Some starting precautions:

1. Vacuum filter all buffers.
   - Removes any large particles/debris that may clog your column
   - De-gases the buffers
2. Clarify lysates first by centrifugation and then filtration using a 0.22µm membrane
   - Removes any large particles/debris that may clog your column
3. Be aware of the limits of your respective column!
   - Parameters to be aware of
     - Pressure limits
     - Flow rates
     - Chemical compatibility
     - Column volume
4. All connections must be finger tight only! Over tightening connections will break the connectors or portions of the instrument.
5. The system is stored in 20% ethanol. Make certain that you flush the system with water before working with buffers or proteins.

Getting Started:

1. Turn on the Akta primeplus. The switch is located at the back of the instrument on the left hand side.
2. Turn on the computer and log into Windows
3. Launch the Primeview software
   - Beware! Don’t launch the Primeview evaluation software.
   - You cannot control the instrument from the software. All user commands are inputted through the interface on the front of the instrument itself.
Flushing the Lines:

1. Place a black cap onto the top of a bottle of filtered MilliQ water or water and insert lines A1 and B through the cap and into the water or buffer as appropriate.
   - **Flush the lines any time that you transfer the lines from one bottle to another. This will prevent any air from reaching your column and damaging it.**
2. Make certain that there is a small bubble in the very end of each line. You can trace the path of the bubbles through the lines to confirm that the lines were primed correctly.
3. Make certain that the ends of each line touch the bottom of the bottle and do not curve up the sides of the bottles.
4. Use the up and down arrows to scroll through the menu until you reach “templates”
5. Scroll to application template
6. Scroll to system wash method
7. At this point, you can select the line(s) you will be using and flush all of the lines at once. The default is lines A1 and B so we won’t change anything. Use the up/down arrows to scroll over to OK then press the OK button.
8. The message “PRESS OK TO START RUN” will be displayed on the LCD screen on the instrument. Press OK and the system will start.
   - The system may prompt you to check tube position. If it does so, press OK to start the run and pause/cont. to begin.
9. The system will run at 50 mL/min for a total of 75 mL for two lines. As the method progresses make certain that the bubbles are pushed all the way through the lines. You may have to flick the lines to dislodge any bubble that may be clinging to the interior of the lines.
10. Once the volume has reached 75 mL, the flow rate will drop to 0.5 mL/min. you can end the system wash at this point.
11. Press end. The system will display the message “END RUN? YES NO”. Make certain that the underscore is below the word YES and press OK
12. The system will then display the message” MEMORY PRINT OUT? YES NO”. Make certain that the underscore is below the word No and press OK. **NEVER** select yes for the memory print out.
Attaching the column and washing the ethanol from the column:

1. Flush the lines with water (See step 4).
2. Once the lines have been washed with water, you will need to start a manual run before attaching your column to the system.
   - When attaching or removing a column **ALWAYS** have water or buffer moving through the system.
3. At this point the screen should display TEMPLATES, use the up/down arrows to scroll through the menu until you reach the option that says MANUAL RUN. Press OK.
4. You are now at a point where you can begin to enter the run parameters.
   - Method base: mL
   - Flow rate: 1.0 mL/minute
   - Pressure limit: column pressure limit
5. Scroll down to START RUN and then press OK.
   - Monitor the system back pressure without the column first for several minutes before attaching the column. It may be necessary to set the pressure limit higher than the column limit due to system back pressure.
6. Unscrew the connectors from the UV detector. Either of the top two connectors will be appropriate to attach the tubing from the injection valve to your column. Open the top of your column and allow water to drip into the opening then attach the connector to the top of the column. Do not tighten completely.
7. Once the top has been loosely attached to the connector, remove the bottom cap and attach the column to the bottom connector and tighten firmly. Do not attach the column directly to the detector. The plastic encasing the resin is soft and can break off inside the detector.
8. Tighten the top connector and wipe away excess water. The idea is to have every connection to be finger tight but not leaking.
9. Allow approximately 1 column volume (CV) to flow the column at a flow rate of 1 mL per minute then using the control panel on the front of the instrument scroll until you see the prompt for flow rate and increase the flow rate. Allow water to flow through the column for 5 – 10 column volumes.
10. Once you have washed the column with water end the manual run.
Equilibrating the column into Buffer:

1. Transfer Line A1 into Buffer A and Line B into Buffer B
2. Flush the lines with buffer
   - Make certain that there is a small bubble in the very end of each line. You can trace the path of the bubbles through the lines to confirm that the lines were primed correctly
   - Make certain that the ends of each line touch the bottom of the bottle and do not curve up the sides of the bottles.
3. At this point the screen should display TEMPLATES, use the up/down arrows to scroll through the menu until you reach the option that says manual run. Press OK.
4. You are now at a point where you can begin to enter the run parameters.
   a. Method base: mL
   b. Flow rate: 1.0 mL/minute
   c. Pressure limit: column pressure limit
5. Scroll down to start run and then press OK.
6. Allow approximately 1 column volume to flow the column at a flow rate of 1 mL per minute then using the control panel on the front of the instrument scroll until you see the prompt for flow rate and increase the flow rate. Allow buffer to flow through the column for 5 – 10 column volumes. Once you have a stable baseline and while the column is still equilibrating, use the up/down arrows to scroll to AUTOZERO UV and press OK.

Preparing your sample loop/super loop:

This can be done while the column is being equilibrated.

Sample Loop:

1. Flush the sample loop first with water then with Buffer A.
2. Attach one end of the loop to port #6 and the other to port #2. You can identify the ports by the numbers etched on the metal ring of the injection valve. Alternatively, port #6 has been marked on the front of the injection valve with a T as shown in the image below and port #2 is directly opposite port #6.
3. Once the sample loop has been attached it is a good idea to inject buffer through the loop to confirm that there are no leaks.
4. Scroll down and decrease your flow rate to 1 mL/minute.
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5. Scroll down until you see the message SET INJECT VALVE POSITION (LOAD). Press OK.
6. Use the up/down arrows to move the underscore from beneath the word LOAD to beneath INJ. Then press OK.
7. This changes the system from loading to injection which puts the sample loop in line with the column. The system will begin to push buffer through the sample loop. Check carefully to be certain that there are no apparent leaks. If any connector is leaking adjust/tighten the connectors until the leaking stops.
8. Once you have allowed several milliliters to be injected onto the column. Use the up/down arrows until you see the message SET INJECT VALVE POSITION (INJ). Press OK.
9. Use the up/down arrows to move the underscore from beneath Inj to beneath LOAD. Then press OK.
10. This changes the system from injection to load and bypasses the sample loop.

Super Loop:

1. The super loop is used to inject larger volumes onto the column.
2. Carefully remove one of the black caps of the super loop and set aside. Slide off the plastic outer casing. Hold tightly to the glass super loop and remove the white plastic plug from the end of the loop. Allow the water to drain from the end of the super loop.
   - To improve your grip on the glass portion of the super loop, hold the loop with a dry paper towel.
3. Remove the remaining black cap and white plug from the opposing end of the super loop. Again allowing the water to drain from the super loop.
4. Rinse each side of the super loop and the white caps with water 3 times.
5. Rinse each side of the super loop with Buffer A 3 times.
6. Fill each end of the super loop with Buffer A and replace the white plastic plugs.
7. Attach a black plastic cap to one end of the super loop and insert the glass super loop into the plastic outer casing.
8. Attach the remaining black plastic cap to the super loop and make sure that both caps are firmly tightened.
9. Insert the super loop into one of the clips attached to the side of the Akta.
10. Obtain two lines with adaptors suitable to attach to the super loop and using a syringe without a needle flush the lines first with water then with buffer A.
11. Attach one line to the top of the super loop and from there to port #6.
12. Attach the second line to the bottom of the super loop and from there to port #2.
   a. You can identify the ports by the numbers etched on the metal ring of the injection valve. Alternatively, port #6 has been marked on the front of the injection valve with a T as shown in the image above and port #2 is directly opposite port #6.
13. Once the sample loop has been attached it is a good idea to inject the buffer contained in the lower portion of the super loop onto the column to confirm that there are no leaks and to speed things along.
14. Scroll down and decrease your flow rate to 1 mL/minute.
15. Scroll down until you see the message SET INJECT VALVE POSITION (LOAD). Press OK.
16. Use the up/down arrows to move the underscore from beneath the word LOAD to beneath INJ. Then press OK.
17. This changes the system from loading to injection which puts the super loop in line with the column. The system will begin to push buffer into the upper portion of the super loop causing the plunger to descend. Check carefully to be certain that there are no apparent leaks. If any connector is leaking adjust/tighten the connectors until the leaking stops.
18. Allow all of the buffer contained in the lower portion of the super loop to be injected onto the column. Halt the injection when the small bubbles contained in the super loop just begin to compress.
19. Use the up/down arrows until you see the message SET INJECT VALVE POSITION (INJ). Press OK.
20. Use the up/down arrows to move the underscore from beneath INJ to beneath LOAD. Then press OK.
21. This changes the system from injection to load and bypasses the sample loop.
22. Once the column has finished equilibrating end the manual run.

