FPLC – User instructions

- Turn on FPLC
- If necessary, switch on light (third button on the left under temperature display) of the FPLC-fridge
- Start up Computer LOGIN: Password: Jcns.Biolab
- Open UNICORN with double-click LOGIN: ID: default
   Password: default

Before strating a run, the system has to be equilibrated:

The whole system has to be kept free of air-bubbles!

Control on the instrument if every connection is sealed, remember to empty the waste if necessary

- Control which Pump the Eluent is connected to
  (to change: *in Tab "Systemcontrol:* "Manual" -> "Pump" -> adjust *gradient in % of Eluent B (Eluent A is therefore the difference to 100%)* -> "Execute")
- Adjusting which compartment is rinsed by changing the flowpath:

  →*Tab "System Control"* → "Manual"→ "Flowpath" →:   "WASTE" (Eluent doesn't run over pillar?loop & goes in top waste)
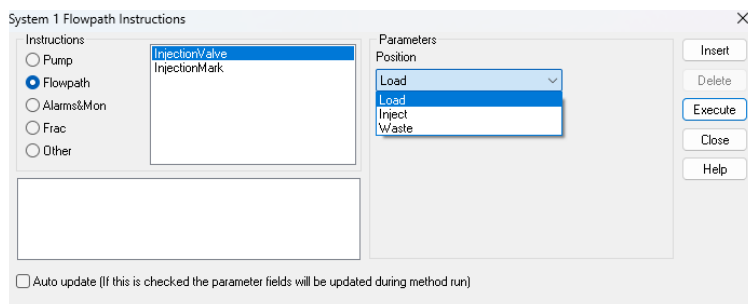
  "LOAD" (Eluent runs through pillar, Loop isn't connected & can be rinsed with syringe)

  "INJECT" (Eluent runs through loop & pillar)

  → "Execute"
- *Tab "System Control"* → "Manual" → "Pump" → *Flow: 0.5ml/min (e.g.., but max 2 ml/min)* →"Insert" → "Execute"
- max. pressure shouldn't exceed 0,5 MPa

Construct method:

In the window "Method editor" choose "File" -> "Method Wizard…", this opens a run-through program in which all parameters are queried:

- Select a chromatography technique in the Main Selection list field: e.g.: Size Exclusion
- Select a column in the Column list field
- Select Flexible Flow Rates

Click NEXT.

- Start Concentration: 0 (-> refers to the pillar volume CV, e.g. 120ml))
- Equilibration: 1 (-> i.e. 120ml rinsing volume, before the sample is injected)
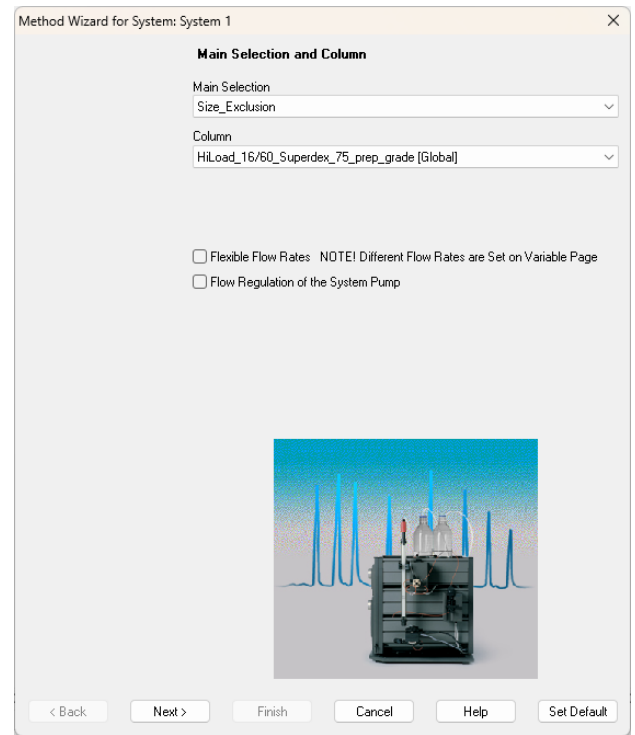- UV Watch: off

Click NEXT.

- Sample Injection:
  ➔ Injection Technique: manual
  ➔ Empty Loop: 0,5 ml

Click NEXT.

- Eluation Fract.: (For collecting the sample)
  ➔ Frac 900
  ➔ Fixed Volume Frac.
  ➔ Fraction Volume (how many ml are supposed to be collected per test tube): 5 ml

Click NEXT.

- Elution
  - → Elution Technique: Isocratic with delayed Fractionation
  - → Length of Elution before Frac. (how many ml will run through before collecting the sample, referring to the pillar volume calculating: volume till begin of peak [ml] : 120 = X)
  - → Length of Elution with Frac. (How many ml should be collected in total, referring to the pillar volume calculating: volume length of peak [ml] : 120 = X)

Click FINISH.

To calculate how many test tubes are needed:

V(length of peak in ml) : V(collection volume in ml)= number of required testing tubes

All parameters will be summarized.

- → Adjust all flows (ml/min)
- → If necessary, increase pressure limit to approx. 0,7

Go through all riders individually

Enter method name on rider "Result name", by clicking on "NAME" and save by pressing "Save As…" in the menu bar, optimally with the same name.

Open UNICORN MANAGER afterwards

The newly constructed method is listed in the method list

- Open methode: right click than click RUN FILE and RUN in the symbol bar
  - → SYSTEM CONTROL rider blinks
    The overview of parameters will show again
  - → Control all the parameters again, on "Method Duration" the total volume and duration of the procedure is shown
  - → Click START

ASCII-export of the eluation diagram
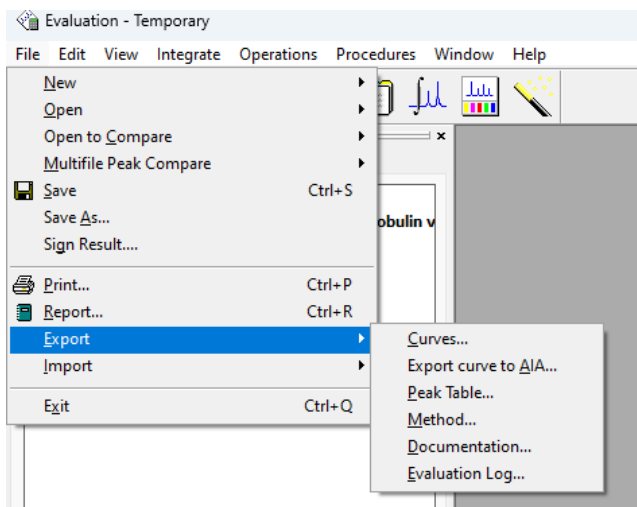
Open window "Evaluation"

Export of the Graphs:

"File" -> "Export" -> "Curves" -> choose desired curves -> "select>>" -> "Export…"

Export of the experiment parameters:

"File" -> "Export" -> "Documentation" -> choose desired data -> "Export…"

Shutting down:

Window "UNICORN Manager"

      "File" -> "Quit Program" ->

Window "System Control 1"

      Pop-up Leavesystem:

            "Locked"

      Enter PW: "default"

      "OK"