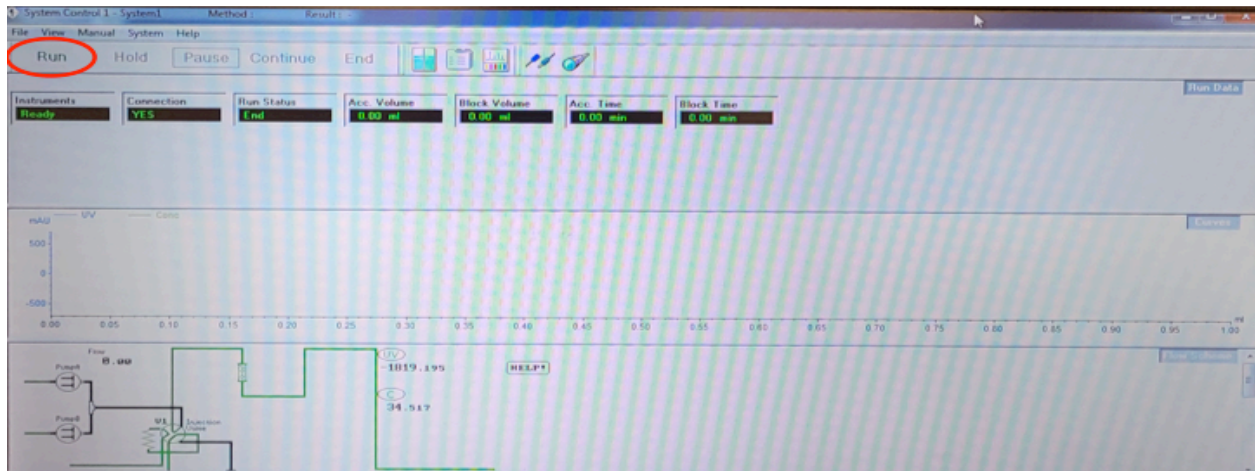
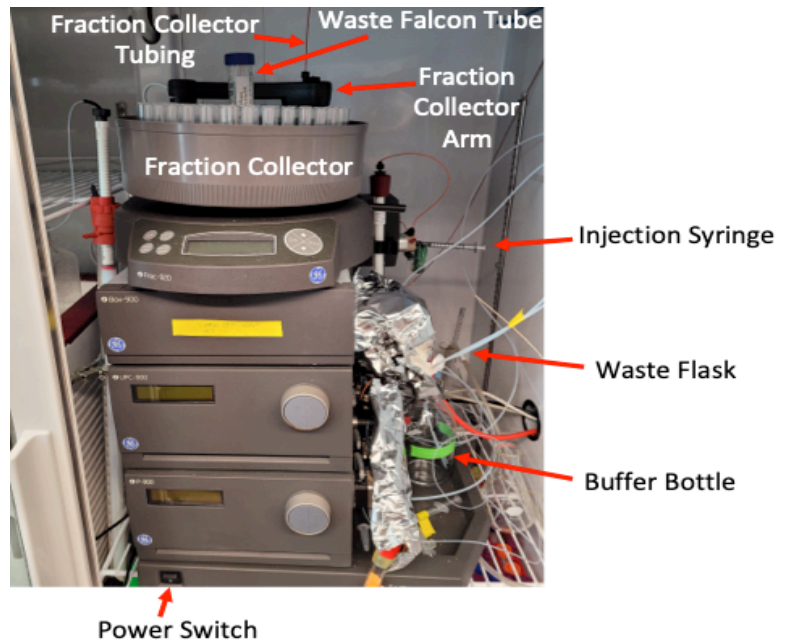


