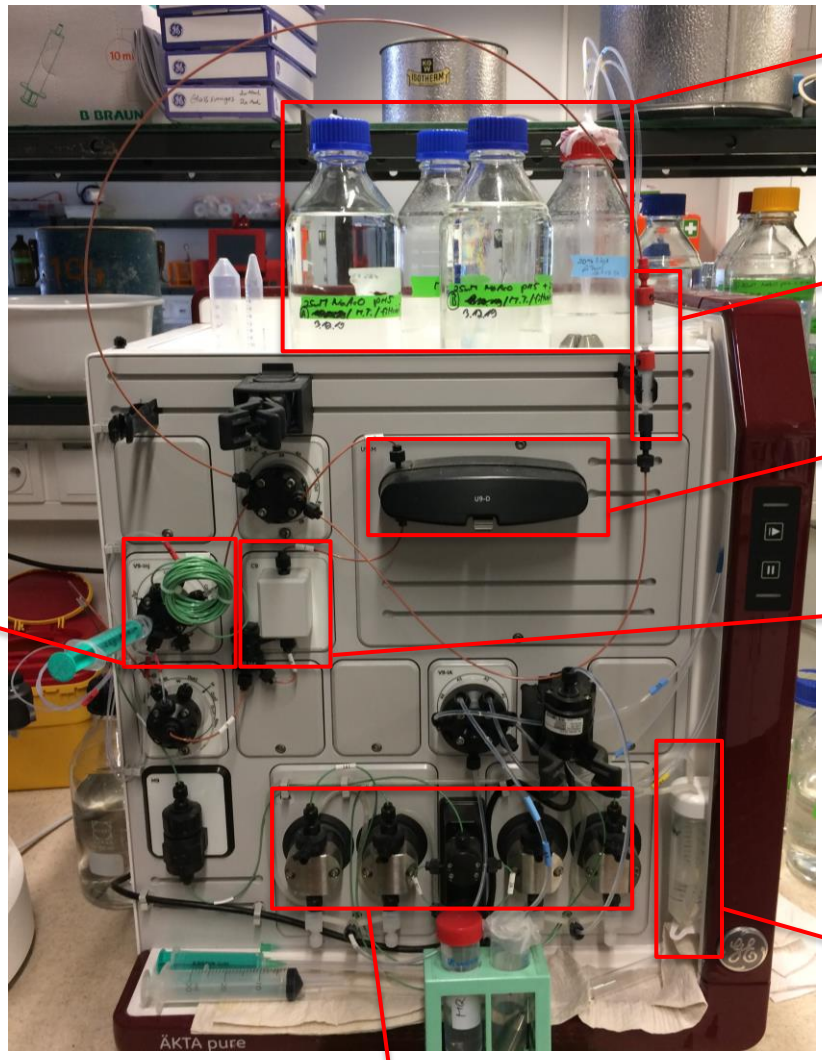
Michael Tully/05.10.21

ÄKTA pure 25 FPLC



General

- For the most important bullet points, check the cue-cards for ÄKTA 25, additionally
- Detailed procedures (purging, setting up a method, data evaluation etc.) can be found in the ÄKTA Manuals
- **Always use filtered and degassed solvents!!!**
- Column connected in manual run: always **set pressure limits**!
- In case of any problems, please contact the responsible person

Using the System for Size-Exclusion Chromatography

In general, preparation of the system follows the order **20% ethanol (storage solution)→Milli Q→buffer** and vice versa for storage.
For Size-Exclusion Chromatography, the ***blue capillaries*** (inner diameter 0.25 mm) are used. Therefore, the correct delay volumes need to be set. In the Unicorn software, click on "system"→"settings"→"Tubing and delay volumes" and implement the correct values. Those are for the blue capillaries: Monitor to Outlet valve: 66µL; Monitor to frac: 86µL;
For more details on that, please check the **ÄKTA pure User Manual** (page 501 and 540–545) in the Wiki.

