

PROTOCOL		
Date: 24-08-09	Written by: Chen Guttman	Laboratory: Raz Zarivach
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FPLC standard operating procedure – AKTA system (GE)

Note: First-time users must coordinate an introduction with Natalie (#28447) before planning their first FPLC run

Before any run should commence, please fill in the blanks @ 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 Zarivach laboratory.

Materials & Equipment

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

Experiment procedure

Pre-run checklist

1. Check that the main waste bottle is empty (lower refrigerator chamber).
2. Check that the pump waste bottle is empty (placed at the left side of the FPLC, with two waste tubes).
3. Check that the fraction collector base unit status light is green colored and that the carousel is positioned securely in its place.
4. Check that there are enough clean tubes in fraction rack, with the “A” row filled (the system’s default choice).

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- Check there is sufficient volumes of buffers, MQ and EtOH 20% for the coming run(s).
- Check pump and 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.

Preparing the system for a run

Note: In order to simultaneous execute several commands at the control panel, press “insert” and at the end of the command listing press “execute”.

1. Switch on PC and initiate the “Unicorn” software package (see desktop icon); four windows will open.
 - a. Login info ⇒ **User:** default
Password: default
2. In the Unicorn software, prompt the system control window; Go to Manual in the menu items (or press Ctrl+M).
3. PC monitor checklist:
 - a. Choose Flowpath:
 - Check that “injection valve” is set to load
 - Column position ⇒ check that it is set at “Position1bypass”
 - Buffer ValveA1 ⇒ Check that the pump inlet is set to A11
 - Pump inlet ⇒ A1
 - b. Choose “Pump” and check the following before continuing:
 - Flow rate ⇒ should be set to the appropriate flow rate of the column (consult column’s accompanying leaflet for the recommended flow rate)
 - Gradient ⇒ Check that inlet %B is set to “0”.

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- Alarm & monitor $\Rightarrow$ set alarm pressure to 0.25MPa
4. Wash system's A1 pump:
 - a. Go to "pump" $\Rightarrow$ "PumpWashExplorer", Choose "Inlet A11". In this automatic program, the pump is washed for 4 minutes with MQ pumped from inlet A11.
 5. If pump B1 is to be used, wash pump B1 with MQ (wash and transfer tubing B11 to MQ bottle).
 6. Wash B11 tubing with it's appropriate buffer (if it's MonoQ it is usually high salt buffer).
 7. (Optional: if lysate or large volume of protein solution is to be used wash inlet A18 tubing):
 - a. Verify that the system is at standby (check to see that flow rate is "0.00").
 - b. Rinse A18 tubing end thoroughly with MQ and place in 50ml flacon with 30ml of freshly filled MQ.
 - c. Change flow path $\Rightarrow$ "BufferValaveA1" $\Rightarrow$ "A18"
 - d. Wash tubing with 20ml of MQ at flow rate $\Rightarrow$ 10ml/min
 - e. Set flow rate $\Rightarrow$ 0ml/min
 - f. Fill falcon tube with 30ml of protein's buffer
 - g. Wash tubing with 20ml of protein's buffer at flow rate $\Rightarrow$ 10ml/min
 - h. Set flow rate $\Rightarrow$ 0ml/min
 - i. Port A18 is ready for injection.
 8. Wash inlet A12 tubing with MQ:
 - a. Verify that the system is at standby (check to see that flow rate is "0.00").
 - b. Rinse A12 tubing end thoroughly with MQ and place in MQ bottle
 - c. Change flow path $\Rightarrow$ "BufferValaveA1" $\Rightarrow$ "A12"
 - d. Set flow rate $\Rightarrow$ 10ml/min
 - e. Wash in 20ml of MQ

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9. (optional) Preparation of loop for small volume injection (up to 5ml):
 - a. Connect loop to injection valve via ports 2 and 6
 - b. Wash loop with MQ - Fill 10ml syringe with MQ, connect syringe to the injection port (screw-type) and pump the MQ while monitoring the outlet tubing drizzle (if it is not constant there might be air bubbles in the system; continue pumping till drizzle is constant)
 - c. Repeat wash with protein's buffer (according to the column used).
 - d. Injection valve is ready for run.
10. Connect column:
 - a. Remove plastic capping from the column ends and mount column on column arms.
 - i. If it is a small column, it is possible to connect the column directly to the pump valve#3 at any available position.
 - b. Connect column's lower part to pump valve#3 (upper left module) via tubing on position# 8
 - c. Connect the column's upper port to pump valve#2 via position#8 in a **drop-to-drop** style (this step ensures that no air bubbles will enter the column):
 - i. Go to Flow path $\Rightarrow$ "column position" $\Rightarrow$ "A18"
 - ii. Check Flow path $\Rightarrow$ "BufferValaveA1" $\Rightarrow$ Choose appropriate tubing
 - iii. Go to Flow rate $\Rightarrow$ $0.5\text{ml}/\text{min}^1$ (make sure the system is at "Run" mode)
 - d. The inlet tube edge should start to drizzle – fill the tubing socket with liquid and only then screw in the connector while pressing down.
 - e. Monitor the curve on screen and see that the curves stabilize after several minutes.