Gel Filtration using the old AKTA Purifier FPLC

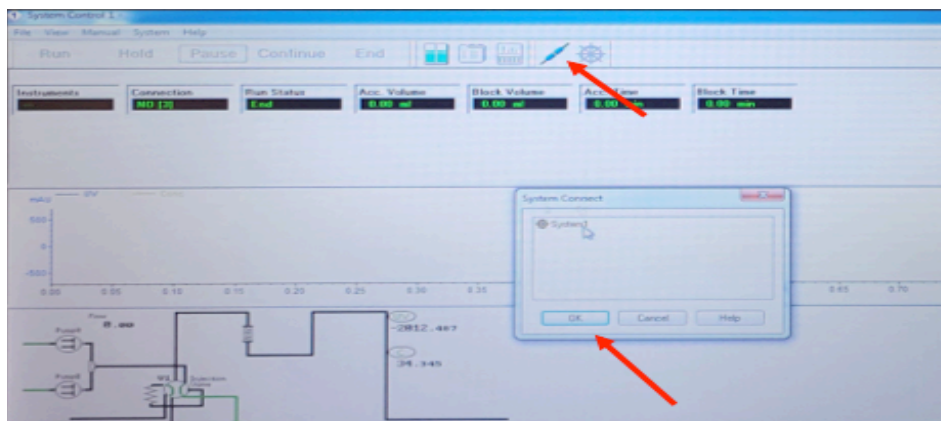
Turn on the FPLC. The power switch for the FPLC is located on the bottom left of the front of the instrument.

If the software is not open, double click the Unicorn 5.31 icon found on the Desktop. Several windows should open (System Control, Method Editor, and Evaluation windows, which will likely be useful for your run).

If the system is connected, the Run button (circled in red below) will be clickable. If it's not connected, it will be grayed out.



If the instrument is not connected, select the plug icon at the top of the screen, shown with a red arrow below. Select System 1 and click OK.



If it won't connect, you will have to restart the FPLC and software.

If you need help, please get Daniela.

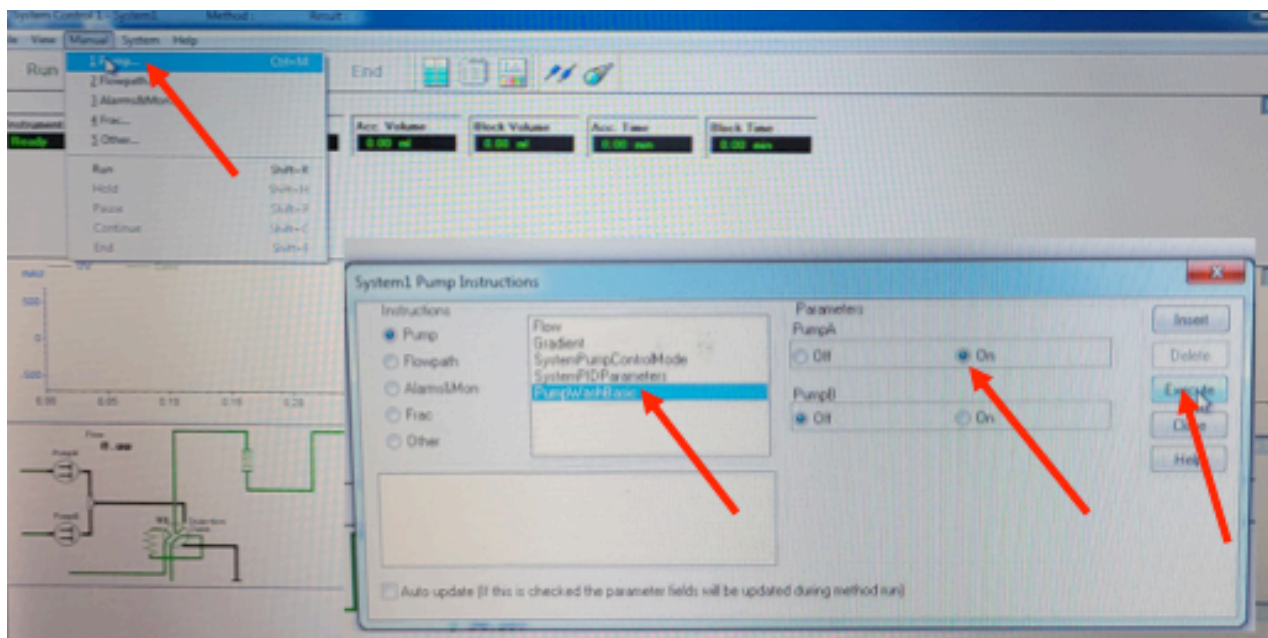
Updated 05/11/21

Before you start, check the waste bottle or flask to make sure it is not full. If it is full, or close to full, empty it and put the tubing back in.