The overall preparation of the system consists of the following steps:

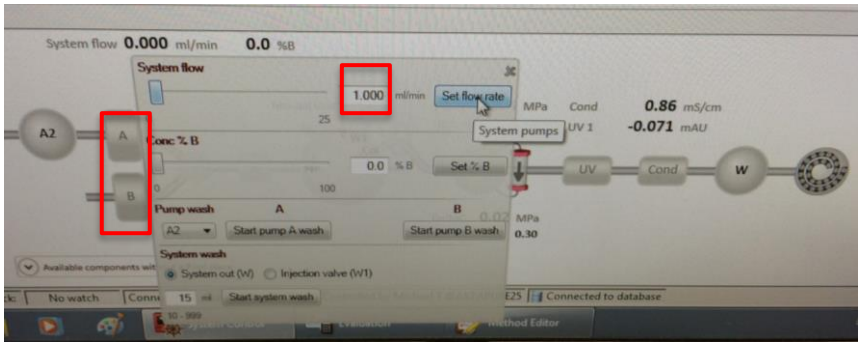
- Replace pump rinsing solution and purge its tubing
- Prime inlet A with Milli Q water
- Purge both pump heads of pump A
- Set column pressure limits
- Connect your column
- Flush the column with Milli Q and subsequently with buffer
- Flush sample loop with buffer

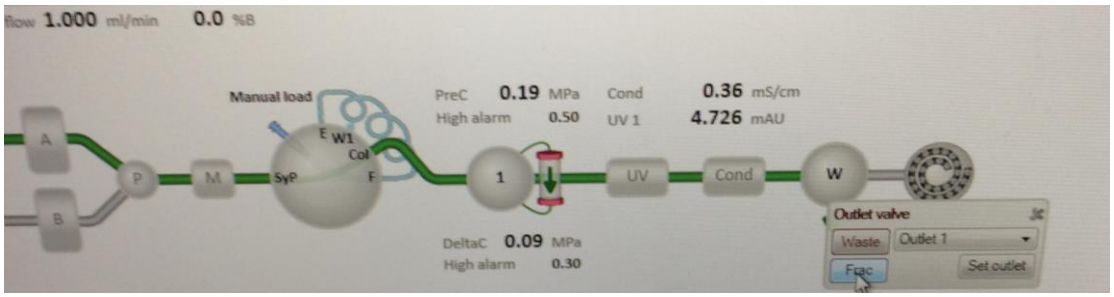
Replace pump rinsing solution and purge its tubing

- On the right side of the ÄKTA, there´s a falcon tube containing 20% ethanol as rinsing solution for the pump heads
- Replace the solution with fresh 20% ethanol and fill it completely
- Remove one end of the tubing and connect it to a syringe; gently pull some liquid with the syringe to remove air from the tubing
- Remove the syringe and put the end of the tubing back into the falcon tube;
- Make sure there is **at least 30mL** of rinsing solution left in the falcon tube!

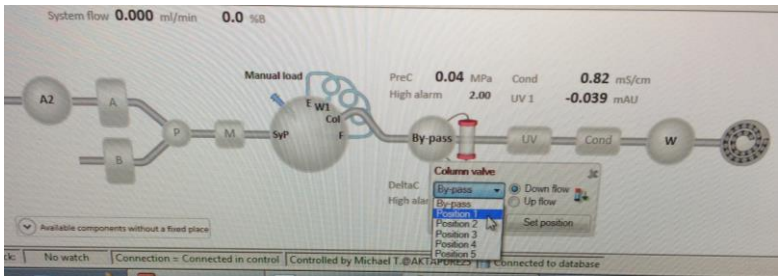
Prime inlet A with Milli Q water

- Put the inlet A you want to use into Milli Q
- In the ***process picture***: select the correct inlet A (A1-7), switch from "by-pass" to "position 1" and click "set"; switch from "W" to "Frac"; then click on the system pump "A" and set a flow rate of 1 mL/min for around 5 min
- !! Put the end of the fraction collector into the waste bottle before!!





The green filled tubing in the process picture indicates the current flow of the solvent.

