set the aimed length, usually 40'. ⇒ "Set Target" set the end %concentration of "B", usually 40%.
16. Step gradient ⇒ to the system's menu ⇒ "Manual Run" ⇒ "Set %Concentration of B".

Ending a run & Wash system

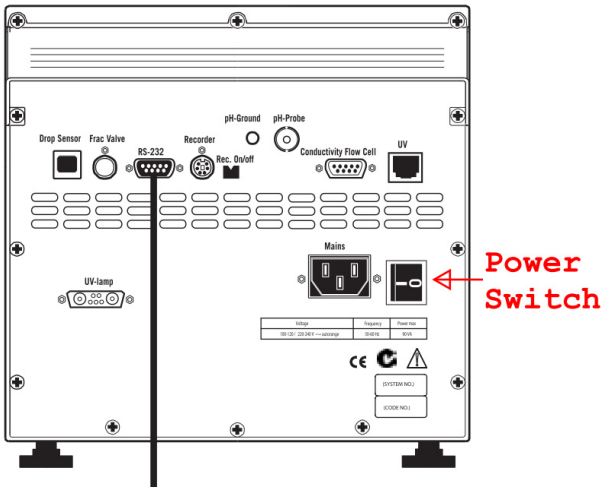
1. Press the "End" button and confirm with the button "OK".
2. Wash the system with MQ as described in steps 4-6.
3. Wash the column as described at steps 11-13, with MQ.
4. Wash the system with 20% Ethanol as described in steps 4-6.
5. Wash the column as described at steps 11-13, with 20% Ethanol.
6. Unless there is another user working with the system the same day, shut down the system by switching the main switch to "off" position.

