FPLC protocol

The important things:

A) All the buffers and solutions used with FPLC must be filtered with 0.22uM filter and degassed first.
B) Always set the correct pressure limit for each column when you are using a column.
C) Always insert commands before execute them together.
D) Always make wet connections and avoid any bubbles in the system.

Start up “Unicorn” program and set up method for each column. In our case, the methods have been setup and saved.

1) Pump Wash

Place both lines "A1 & B1" in to water bottle (careful not to introduce air bubbles into line)→set flow rate to 10ml/min→choose PumpWashBasic, click pump A and B on→set valve position to waste→set stop volumn 40ml→execute

2) Wash the column

Place both lines "A1 & B1" in to buffers to use (For gradient, put A1 in low gradient buffer and B1 in high gradient buffer) → set flow rate to 0.5ml/min→ set valve position to load→set column position (In our case, Ni column position 8, MonoQ position 4, gel filtration, position 2) →set pressure limit for each column (

Ni 0.3MPa, MonoQ 4Mpa, gel filtration, 0.3Mpa)→set stop volumn to 5 column volumn→execute

3) Sample loading

a. Large amount of sample loading with a superloop

Assemble the superloop and fill it with 10ml samples→connect the superloop between valve port 2 and 6→set valve position to inject →select the column you want to load the samples to→set up flow rate to 0.5ml/min→set up pressure limit of corresponding column→execute (if you have more than 10ml, repeat it several times.)

b. Small amount of sample loading with 1ml loop

Fill the 1ml syringe with sample→Connect the 1ml sample loop between valve port 2 and 6→Fill the 1ml loop with buffer with valve position at inject→change valve position from inject to load→connect the 1ml syringe filled with sample to the valve port 3→inject the sample to the sample loop→start gel filtration method

4) Run the method

Run the corresponding method for each column. Make sure there are enough tubes for the fraction collection.

5) Clean up

Clean up the tubes and tubing as soon as possible with water to avoid protein precipitation.

6) Column storage

After each use (if you will not use it again within a week), wash each column with 5 column volume of filtered water and then with 5 column volumn of filtered 20% ethanol and store them in 20% ethanol to avoid bacteria growth.

7) Filter change

When the pressure of the system during pump wash is higher than 0.5 MPa, we need to change the filter. The filter can be cleaned in water with sonication for 1h. After that, the filter can be reused.