

AKTA pure 25 New Owner's Intro

The exercise below will give a quick demonstration of how easy and intuitive the AKTA pure 25 will be for you in demonstrating downstream processing to your students.

Steps include:

- Opening the Unicorn software windows for manual control and programmable features
- Purging/priming pumps and tubing
- Loading a sample loop prior to injection on the column
- Editing a method
- Running a method

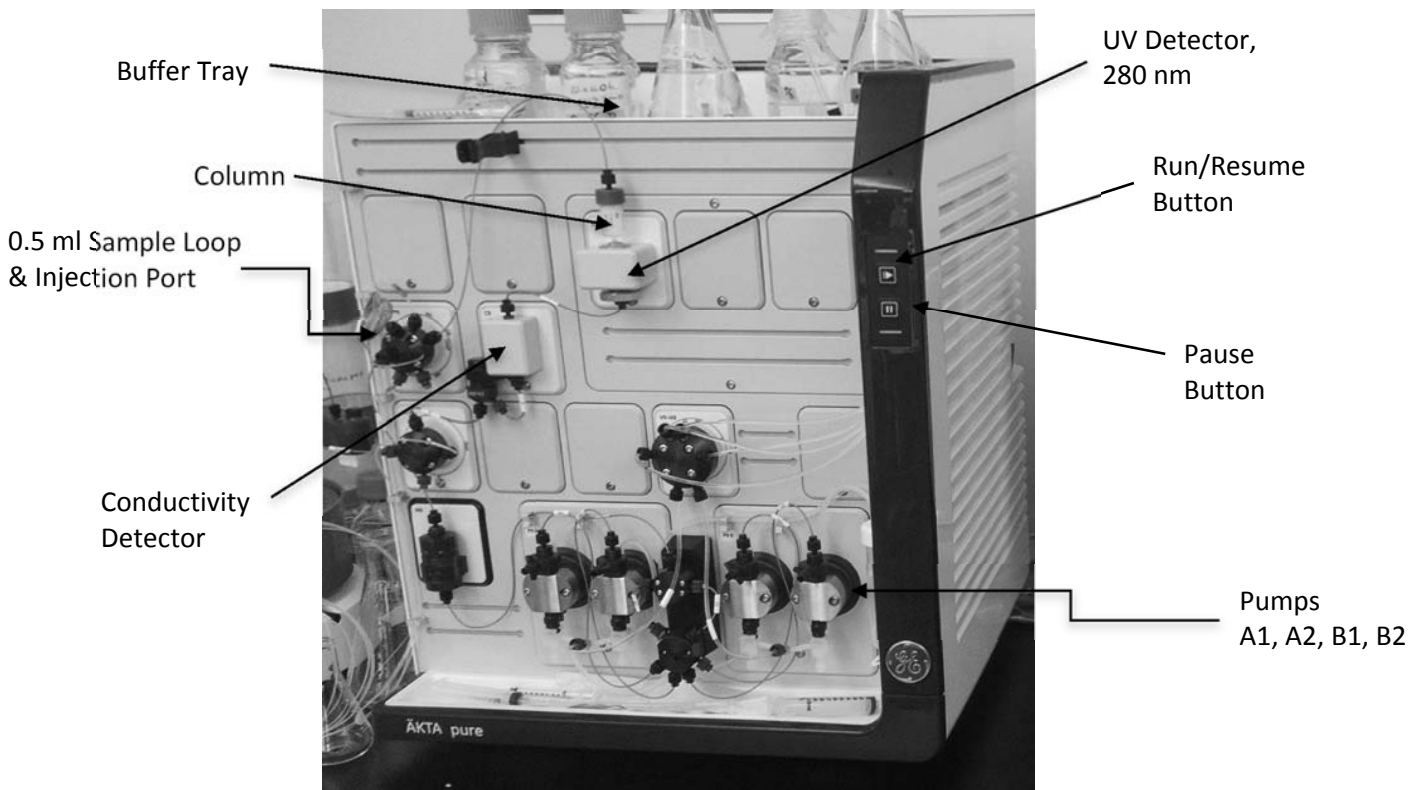
For this intro, a length of (restricted flow) tubing will be used in place of the column. You will also need filtered degassed deionized water or buffers, and a solution of 0.1% acetone in water (the sample).

Definitions:

Manual Load - position of the injection valve that allows the user to load the sample loop with sample by depressing the plunger of inserted syringe full of sample (commonly referred to as injecting, but in this context, injection has a different meaning; see below).

