

**Preparation of Waters 2960/5 systems
Step-by-Step procedure
By Dr. Shulamit Levin**

Preparing the Solvents

1. Remove old mobile phase solutions from reservoirs A and B (if B was used with buffer) and replace them with PURE WATER.
2. Select **100% A** and then: *Direct Function* -> *Wet Prime* for at least 2 minutes at 7.5 ml/min. If pressure is not stable abort the wet prime and perform "Dry Prime". **DO NOT TRY TO FORCE THE SYSTEM TO STABILIZE BY USING ACCESSIVE WET PRIME!**
3. To perform a dry prime when pressure fluctuates: go to *Direct Function* -> *Dry Prime*. Select **A**, then open the divert valve (front) counter-clockwise. Draw the solvent from **A** until the liquid flows freely. Close the valve by pressing *Cancel*.
4. Repeat the *Wet Prime* from **A** until the pressure is stable (2-3% fluctuations).
5. Repeat these steps for reservoir **B with water if it was used for the mobile phase with buffers**.
6. Purge the injector with water: Select **100% A** (which is still with **100% water!**) and then go to *Direct Function*-> *Purge injector*. Let the process complete on its own.
7. Fill reservoirs **A** and **B** with the mobile phase and perform *Wet Prime* one after the other (see steps 2-4).
8. Make sure that reservoirs C and D are filled with the appropriate solvents (water or methanol or acetonitrile, etc.) and repeat steps 2-4 for C and D.
9. Select the appropriate **mobile phase composition** and perform *Direct Function*->*Purge Injector*.
10. Make sure the *Seal Wash* inlet is in a water enriched solution, go to the *Menu* screen (initial screen) and select *Diag*-> *Seal Wash*. Check that the liquid flows properly in the tubing around the pump heads, and abort.
11. Make sure that the *Needle Wash* inlet is in the appropriate solution (**Enriched with organic solvent, depending on the application**), go to the *Menu* screen (initial screen). Select *Diag*-> *NeedleWash*. Let it finish on its own. Needle wash needs to be repeated if the solvent composition is changed

Stabilizing the system

1. **Connect the column.** Make sure that it is connected properly according to the flow direction (arrow on column). Wash it with water if needed, to remove all organic remains before introducing it to the new mobile phase composition. Begin with a low flow rate then increase the flow to the working flow-rate.
2. Switch the composition to the method's composition and start flowing it through the column at low flow rate.
3. Increase the flow when the pressure is stabilized to the working flow rate. Make sure that at least x20 column's volume was flowing through the column.
4. Watch the pressure and make sure it has stabilized (2-3% fluctuations).
5. Select the appropriate project to work with and Go to "Run Samples".
6. Select the appropriate "Instrument Method" and monitor the baseline until it stabilized. The system is then ready for injections.

Run the Samples

7. *Prepare a Sample-Set-Method in the Sample screen of the Run Samples window.* You can select an old *Sample-Set-Method* and revise it.
8. Select the working instrument method in the appropriate window and click on the *Monitor* icon to verify communication with the detector and to monitor the baseline. **You have to abort the monitoring to be able to begin injecting.**
9. When everything is ready, save the *Sample-Set-Method* and Click on the green light icon to run it. Monitor the pressure, temperature, composition etc. to verify that everything is OK.