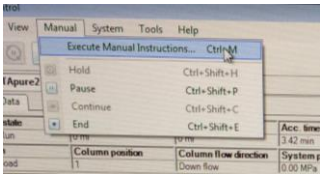


Purge both pump heads of pump A

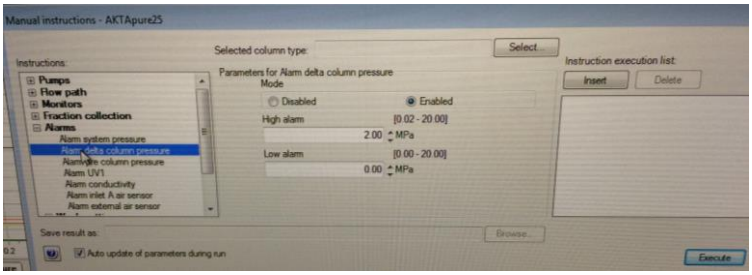
- In the ***process picture***: click on “injection valve” and switch from “manual load” to “system pump waste”
- Detailed description: see **ÄKTA pure User Manual** p. 182-186 on the Wiki

Set column pressure limits

- In the ***process picture***: click on “injection valve” and switch from “system pump waste” to “manual load”
- Click “manual” → “execute manual instructions”



- Alarms: set high alarm values for pre-column pressure→ “insert”; do the same for delta column pressure → “insert” → “execute” (pressure limits are column-specific and need to be looked up in the column data sheets or in the method editor)



- The pressure limits are now embedded in the process picture

Connect your column (see also ***ÄKTA pure User manual***, page 193–197)

- Set a flow rate of 0.3 ml/min
- Put your column into the column holder
- Slightly loosen the lower stopper of your column, but don´t remove it completely
- Remove the upper stopper of your column
- Fill your column ***dropwise*** from the top with solvent from the tubing of the column valve, position 1A, until there is a small meniscus on top; **avoid air bubbles!**

- Put the connector with the tubing of the column valve into the column and fix it tightly
- Quickly remove the lower stopper of the column to avoid overpressure
- Wait until a first drop is eluting from the lower end of the column
- Then tighten the connector with tubing from position 1B at the lower end of the column and fix it tightly

Flush the column with Milli Q and running buffer

- Flush the column with 1 CV of Milli Q water to remove the 20% ethanol storage solution of the column (***check the max. flow rates*** for 20% ethanol to avoid overpressure!!); the column pressure in the chromatogram should decrease over time
- Pause the flow (II Symbol in the Unicorn software) and put your inlet A into the running buffer solution you want to use
- Equilibrate your column with running buffer for at least 2 CV; monitor the equilibration through the increase in conductivity
- When your column is equilibrated, stop the flow

Flush sample loop with buffer

- Flush your sample loop with running buffer at least 3-5 times the volume of the loop; keep the syringe attached and remove it only directly before filling the loop with your sample

Your system is now ready to use!

Preparing the system for storage

After finishing your experiments, you need to flush your column with ***Milli Q and subsequently with 20% ethanol.*** Storage in 20% ethanol is generally recommended to avoid microbial growth, no matter how short the storage period is.
Additionally, the sample loop needs to be flushed with 20% ethanol manually with a syringe; leave the syringe attached after flushing.

The overall preparation of the system consists of the following steps:

- Set column pressure limits (***see above***)
- Flush the column with Milli Q and 20% ethanol
- De-Connect your column
- Flush sample loop with 20% ethanol (***see above***)
- Quit the software and switch off the ÄKTA

Set column pressure limits (*see above*)

Flush the column with Milli Q and 20% ethanol

- Put the inlet A you used into Milli Q
- In the ***process picture***: select the correct inlet A (A1-7), switch from "by-pass" to "position 1" and click "set"; switch from "W" to "Frac"; then click on the system pump "A" and set a flow rate of 0.5–1 mL/min
- Flush the column with 1 CV of Milli Q water or until the conductivity in the chromatogram decreases
- Pause the flow (II Symbol in the Unicorn software) and put your inlet A into 20% ethanol solution
- Flush your column with 20% ethanol for at least 2 CV; monitor the equilibration through the increase in system pressure (***check max. flow rates for 20% ethanol!)***

De-Connect your column

- Set a flow rate of 0.3 mL/min
- Remove the connector from the lower end of the column
- Slightly loosen the upper connector from the column, but don´t remove completely
- Close the lower end of the column with a stop plug
- Remove the upper connector from the column; add solution dropwise to the top until a small meniscus is formed
- Close the upper end of the column with a stop plug
- Connect both connectors of the column valve (Tubing from 1A and 1B) with the "dummy" connector
- Stop the flow

Flush sample loop with 20% ethanol (*see above*)

Quit the software and switch off the ÄKTA

Your system is now ready for storage!