Always Equilibrate the FPLC before use with the buffers you will run your sample in. You will need to wash the pumps and column for this purpose:

A. Wash Pump

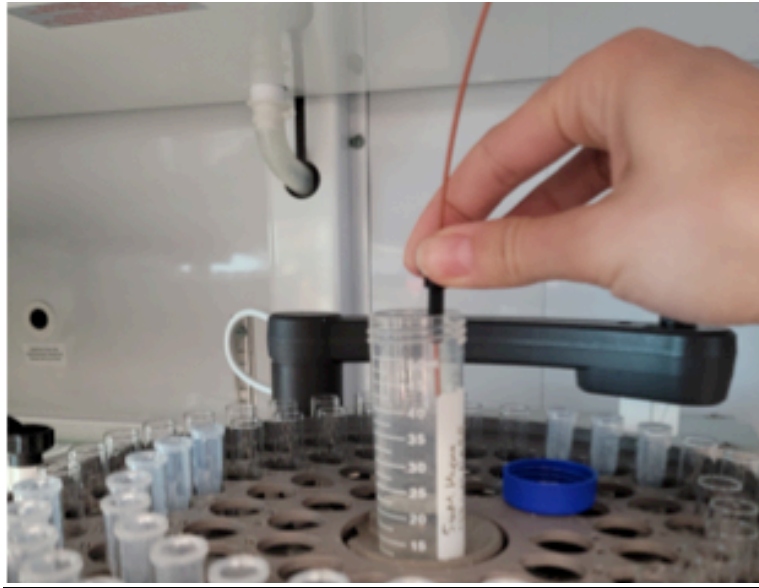
1. Wash Pump. Do this whenever you put the lines into a new buffer. This step is to make sure the line(s) are filled with your buffer before running buffer through the column.
 - ❖ Click Manual > Pump. This opens up a new window (shown in the figure below).
 - ❖ Highlight Pump Wash Basic > Pump A* > 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.
2. When wash pump is finished, click END.



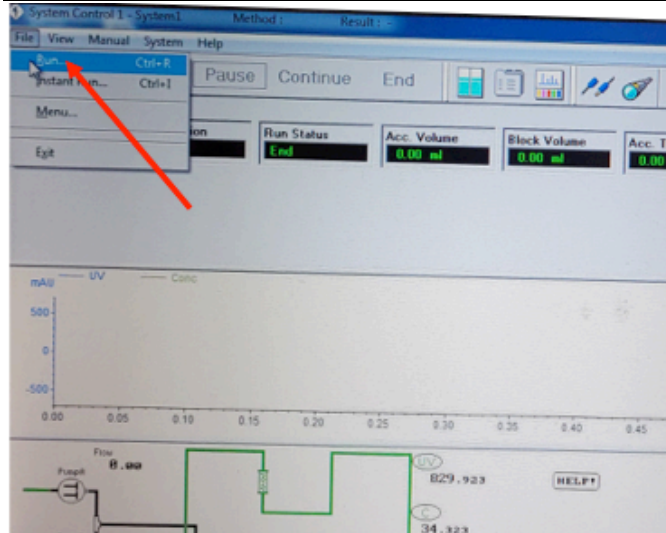
Updated 05/11/21

B. Wash Column

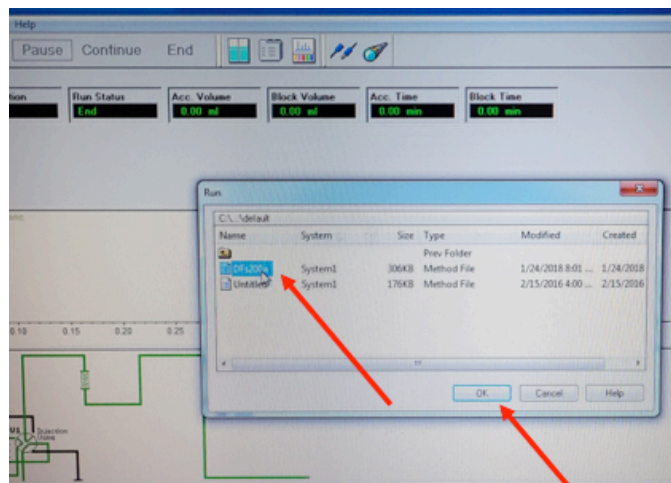
1. Manually put fraction collector line in a 50 mL Falcon Tube in the center of the fraction collector. This will act as waste for the run.