Injection - position of the injection valve that puts the sample loop in the flow path of mobile phase between the pumps and the column, such that the loaded sample flows from the sample loop onto/through the column and downstream path. The amount of sample that is injected onto the column can be specified in the method by editing prior to starting a chromatographic procedure.

Take a moment to identify the labeled components of the system:



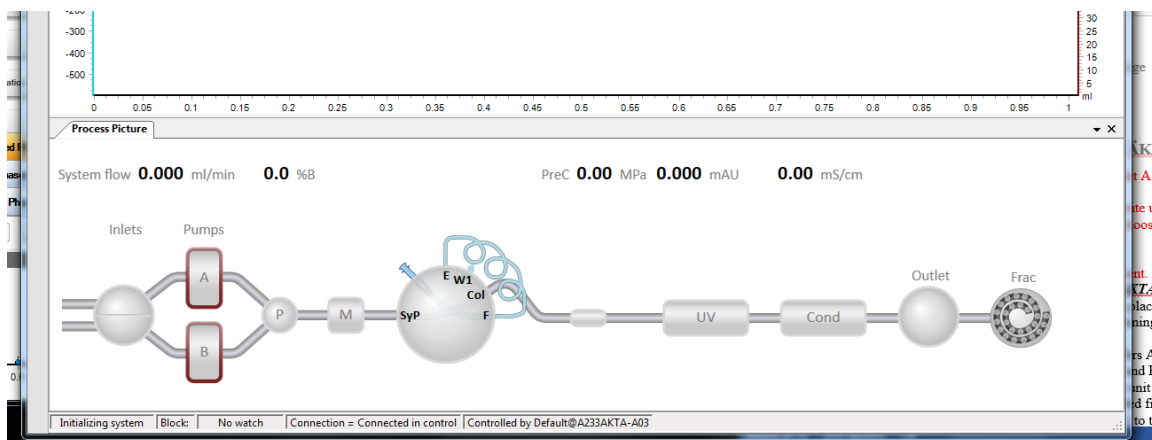
What to do with your new AKTA pure 25.

Great news - you have a brand new AKTApure automated chromatography instrument in your biomanufacturing lab!! Here is a brief introduction that will get you comfortable using most of the important functions of the instrument and allow you to successfully separate proteins in short order.

First the most important rule: never let air into the flow path. Running the pumps while dry will almost certainly damage them. Air space in the column can adversely affect resolution. Air in the us detector may result in uninterpretable A280 traces on the chromatogram. As with any liquid chromatography system air or gas in the system is the enemy, so make sure each of the inlet tubes (which deliver buffer to each of the pumps) is securely resting on the bottom of a buffer container (flask, bottle, etc).

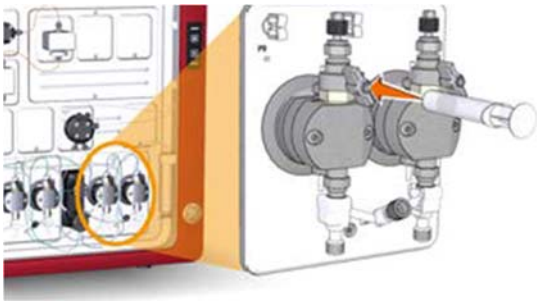
1. Power up. Now you're ready to turn on the instrument (switch is on the right side, toward the rear), and the computer, if it's not already on.

2. Boot up Unicorn and get to know the manual System Controls. Open the Unicorn software windows: System Control, Method Editor, Evaluation, and Administration. Bring the System Control window to the forefront. If it is blank, go to the menu **System** and select **System Connect**. Place a check by the name of the instrument and click OK. You should now see the schematic of the flow path across the bottom of the screen, as shown here:



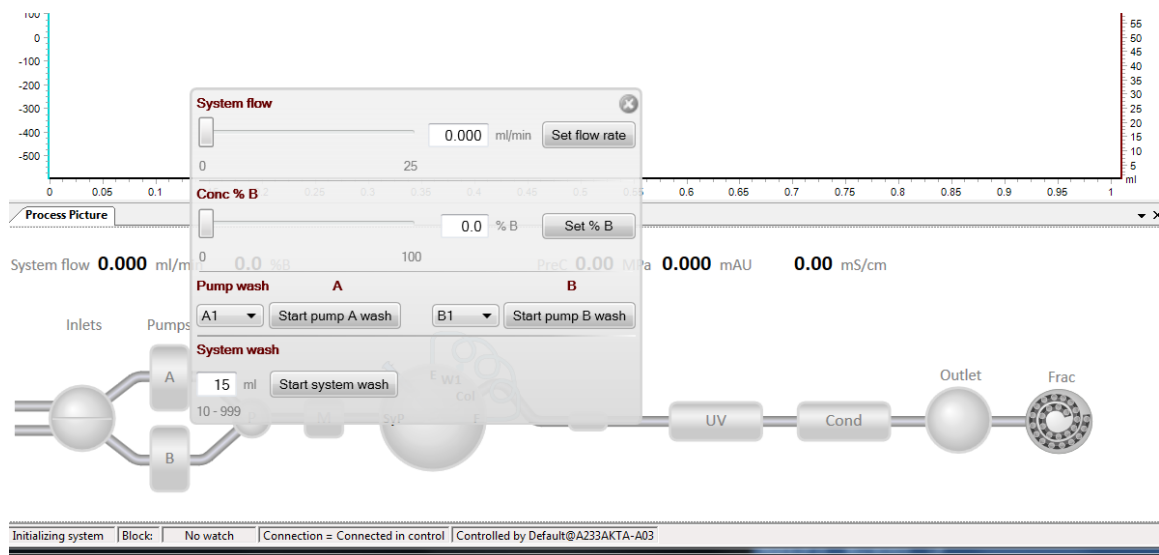
Now, if you click on each component you will see functions or parameters that you can change manually (via the software). For example, clicking the Inlets depicted (on the left side) allows you to switch the A inlet line and pumps between A1 and A2. Similarly you can switch between B1 and B2 .

If the system is equipped with a pH detector, it will be shown between the Cond (conductivity) and Outlet positions. Click it and select the option to take it out of the flow path.



Starting with B1, attach a 10 ml syringe to the pump priming fitting and open the valve one full turn or more until you are easily able to withdraw 10 ml of liquid, which will insure the line is full of liquid and any bubbles are out of the line. Repeat these steps to prime every pump. Note that you will need to use the System Control window to manually switch A1 to A2 and B1 to B2.

Clicking either of the depicted Pumps brings up a very useful menu that allows you to set the flow rate and buffer mix (expressed at %B), as well as quickly flushing the pumps and flow path up to the injection valve. Give it a try now. Use the mouse cursor to move the sliders then click the "Set" button to enter the new settings. *CAUTION [Changes take effect immediately upon clicking Set; if a column is installed and the flow rate is high enough, pressure will quickly rise, possibly damaging the column- so be careful here. This is less of an issue when running a programmed method, which will have pressure limits set to protect the column.]*



3. Wash the pumps. After confirming that you have enough mobile phase (i.e. buffer, water, cleaning solution or storage solution) in each reservoir, open the Pump manual control menu. Pumps should be washed in the order B2, B1, A2 and A1. Select **B2** from the dropdown menu in the **Pump wash** area of the dialog box, then choose the 'Wash Pump B' option. You will hear the injection valve reposition, sending liquid to waste, and the exhilarating sound of the pump working at high speed. This will quickly fill the pump and flow path up to the injection valve, then eluent will go to waste. The wash step stops automatically after one minute. (This operation will not increase pressure on the column, if installed). Repeat with pump B1, A2 and A1.

What you have just done is the same procedure used to change buffers between operations, e.g. from storage in 20% EtOH to water to buffer for a chromatographic separation, or from chromatography buffer to CIP solutions for clean-in-place, CIP to water to EtOH for storage, etc).

4. Equilibrate the 'column' in start buffer. In the manual control panel for the pumps, move the slider to a flow rate of 1 ml/min and click 'Set flow rate'. Pump A will begin to move the

mobile phase at that rate through the injection valve, the 'column', detectors (uv, conductivity and/or pH) and on to the waste receptacle (or fraction collector if specified). After about 5 column volumes (CV), stop the flow, then end the 'run' by clicking the stop icon (a solid square) near the top of the window.

5. Load the Sample Loop. The default position for the Injection Valve is called 'Manual Load', in which the mobile phase flows from the pumps through the valve and then to the column and detectors. The sample loop is isolated from the flow path. It's good practice to rinse and fill the sample loop with start buffer prior to manually loading it with sample. To do so, fill a one ml syringe with start buffer (taking care to eliminate air bubbles) and attach it to the Luer lock fitting on the injection valve. Depress the plunger to force liquid into and through the loop (stopping before any air near the plunger can enter the sample loop). Excess liquid will go to the waste receptacle. Now repeat the process but fill the syringe with 'sample' (0.1% acetone), leaving the syringe attached to the fitting (to prevent siphoning of sample from the loop).