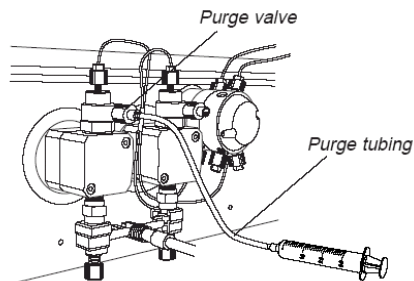
¹ Refer to manufacturer flow rate recommendation; this is the maximum flow rate of the Superdex 10/300

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- b. Set flow rate $\Rightarrow$ 0ml/min
11. Wash column with MQ:
 - a. Verify that the system is at standby (check to see that flow rate is “0.00”).
 - b. Check A12 tubing is placed in MQ bottle
 - c. Set flow rate $\Rightarrow$ 0.5ml/min
 - d. Wash column in one column volume
 - e. Set flow rate $\Rightarrow$ 0ml/min
12. Perform pump wash with appropriate buffer:
 - a. Go to “pump” $\Rightarrow$ ”PumpWashExplorer”, Choose the appropriate buffer inlet (usually A12).
13. Wash column with protein’s buffer²:
 - a. Verify that the system is at standby (check to see that flow rate is “0.00”).
 - b. Transfer A12 tubing to buffer’s bottle
 - c. Set flow rate $\Rightarrow$ 0.5ml/min
 - d. Wash column with two column volume
 - e. Column is ready for sample run.

Purging air from pump

1. Connect purge tubing with syringe to the purge valve.
2. Unscrew purge valve half a turn and connect purge and draw approximately 10ml or until no air bubbles can be seen in the pump tubing and in the purge tubing.
3. Close purge valve and disconnect the purge tubing.



² Refer to column’s data sheet for specific volumes and flow rates (flow rates here are examples)

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Sample run – Loading via buffer selection valve (IV-908 module)

Note: This injection method is suitable for >5ml protein solutions; lower volume should be injected via loop (see detailed procedure below)

1. Make sure the relevant tubings are located at the appropriate buffer containing buffer and that there is enough volume for the expected run.
2. Make sure the flow rate is set to “0 ml/min”, column position is set accordingly (usually at position 8)
3. Transfer the injection tube into your sample tube (should be 50ml flacon) and screw the cap; make sure the tube’s ending is at the lower part of the tube (this position should be maintained along the whole length of the injection step).
4. Set Flow path $\Rightarrow$ Buffer Valve A1 $\Rightarrow$ “A18”
5. Initiate injection by setting flow rate to the recommended by the column’s manufacturer recommendation³; it is adviseable to use a 1ml/min.
6. Monitor the volume of sample; when 2-3ml are left in the tube stop injection by setting the flow rate to “0ml/min”
7. (optional – IEC application):
 - a. Go to flowpath $\Rightarrow$ Set “A12”
 - b. Go to pump $\Rightarrow$ gradient $\Rightarrow$ set “%B” as 40% for 40’ at 1ml/min
 - c. Prepare for sample collection:
 - i. Go to “Frac” $\Rightarrow$ man_fractionation
 - ii. Choose frac size $\Rightarrow$ “10ml”, “Row-by-Row”

Note: Don’t press execute until sample starts to elute!

- iii. Once there is need to move to the next tube, press “Feed tube”
- iv. Fractionation stop is initiated via “Fractionation_stop”
command $\Rightarrow$ notice that flowpath is switched to “Waste”.