2. File > Run >



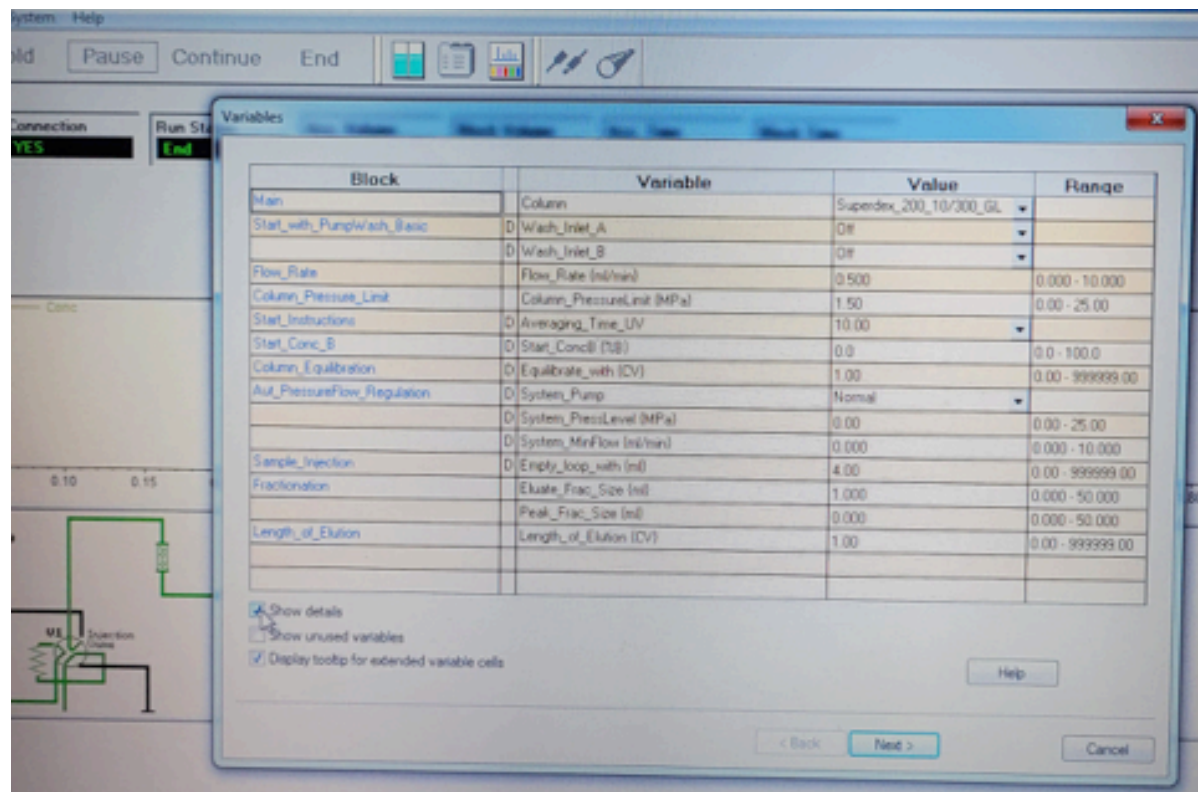
DFs200a > OK



Updated 05/11/21

3. Click Show Details box. A window like the one shown below will pop-up.

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



4. 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.
- a. Instrument will yell at you about the pressure limit, Just select "Clear All".
 - b. Check pressure on front of the machine. It should read about ~1.7mPa. If it is higher, call Daniela.

