**Gel Filtration using the old FPLC**

*Equilibrate FPLC*

A. Wash Pump

1. Wash Pump. Do this whenever you put the lines into a new buffer. This step is to make sure the line(s) are filled with your buffer before running buffer through the column.

* Click Manual > Pump > Pump Wash Basic > Pump A\*> Execute.
* Run will take approximately ~2 minutes.
* Check FPLC to see if wash is working. Screen on the FPLC will say “Washing Please Wait…” and the machine will be making noise.

1. When wash pump is finished, click END.

B. Wash Column

* 1. Put line in waste: 50 mL Falcon Tube in the center of the fraction collector
  2. File > Run > DFs200a > OK

Click Show Details box.

* Change Pressure: 1.5 → 2.2 mPa
* Equilibrate w/ CV: 1 → 0
* Empty Loop w/ mL: 1→ 0
* Eluate Fraction Size: 1→ 0

1. Click Next 4 times until you hit screen that asks you to rename sample. Name the run with the following format: Initials-Column Name- Sample (wash in this case) and date (Month, Date, Year). Use only alpha numerics as the software doesn’t allow for symbols.
   1. Instrument will yell at you about the pressure limit, Just select “Clear All”.
   2. Check pressure on front of the machine. It should read about ~1.7mPa. If it is higher, call Daniela.
2. Wash Loop
3. Take 2 x 3 mL syringes (to wash the 2mL loop twice) and 1 x 1 mL syringe and 1 needle (for the sample).
   1. Note, if using a smaller loop, you can run less buffer through it. As a rule of thumb, wash with at least 2x the loop volume.
4. Open and loosen up syringe by pushing plunger up and down.
5. Aliquot FPLC buffer in falcon tube and draw up ~2.5 mL of buffer into both of the 3 mL syringes. Remove all large air bubbles from syringe by turning it upside down. Hold the plunger and tap on the syringe to remove air bubbles.
6. Screw 3 mL syringe onto adapter on the FPLC machine and slowly push in buffer. Do not push all the way if there were air bubbles at the bottom of the syringe. Adding air bubbles to the FPLC machine will ruin the column. Repeat with the second 3 mL syringe of buffer.

*Running the FPLC with Sample*

1. Loading Sample and Setting up a Run

1. Take line out of the waste Falcon tube and put line into the 1st capless tube.

2. Lift arm up and push out to the side, away from fraction tray. Take the tray out and fill

first 25 glass tubes with plastic capless tubes (located in back of lab in bottom right hand

cabinet).

* Note: if running a large column (like the s200prep, then you would have to fill the entire fraction collector with capless tubes).

3. Place fraction tray back in and align arm to put line in first plastic capless tube.

4. Screw needle on 1 mL syringe and uncap. Draw up sample slowly. Put cap on and unscrew

the needle, discarding the needle in designated needles waste bin.

1. Screw the syringe contained the sample to the FPLC and push slowly. Adding air bubbles to the FPLC machine will ruin the column.
2. Run Sample.

* Click File > Run > DFs200a > OK
* Click Show Details and change the following settings:
  + - 1. Pressure: 1.5 → 2.2 mPa
      2. Equilibrate w/ CV: 1 → 0
      3. Empty Loop with mL: 4 → 5 mL
      4. Eluate Fraction Size: 0 → 1

1. Click Next 4 times until hit screen asking you to rename the run. Rename sample with the following format: Initials-Column Name- Sample (wash in this case) and date (Month, Date, Year). Use only alpha numerics as the software doesn’t allow for symbols.
2. Machine will “yell” at you and notify you of changes to pressure settings. Click Clear All.