AKTA FPLC Instructions for Size Exclusion Chromatography – New FPLC (on left)

If the software is not open, double click the Unicorn 7.1 icon found on the Desktop. Make sure all the boxes are checked before clicking OK. Several windows should open (System Control, Method Editor, and Evaluation windows, which will likely be useful for your run). If the instrument is not connected, select System at the top of the menu, connect systems, and select the system before proceeding. If it won't connect, you will have to restart the FPLC and software. The on/off switch for the FPLC is located on the back right of the instrument. Be careful to not hit the large column if reaching for it. If you need help, please get Daniela.

Before you start, check the waste bottles to make sure they are not full. If they are full, or close to full, empty them 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. <u>**Wash Pump**</u>
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 > Execute Manual Instructions > Pumps > Pump A wash > On > Execute
     - ○ If doing ion exchange (IEX), you would click "insert" instead of Execute after selecting On for Pump A and then click insert after selecting On for Pump B. Once the two items are listed in the window on the right, you can click Execute.
   - ❖ Run will take approximately ~2 minutes (4 minutes if washing two pumps).
   - ❖ Check FPLC to see if wash is working -- the machine will be making noise.
2. When wash pump is finished, close the window and hit the "stop button" on the System Control Window.

B. Wash Column
1. File > Open > select file you will run > OK
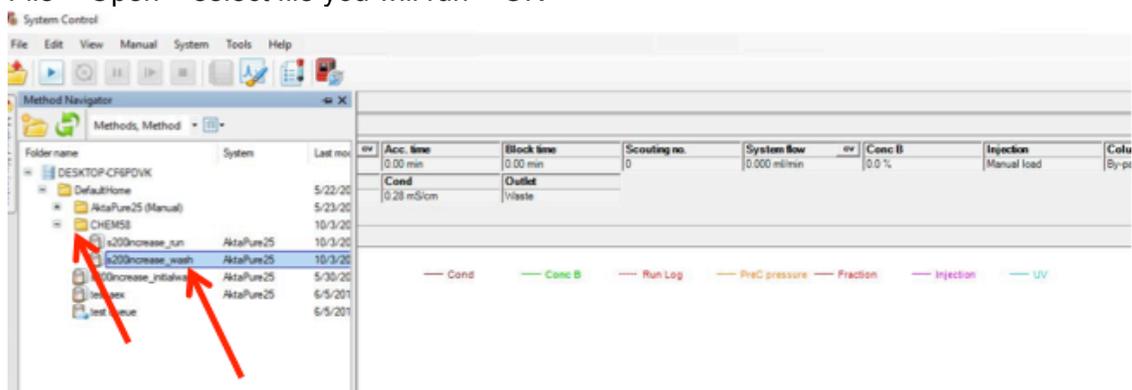
2. Expand details. Check the following (and adjust as needed):
   - equilibration volume (CV) is set to 1.0
   - empty loop with mL is set to 0.0
   - frac volume elution (mL) is set to 0.0
   - isocratic elution length (CV) is set to 0.0
3. Click Next until you hit screen that asks you to rename sample. Then start the run.
   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, please let Daniela know as the system filters may need de-clogging.
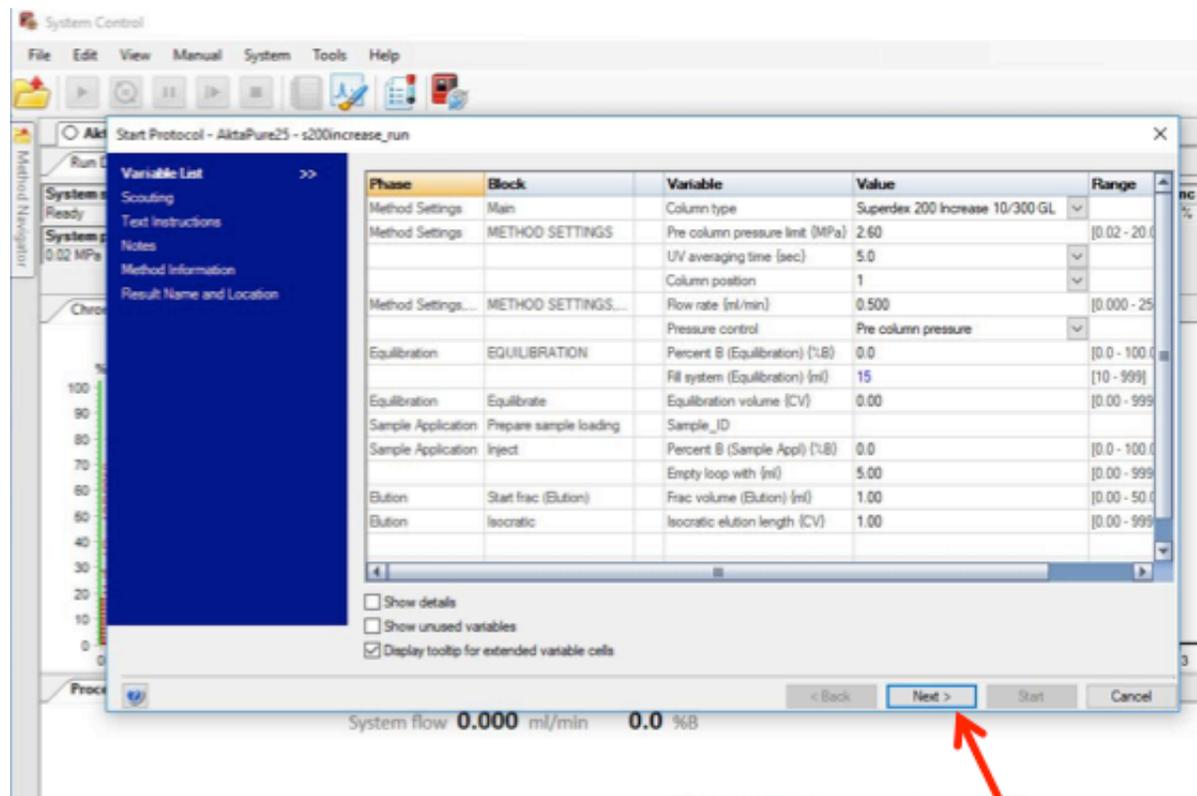
Before adding your sample, you want to make sure that your buffer of interest is in the sample loop:

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 (located in back of lab in bottom right hand cabinet).
   1. To do this, first lift the arm up and push it out to the side, away from fraction tray.
   2. Then 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.
   3. Fill the fraction collector with at least 25 capless tubes if using one of the smaller gel filtration columns or fill the entire fraction collector if doing ion exchange chromatography or running the large s200prep column.
   4. Push the rotor on the back left part of the fraction collector away again and put the fraction collector back in.
   5. 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. File > Open > select file you will run > OK (similar to part B above)

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9. Expand details. Check the following (and adjust as needed):

    - equilibration volume (CV) is set to 0.0
    - empty loop with mL is set to 4.0 (for a 1mL loop or 5.0 for a 2mL loop)
    - frac volume elution (mL) is set to 1.0 for small column or IEX; 1.4 for s200prep
    - isocratic elution length (CV) is set to 1.0

10. Click Next until you hit screen that asks you to rename sample. Name the run with the following format: Initials-Column Name- Sample and date (Month, Date, Year). Use only alpha numerics as the software doesn't allow for symbols.

    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.
    b. To stop the fraction collector (once desired protein has eluted), Click Manual > Execute Manual Instructions > Fraction Collection > Fractionation > change to 0.0 mL. Then wait until the drop start going to waste before removing the fraction collector wheel (it could take a minute).