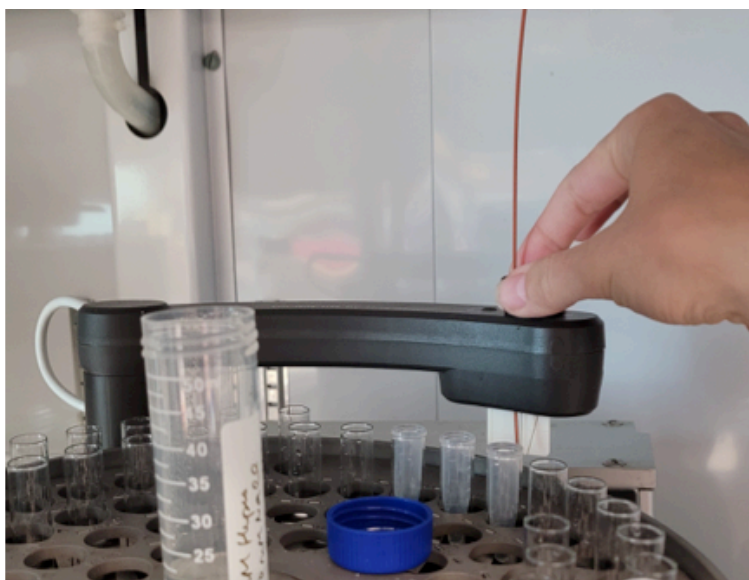
C. Wash Sample Loop

1. Take two 3 mL syringes (to wash the 2mL loop twice with buffer).
 - a. 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.
2. Loosen up syringe by pushing plunger up and down.
3. 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 so the open side is face up. Hold the plunger and tap on the syringe to push air bubbles to the top and then push the plunger to expel them from the syringe.
4. Make sure there is a positive meniscus of buffer on the syringe tip and then screw the syringe onto the adapter on the FPLC machine. Slowly push in buffer but 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.

D. Prepare the fraction collector

You will need to take the fraction collector out to fill it with capless tubes.

1. First, take the line out of the waste Falcon tube and put it back into the arm of the fraction collector



2. Then lift the arm up and push it out to the side, away from fraction tray.
3. Push the rotor on the back left part of the fraction collector away from the wheel (do this whenever you turn the wheel, take it out, or put it back in) and take out the wheel.
4. Fill the fraction collector with at least 27 capless tubes (located in back of lab in bottom right hand cabinet).
5. Push the rotor on the back left part of the fraction collector away again and put the fraction collector back in.
6. Align the first tube so that it is centered against the vertical line below the fraction collector tubing.

E. Run your Sample Through the Column

1. Take a 1mL syringe (or larger if you have more than 1mL of sample) and connect a needle to it.
2. Loosen up syringe by pushing plunger up and down.
3. Using the same hand you are using to hold the syringe, push the needle cap off.
4. Insert the needle into your protein solution (which you should have spun down for 10 minutes at 14,000 RPM in the cold room) and draw it up slowly, being careful to not draw up any visible precipitate at the bottom of the tube.
5. Cap the needle and take it off. Discard the needle in the sharps container on the bench across from the FPLC.
6. Hold the plunger with the tip face up and tap on the syringe to push air bubbles to the top. Push the plunger to expel air bubbles from the syringe.
7. Make sure there is a positive meniscus of protein sample on the syringe tip and then screw the syringe onto the adapter on the FPLC machine. Slowly push in the solution but 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.
8. Now you are ready to run the sample.
 - a. Click File > Run > DFs200a > OK (similar to part B, step 2 above)
 - b. Click Show Details and change the following settings (different from part B above):
 - Pressure: 1.5 → 2.2 mPa
 - Equilibrate w/ CV: 1 → 0
 - Empty Loop with mL: 4 → 5 mL
 - Eluate Fraction Size: 0 → 1
9. 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.
10. Machine will "yell" at you and notify you of changes to pressure settings. Click Clear All.
 - a. Check pressure on the computer screen; keep track of this in your notebook as you use the same column. If you ever notice a drastic increase in pressure, let Daniela know as the system filters may need de-clogging.

When you are finished, turn the power switch off on the FPLC or else the UV light will burn out!