Appendix A – AKTA prime plus system

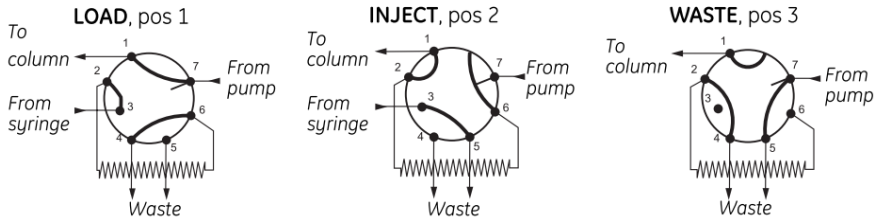
Component Description



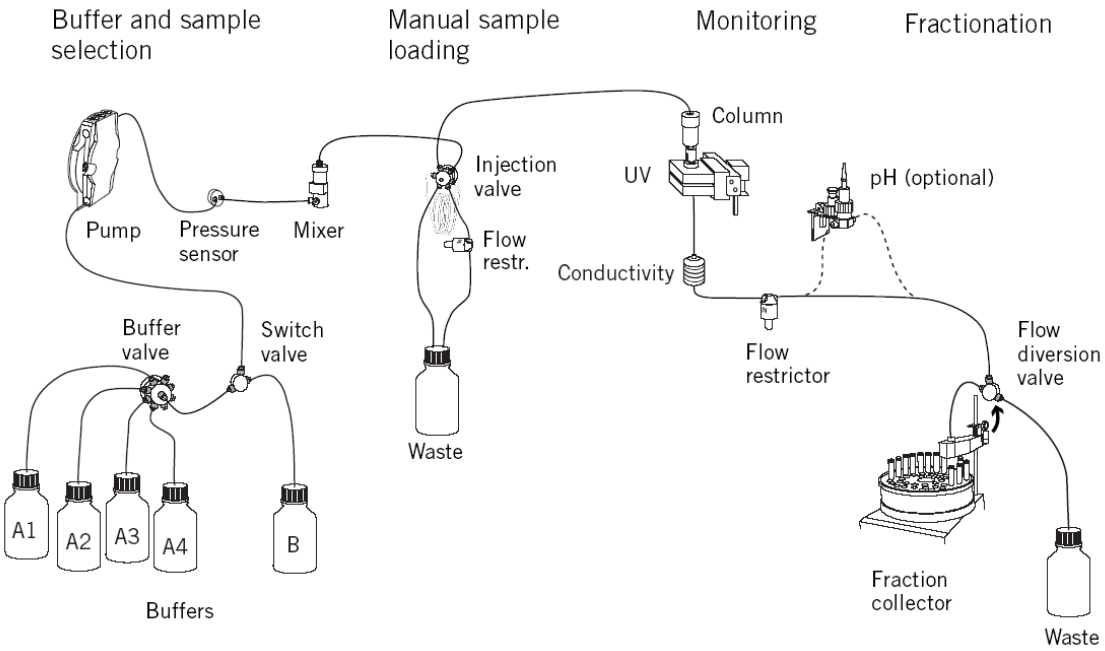
Location of Power switch



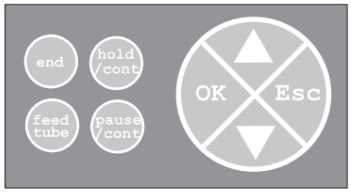
Injection valve positions



Liquid flow overview

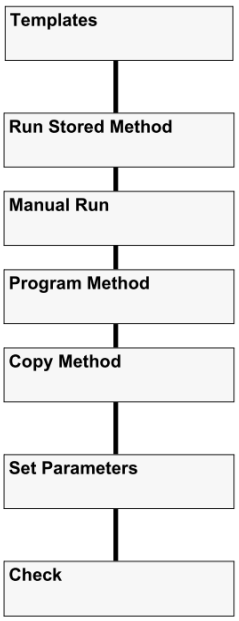


System's control panel



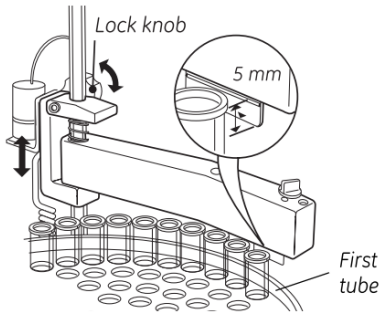
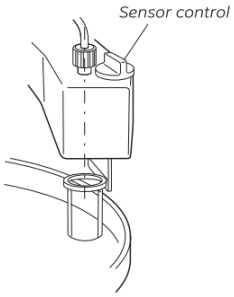
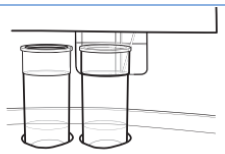
- △ or ▽: Find a specific menu option.
- **OK**: Enter a menu.
- **Esc**: Return one menu level.

Main menu items



- Used to run pre-made application templates and method templates. This menu appears after the self-test when turning on the system.
  See sections 5.6 Starting a run and 5.8 Running a method template.
- Used to run methods that are programmed by the user.
  See section 5.9 Running a stored method.
- Used to run the system manually without using methods.
  See section 5.10 Running the system manually.
- Used to program user-specific methods.
  See chapter 6 Method programming.
- Used to copy methods between ÄKTAprime plus and an external computer connected to the serial interface of the system.
  See section 6.5 Copying a method.
- Used to calibrate and set parameters, for example for UV, conductivity, temperature, pressure and flow rate.
  See sections 9.20 Calibrations and 11.2.1 Set Parameters menus.
- Used to check system parameters, such as serial number, pump run time and lamp intensity.
  See section 11.2.2 Check menus.

Appendix B – Preparing the fraction collector

Steps	Scheme
1. Fill the fraction collector with 18mm tubes.	
2. Adjust the height of the delivery arm using the lock knob as shown in the scheme, making sure the top rim of the tubes are located under the horizontal line on the tube's sensor.	
3. Adjust the nozzle alignment according to the center of the fraction tube using the sensor control	
4. Rotate the carousel rack by hand till the <u>rear half</u> of the tube sensor is aligned against the first tube.	
5. Press the "Feed tube" button and test the movement of the fraction collector and the alignment of the tubes.	

Appendix C – Columns selection[5]

Column/Resin ID	Cat#
Q Sepharose FF	17-0510-01
DEAE Sepharose FF	17-0709-01

[5] Zarivach lab, Jan 2010

IMAC Sepharose HP	17-0920-07
Benzamidine Sepharose FF	17-5123-10
HisTrap HP	17-5248-02
HisTrap HP	17-5247-01
HiTrap IEX selection kit	17-6002-33
HiTrap Q HP	17-1154-01
HiTrap Butyl FF	17-1357-01
Mono S 5/50GL	17-5168-01
Mono Q 4.6/100 PE	17-5179-01
Mono Q 5/50GL	17-5166-01
Superdex 200 10/300GL	17-5175-01
Superdex 75 10/300GL	17-5174-01
Superdex 200pg HiLoad 26/60	17-1070-01
Superdex 75pg HiLoad 26/60	17-1071-01