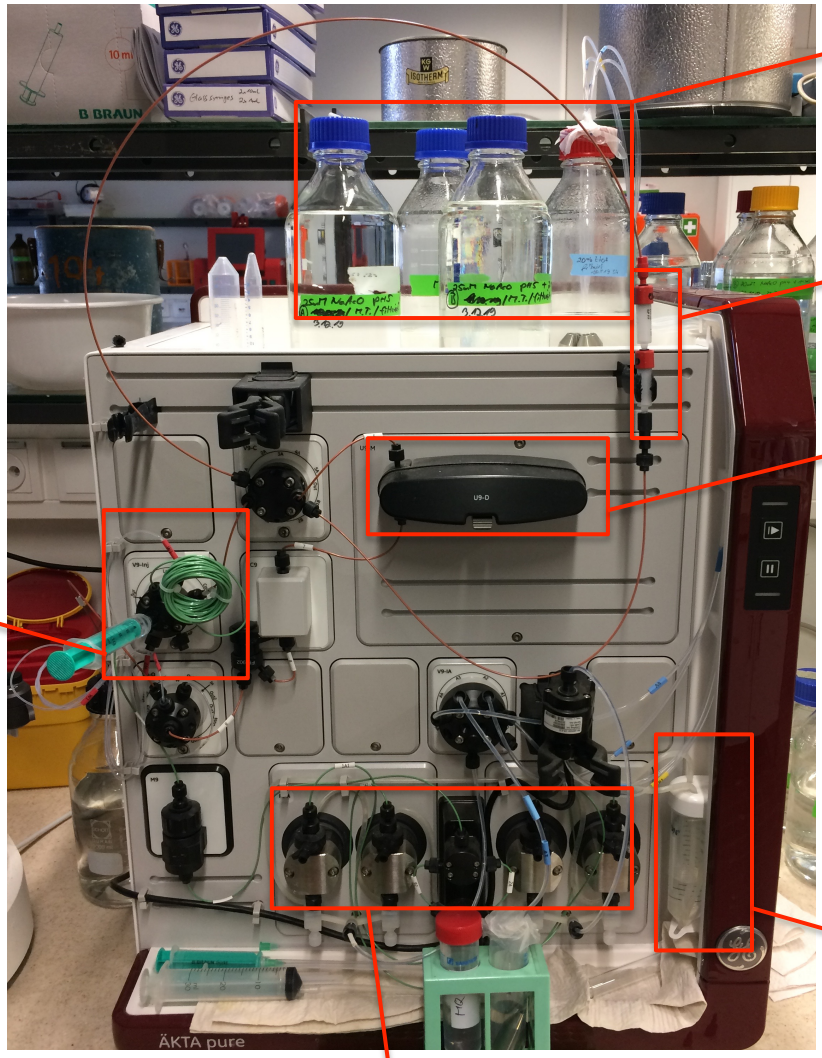
erstellt von Michael Tully am 20.04.2020

