Protocol for Shimadzu HPLC and Fluorescence Size Exclusion Chromatography (FSEC)

Six modules of the instruments are:
LC-20AD (the pump)
SIL-20AC (auto sampler)
RF-10A (fluorescence detector)
SPD-20A (UV/Vis detector)
FRC-10A (fraction collector)
CBM-20A (communication bus module)

Start the instrument and software:

1. Turn on the powers of five parts of the Shimadzu HPLC at the bottom left corner of each (no power bottom on FRC-10A).
2. Open the EZStart 7.4 software from Desktop.
3. If the software is connected with the instruments, there should be a beep sound, and the "connect" LED on CBM-20A should be lit.
4. The "remote" LED on LC-20AD, SIL-20 and SPD-20A should be lit. The "remote" LED on RF-10A should be blinking.
5. If the RF-10A fluorescence lamp is on, the "lamp" LED is lit.
6. If the SPD-20A US/VIS lamp is on, "D2" is shown on the display.
7. To turn the lamp on, open and download a method with lamps on in the method.

Before your run, create or edit your method:

To edit a method, open a method and change the parameters in Pumps, SPD-20A, and RF-10A panels. Modifying a method from the Lab Method folder is recommended!

1. In the Pumps panel, flow rate should be less than 0.5 ml/ml and pressure is less than 1.5MPa.
2. In the SPD-20A panel, check the box "acquisition channel 1 on", set the wavelength channel 1 = 280nm and switch the lamp from off to "D2" to turn on the lamp.
3. In the RF-10A panel, check the box "acquisition channel 1 on", set the wavelength corresponding to your type of GFP. **Excitation at 398nm and emission at 508nm for common GFP or excitation at 488nm and emission at 512nm for EGFP, excitation at 587nm and emission at 610nm for mCherry and switch the lamp from off to "on" to turn on the lamp**.

Equilibration of the column:

To wash the pump:

1. Put the Pump A in your buffer.
2. Open the "drain" on LC-20AD.
3. Turn off the pump by pressing "Pump" on the LC-20AD
4. To start purging, press the "Purge" bottom on both LC-20AD and SIL-20AC. (You can press the same bottoms again to stop purging.
5. When purging on LC-20AD is done, press the "Purge" bottom on SIL-20AC to stop purging.
6. Close the "drain" on LC-20AD.

To equilibrate the column:
Option 1:

1. Download the desired method and run for at least one column volume. (0.4m1/ml flow rate for 70 min)
2. **Stop the pump manually after the wash. (Remember the HPLC will not turn off the pump automatically!!!)**

Option 2: run the column wash.seq in the Lab Sequences folder. **(This sequence will stop the pump automatically.)**

To start a single run:

To run a single run, click on single run bottom, choose a method, define the volume and vial number, and click start.

To run a sequence:

1. Open a .seq file from the Lab Sequence Folder and modify it. Define methods, sample volume and vial numbers of each run. Save the .seq file.
2. **Remember the pump will not be turned off by itself, so it's recommended to add a shutdown run with the shutdown method in the last run of the sequence.**
3. To run a sequence of multiple samples, click on sequence run bottom, choose a sequence, and click start.

After your run:

1. Wash the column by putting Pump A into ddH2O. Turn on the pump and wash for more than one column volume or run the column wash.seq.
2. Turn off the instruments by pressing the power bottoms on each component.
3. Check the results of your run by using EZStart_offline.

Maintenance:

Lamp life: D2 lamp: 2000 hours. W lamp: 2000 hours. Xenon lamp: 500-750 hours (Do not use it over 1000hours)

To check lamp hours, press "function" bottom until lamp hour is shown on the panel of SPD-20A or RF10A.

To replace D2 or W lamps, follow instructions on SPD-20A manual page 8-11.

To replace xenon lamp, check RF-10A manual page 4-12.

To open data In the HPLC (Offline)

(option) If you compares A graph with B graph

1. After you click mouse right button, you can click "multiple traces".
2. As you click "Add" button, open your data from your data from your folder.
3. Change SPD-20A ch1-280nm to RF-10Axl in Trace and Click "Open" button.

(option) If you change color in the graph

1. After you click mouse right button, you can click "Appearance" or you can click "properties - Appearance "
2. After you click item and color and size of graph, change both.

(Other option)

1. After you click mouse right button, you can click "properties".
2. As you click "show" and "land" button, you can disappear your peak and name.
3. If you want to change your Left Y-axis scale, go to "Axis Setup - Left Y-Axis" and change min and max number what you want.