Loading your sample into the Sample/Super Loop and Injecting onto the Column:

1. Start a manual run and set the following parameters
   - Method base: min
   - Flow rate: 1.0 mL/minute
   - Pressure limit: column pressure limit
2. Load your sample into the sample/super loop by first loading it into a luer lock syringe and then attaching the syringe to the connector on the face of the injection valve. Slowly depress the plunger to push the sample from the syringe into the loop.
   - Make sure that there are NO bubbles in the syringe before you attach the syringe to the connector.
   - The largest syringe volume you can use is 25 mL. If you are loading a larger volume into a super loop, you can iteratively fill the syringe and load into the super loop.
3. Change the injection valve position from load to inject. Stop the injection before any bubbles reach the injection valve.

4. Wash the column at 1 mL/minute until UV trace drops to approximately 1200 mAU. Increase the flow rate and continue to wash the column until the UV trace reaches baseline.

5. You can then elute your protein in a number of different ways.
   a. Gradient elution
   b. Step elution
   c. Isocratic elution

**Setting a gradient elution**

1. Using the control panel scroll down to set gradient. Press OK and set the following parameters
   a. Gradient length: 60 minutes
   b. Set Target: 100% buffer B

   Once the parameters are set, press OK. The system will begin a gradient elution. This is a good starting point but the parameters can be altered to improve the resolution of the elution.

2. Scroll down to set fraction size. Press OK. Set fraction size to 1.0 mL. The fraction collector will begin to collect 1 mL fractions.

**Setting a Step elution**

1. Using the control panel scroll down to Set Concentration %B (0%B). Press OK. Use the up/down arrows to set the desired percentage of buffer B and then press OK.
   a. Each individual step will need to be adjusted in this manner.

2. Scroll down to set fraction size. Press OK. Set fraction size to 1.0 mL. The fraction collector will begin to collect 1 mL fractions.
1. Sample is pushed from a Luer Lock syringe and into the injection valve

2. Sample is pushed from the injection valve into the bottom of the sample loop

3. Buffer is pushed from the top of the sample loop to waste

**LOADING** into the sample loop

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1. Buffer is pushed into the top of the sample loop

2. Sample is pushed from the sample loop and into the injection valve

3. Sample is pushed from the injection valve and onto the column

**INJECTING** onto the column
Saving Your Chromatogram

The Akta system by default saves your chromatogram data as Manual run 1, 2, 3 and so on. It is recommended that you set up a file to save the data from individual purifications. If you have already created a folder for your chromatogram data you can ignore the next several steps.

- Open Primeview Evaluation
- Click file (in the upper left hand corner)
- The open result window will open
- Right click inside the open result window and a small menu will pop up
- Click on New Folder
- Name your folder.

To save the file make note of the manual run number. It will be displayed at the top of the screen in Primeview.

- Open Primeview Evaluation
- Click file (in the upper left hand corner)
- The open result window will open
- Scroll down until you see a folder named Manual Runs (prime)
- Double click to open the folder and locate the manual run that you want to save
- Double click on the file name to open the file and you will see your chromatogram displayed on the screen
- Go to file
- Click on save as and the save as window will open
- Navigate through the files until you reach your folder and double click on the folder
- Re-name your chromatogram and save your chromatogram to your folder.