ÄKTA 25 FPLC (GE Healthcare)
Quick starting-guide



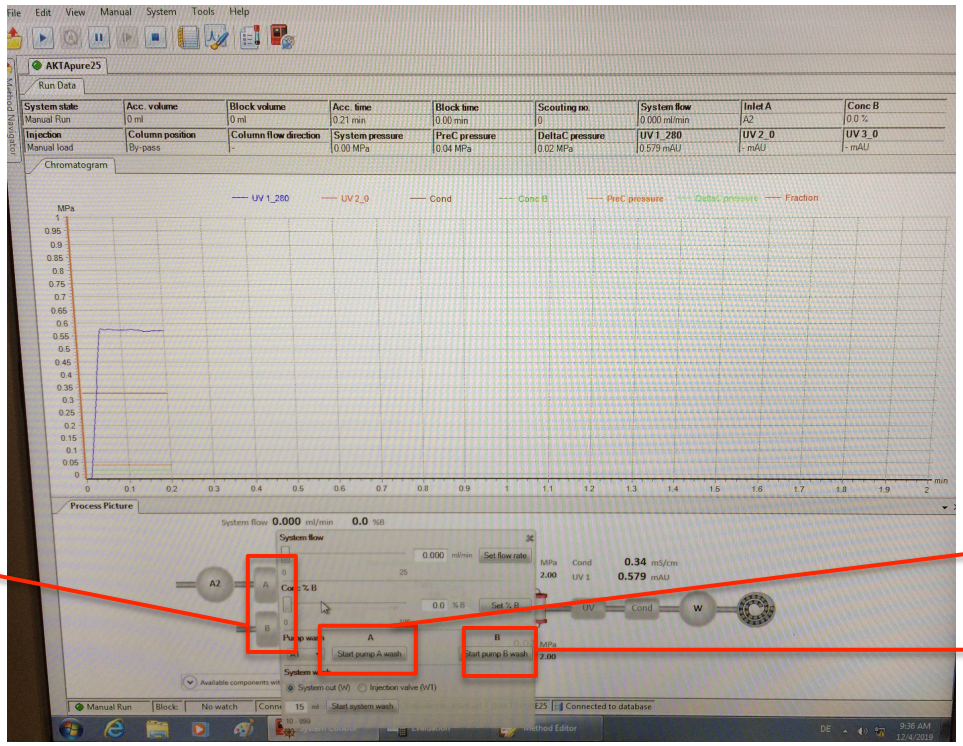
General

- for the most important bullet points, check the cue-cards for ÄKTA 25 (in the lowest drawer)
- practical and software manuals for detailed procedures and descriptions (purging, setting up a method, data evaluation etc.) can be found in the lowest drawer or in the Bio-Wiki
- whenever you rinse your columns: don´t forget to **set pressure limits** to avoid damaging the column!
- **always use filtered and degassed solvents!!!**
- in case of any problems, please contact the responsible person(s)

Getting your system ready to work (steps A-C)

A) Prime sample loop (if used; rinse manually via syringe) and all inlet tubings you want to use (inlet A1-6, inlet B) with **MilliQ**. Therefore put all the inlets into the MilliQ bottle (on top of the ÄKTA). Then repeat the same procedure with your **buffer of choice**.

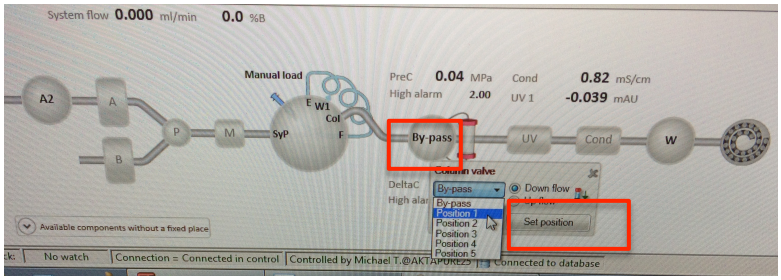
1) click on system pump to open pump screen



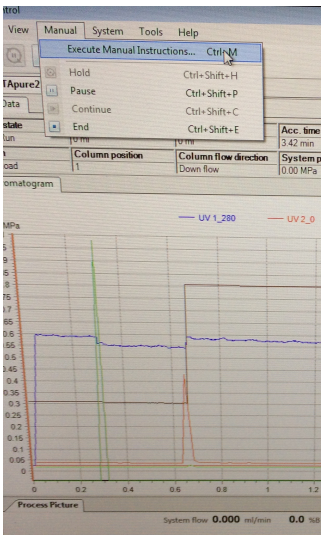
2) click pump A wash (for the **selected** A-inlet) or pump B wash (for B-inlet) and flush

B) **Purge** both pump heads (see ÄKTA pure User Manual **p. 162-166**)
If system hasn´t been used for >7 days: replace **rinsing solution of pump heads** in the falcon tube on the right with fresh 20 % EtOH

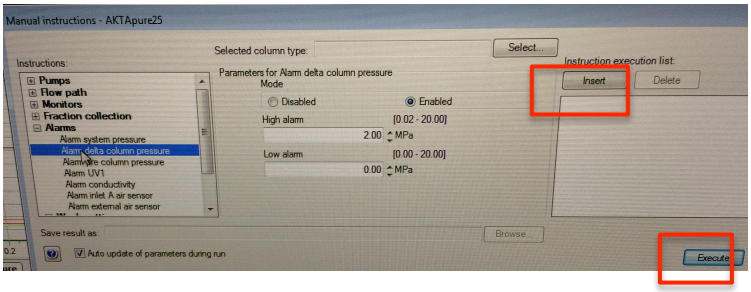
C) **Connect your column**: Overview: set column position 1 --> set pressure limits --> apply flow --> drop-to-drop connection



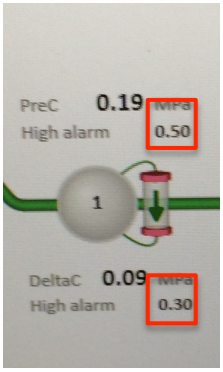
1) click on "by-pass" --> change column valve to "position 1" --> click "set-position"
The solvent will now pass the column. However the system is NOT running yet, so don´t panic ;-)



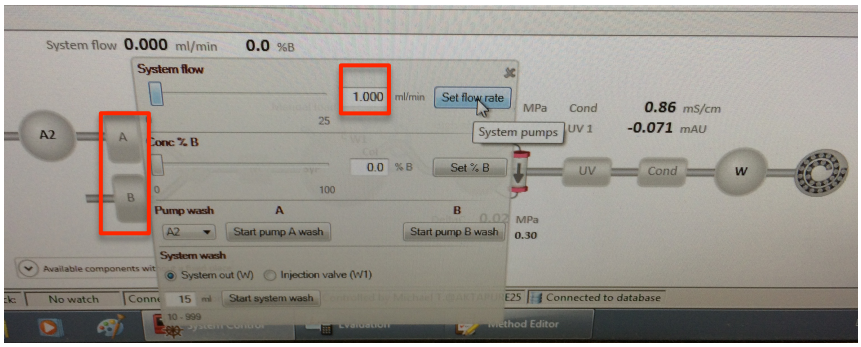
2) click "manual"
--> "execute manual instructions"