³ Depends on the ideal flow rate of the specific column; refer to appendix C

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Sample run – Loading via loop (INV-908)

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

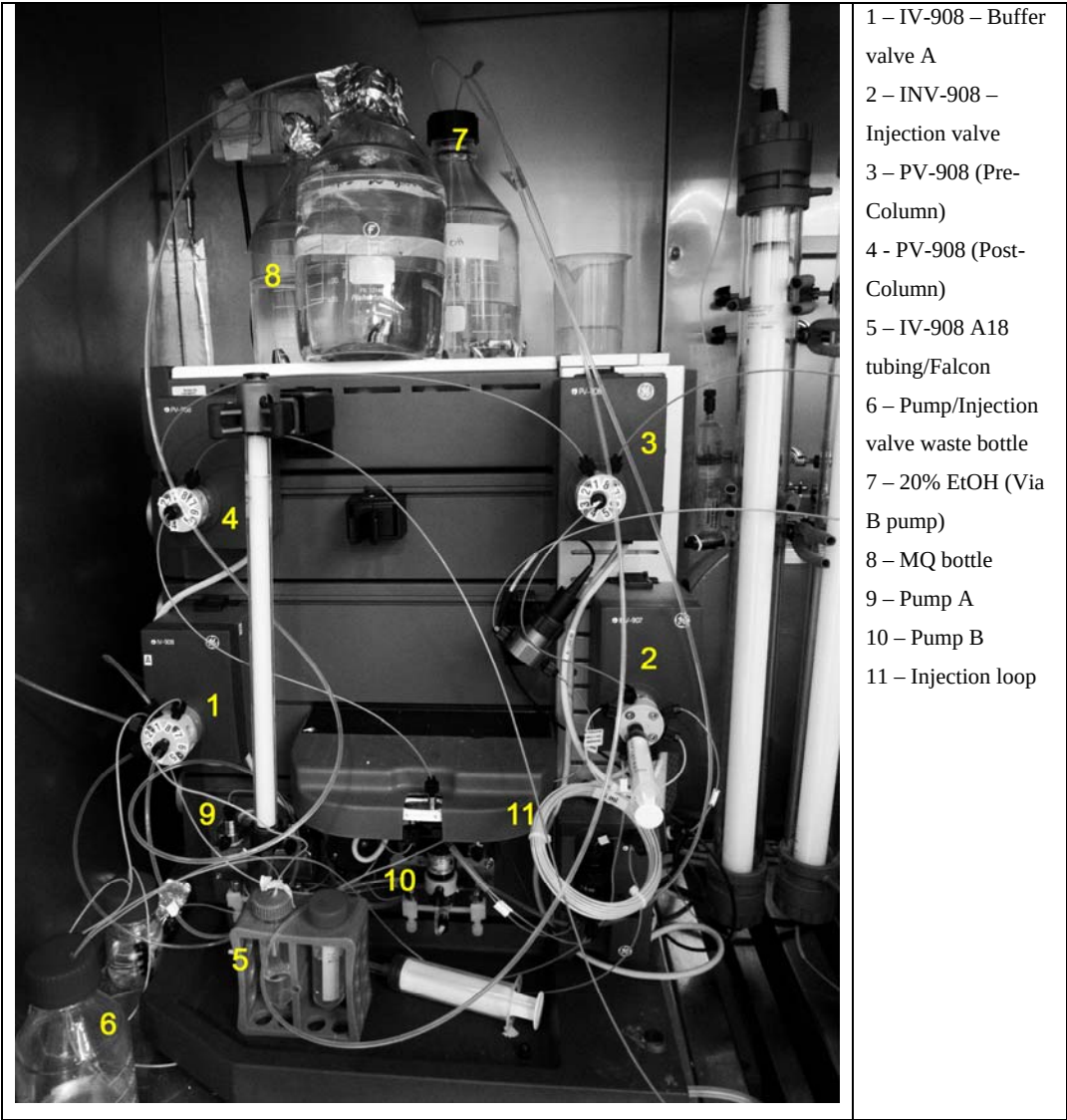
1. Connect loop via ports 3 and 6 on INV-908.
2. Wash loop with MQ, at least x2 loop volume.
3. Repeat step 2 with protein's buffer.
4. Connect needle to appropriate volume syringe and wash needle with one syringe volume of MQ and one syringe volume of protein's buffer.
5. Draw protein sample into syringe and remove any bubble formed.

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 and that the injection valve is set to “load”.

6. If using filter, connect filter to syringe and connect the filter nozzle to the injection port.
7. Inject your sample at half the loop volume and set injection status to “Inject”.
8. Wait at least x2 loop volume and then re-set injection valve to “Load”.
9. Monitor progression and collect samples.

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Appendix A – AKTA purifier customized system scheme



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Appendix B – Default tubing connections

Pump A1	Pump B1
A11 – MQ	B11 – Free
A12 – Free	B12 – EtOH 20%
A13 – NaOH	
A14-A18 – Free	

Appendix C – Columns selection⁴

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

⁴ Zarivach lab, Jan 2010