1. prep-HPLC instrument

① Computer
② Solvents
③ UV detector
④ Pumps (A and B)
⑤ Injector
⑥ Column
⑦ Fraction collector and rack
⑧ Storage drawer for columns and syringes
2. Before turning on the software

a) Check waste container
- Make sure the waste container under the flame area has enough space and the waste line is connected to it (see right picture).

b) Prepare solvents and column
- You can find solvents in the cabinet under extraction hood.
- We have a C18 column for prep, and a C18, C8, phenyl-hexyl, amide, hypercarb, and silica column for semi-prep.
*Dr. Kim’s Tip: I usually use acetonitrile/water both with 0.01% TFA for isolation of natural products.

c) Prepare rack(s) with tubes for using fraction collector or 2L flask for manual collection (see right picture)
- Use 16 x 125 mm tubes (this is a default setting)

3. Equilibration

a) Turn on the software
- Click the icon ‘Prep LC (online)’. Offline icon is for processing after run.

b) Select/create method
- Load your method by clicking ‘Method’ and ‘Load Method’.
- To change the method, click ‘Instrument’, ‘Set up Instrument Method’, and make your own method.
*Dr. Kim’s Tips: For semi-prep column, I set the flow rate of 4 mL/min, and for prep-column, 8 mL/min.

c) Set your sample name
- Click ‘RunControl’ and ‘Sample Info’, and change ‘Subdirectory’ and ‘Sample name’
- If you use fraction collector, you can change the starting position of tube on the rack by changing ‘Fraction start location’. Default setting is R1-01, which means collection starts from the first tube in the first rack.
d) Set the UV detection wavelength
- Click ‘Change’ button at the bottom and add the UV wavelength you want to see.

e) Turn on the pumps and DAD
- Click the ‘On’ button. It takes 15 min to equilibrate.
- After equilibration, click ‘Balance’ to set the UV signals to zero. And you’re ready to inject the sample.
4. Injection

- Click ‘Single Sample’ and wait a few seconds. When the message ‘System ready for injection’ under the ‘Flow rate’ pops up, inject your sample.

- For injection, turn the injector counterclockwise, inject sample with syringe, and turn the injector clockwise.

- Then the software automatically starts your run.

5. During run

- If you use a fraction collector, make sure your sample is collected well in each tube.

*Dr. Kim’s Tip: If you have a target peak, know its UV trace, and decide to collect manually, click ‘View’, ‘Online Spectra’, and ‘DAD’. By doing so, you can see the UV trace you are collecting on-line which makes sure you are collecting your target.
6. After run

a) Wash the column
   - Wash with proper solvent for more than 15 min. If you use acetonitrile/water with 0.01% TFA, using 100 % acetonitrile with 0.01% TFA should be fine. You can change the solvent ratio and flow rate directly by right click on ‘Pumps’ window and click ‘Direct control’.
   
b) Turn off the pumps and DAD
   - Click the ‘Off’ button.

7. Sample preparation

a) Dissolve your sample with the starting solvent mixture you set in your method. If the sample is not dissolved in that solvent, you can add a little amount of other solvent (e.g. methanol), which might affect retention time and resolution.

b) Filter or centrifuge the sample and inject only clear solution.