3) Alarms: set high alarm pre- and delta-column pressure limits (e.g. 0.5 and 0.3 MPa)
--> click "insert" --> confirm with "execute" (pressure limits need to be set for each column individually; for specific values check column data sheets)

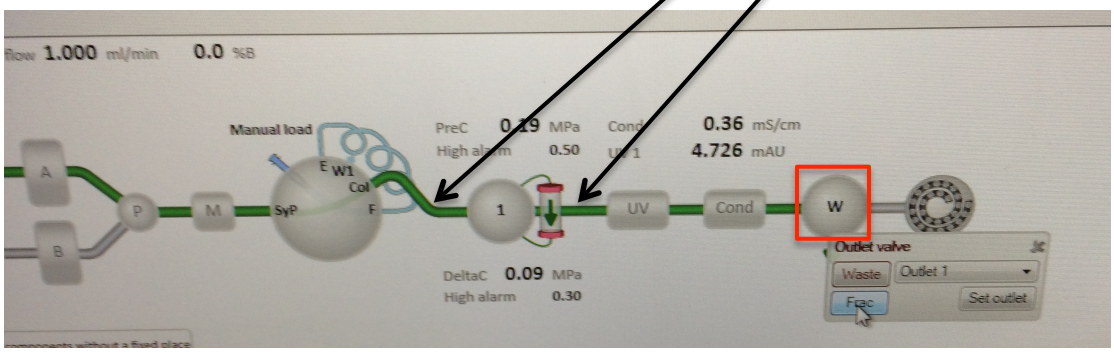


4) pressure limits are now embedded in the process picture



5) click on system pump A or B to open pump screen
--> apply a flow of 0.2 mL / min
--> click "set flow rate"

Now the system is RUNNING and solvent is flushing through your column. This can be seen from the filled **green tubings** in the process picture, that show the **flow path**.



6) To also rinse your fraction collector, click on outlet valve "W" --> click on "Frac" --> now the solvent flushes your fraction collector tubing. (remember to put the outlet of your fraction collector tubing into the waste beforehand, so that you don´t flush the lab bench!)

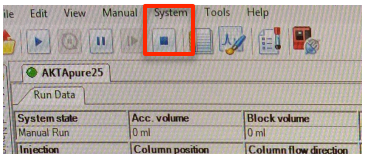
7) Now **connect your column** via drop-to-drop connection (see also ÄKTA pure user manual, **page 172**)

- remove upper and lower stopper of your column
- fix your column in a column holder
- fill your column dropwise from the top with solvent from the tubing and tighten connector when column is "filled"; **avoid air bubbles!**

- connect tubing to the column outlet

8) Monitor the flushing of your column through **conductivity** detector to see when your buffer reaches the column. **Flush at least with 5CV!!**

9) When your column is equilibrated, stop the flow:



Your system is now ready to use, either manually or for applying a method.

Preparing your system for storage

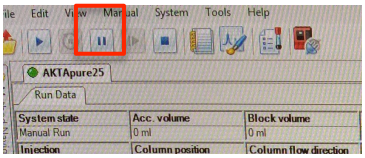
After finishing your experiments you need to **rinse** all the used **inlet tubings/pumps, sample loop, column, By-pass and fraction collector tubing** first with **Milli Q**, and (if stored for > 1 day) subsequently with **20 % Ethanol**.
Storage in 20 % Ethanol is generally recommended to avoid microbial growth, no matter how short the storage period is.

1) Put all your used inlet tubings into the MilliQ bottle and repeat steps A1+A2 as described above.

2) Put inlet tubing B and all A-inlets (**except for one)** into the 20 % Ethanol bottle and repeat step A1+A2 for the pump heads A and B.

3) Follow the steps C1-C6 and rinse your column via **the A-inlet that is still in MilliQ bottle** at a flow rate between 1-5 ml/min (depending on column) until conductivity decreases.

4) Pause the flow and put the A-inlet from MilliQ to 20 % Ethanol bottle.



5) Flush your column with **at least 5CV!** Monitor pre- and delta-column pressure! Pressure increases due to higher viscosity of 20 % Ethanol.

6) Set a flow of 0.2 mL / min, remove your column and seal column with stoppers.

7) Finish the cleaning procedure with rinsing **By-pass** (step C1), **fraction collector** tubing (step C6) and **sample loop** (manually with syringe, first MilliQ, then 20 % Ethanol, keep syringe filled with 20 % Ethanol at the port of the sample loop).

8) Stop the flow, quit software and switch off the ÄKTA pure 25.