6. Select, Edit and Run a Method.

Select: In the Unicorn software, bring the Method Editor to the forefront. Under File, Open a method of your choice, for example pretend to have installed a 1 ml ion exchange column; select a pre-programmed method for anion or cation exchange.

Edit: When the method opens, you will see each of the steps in the method (general settings, equilibration, sample injection, column wash, elution, re-equilibration and perhaps more). Clicking on any of the steps (called Phases) will result in the display of those parameters in the right pane. The Method Settings allows one to choose the appropriate column from the myriad available from GE, or to input custom column dimensions and characteristics. For this exercise, select a one ml ion exchange column. You will see the recommended settings for back pressure limit and flow rate. Here you may also indicate which pumps will be in use (A1, B1 are defaults), the measurement used in each setting (ml, min, or CV - column volumes) and which detectors are to be employed.

For each phase you will be able to designate the proper pump, % B in the eluent, override the flow rate, and set fraction collection details. Set the fraction collection ('Output') to waste for this demonstration. Changing the settings in one phase does not effect other phases, so you will need to set the output in each phase.

The elution phase will probably include a salt gradient; you may choose linear or step, and the initial and ending %B. Multiple step elution programs are also available.

After making the desired changes, choose Save As in the File menu, then rename the program and save it.

Run: Return to the System Control window. Under the File menu, choose Open, then double click the name of the method you just saved. The run will begin immediately, unless there is something amiss, e.g. the absence of tubes in the fraction collector, or the fraction collector is not in the ready position (which may not appear until the phase is initiated that calls for collection of fractions).

You can monitor the progress of the run in the System Control window. There should be an absorbance peak evident when the acetone sample passes through the uv detector.

7. Return to Storage. Upon completion, the system should be washed through with water, then 20% EtOH if long term storage (one week or longer) is desired. If you've run this demo with water, you can stop here, for short term storage. Repeat the initial pump wash step to change over to 20% EtOH, then run the pumps at 50%B for about 5 min at 5 ml/min. Make sure there is a large excess of 20% EtOH prior to starting.

8. Open the Evaluation window and the Results Navigator. The most recent chromatogram (yours) will be at the bottom of the list. Double click it to open. You can print it, or choose to save it as a pdf.

For more information, please see the library of documentation on the GE Life Sciences website.

The one page version:

Quick steps to acquaint you with the AKTA pure.

1. Check the buffer reservoirs to insure that adequate volume is present, and tubing is resting on the bottom of the vessel(s). Use water for this operation or buffers of your choice.
2. Turn on the instrument and computer. Open the Unicorn software System Control window.
3. Use the manual control panel to wash pumps B2, B1, A2 and A1.
4. Flush the sample loop with start buffer by attaching a filled syringe to the Luer lock sample loading port.
5. Fill the sample loop with 0.1% acetone in start buffer (or water).
6. Open the Method Editor, then open a program for SEC (simplest, isocratic elution).
 - a. Method Settings - choose a small column volume, e.g. 1 ml;
 - set the flow rate = 1 ml/min;
 - unit of measure = CV
 - detectors = A280, conductivity;
 - pH detector OFF; restrictor only position
 - default pumps = A1, B1
 - %B = 50
 - b. Equilibration
 - flow rate 'use system setting'
 - %B = 50% or your choice
 - Outlet = waste
 - c. Injection
 - Volume to inject = 0.6 ml
 - Wash loop with 1 ml
 - Outlet = waste (or for fun, collect some fractions; check out the options)
 - d. Elution
 - 10 CV
 - Use system settings
 - e. Save the method with a new name
7. Run the program: open System Control window; File\Open\your new file DOUBLE CLICK. Click through the windows ("Next") until you arrive at the final one with a 'Start' button. Accept the default name and click Start. Away you go. Upon completion of the run, the system will stop automatically.
8. Open the Evaluation window and the Results Navigator. The most recent chromatogram (yours) will be at the bottom of the list. Double click it to open. You can print it, or choose to save it as a pdf.
9. Return the instrument to the storage state, by flushing pumps and flow path with 20% EtOH. Monitor to insure the pumps do not run dry.