

AKTA 10 Purifier Instructions, Check-list and Trouble-shooting

Start-up

1. Restart computer.
2. Power on the AKTA unit.
3. After self-tests on the AKTA unit are complete, launch Unicorn.
4. If communication with the AKTA unit is not established (i.e. you get an error message along the lines “Informational: unable to establish communication”), quit Unicorn, restart computer and re-launch program.
5. Sometimes you may get the following error on the P-900 unit: “Synchronizing pumps: Waiting for start” (note: this message is *not* displayed on the computer). Solution: do power cycling. The power switch is located at the back of the unit.

Do's and Don'ts

1. Always inspect lines for leaks.
2. Always keep an eye on the pressure, pH, flow-rate and conductivity.
3. The pH meter should never be kept on-line when organic solvents (e.g. 20% ethanol) are used.

Before starting a run

1. Fill two 500 ml flasks with UV/UF water. Transfer all four inlets to UV/UF water flask #1. Start “pump wash” and purge pumps. Transfer all four inlets to UV/UF water flask #2. Perform another “pump wash”.
2. Bring column on-line. Columns are usually stored in 20% ethanol (in UV/UF water). Always wash column with UV/UF water (be sure to use the correct flow-rate; flow-rates vary for different columns) in the “Manual” mode. Wash for at least 5 column volumes. This would be a good time to bring the pH electrode on-line. Be sure to wash electrode well with UV/UF water and calibrate.
3. Transfer inlets to respective buffers. Switch injection valve to “waste” and pump ~5 ml of buffer from each inlet. This is to ensure each inlet-line is saturated with current buffer. Be sure all the buffers are compatible (i.e. do not pump a buffer containing Zn/Ca salts and follow-up with a phosphate buffer). Always pump the start (or column equilibration) buffer last in this series.

Superloop set-up

1. Remove the lines connecting the superloop. Remove the end-fittings. Decant buffer (if any) from the loop. Visually inspect the inner chamber for any precipitated protein, etc. Wash thoroughly with UV/UF water. Wash with 20% ethanol. Wash again

thoroughly with UV/UF water. Use Kimwipes and wipe the inner chamber dry. Repeat process with the end pieces and the movable seal.

2. Slightly wet the end pieces and movable seal and assemble superloop. Re-connect lines labeled "SL Top" and "SL Bot" with the top and bottom ports of the superloop. To fill superloop, disconnect "SL Bot" with injection valve port 2. Change injection valve position to "Inject". Start pump at flow-rate of 10 ml/min. Stop pump when superloop is full and return injection valve to "Load" position. Re-connect "SL Bot" with injection valve port 2 and disconnect "SL Bot" from the superloop. Wash injection port in the injection valve. Re-connect "SL Bot" to the superloop. Inject sample through the injection port (be sure to not introduce any air bubbles during injection). Clean superloop when done (described above).

After performing a run

1. Be sure all proteins from column were flushed out or the column regenerated during the final step of the run. In the case of:
 - a. Ion-exchange chromatography, this will involve a column wash with a buffer of high ionic-strength.
 - b. Size-exclusion chromatography, this will involve a column wash with the eluent for at least 2 column volumes.
 - c. IMAC using Talon resin, this will involve a column wash with CoCl_2 .
 - d. GSH-Sepharose chromatography, this could involve a column wash with reduced glutathione.
2. Remove pH electrode and replace with dummy electrode. Wash the pH electrode with UV/UF water and store electrode in electrode storage buffer (be sure the electrode tip is in contact with buffer).
3. Transfer inlets from various buffer solutions to UV/UF water flask #1. Perform a "pump wash". Transfer inlets to UV/UF water flask #2. Perform another "pump wash".
4. Wash column with at least 5 column volumes of UV/UF water. Transfer inlets to 20% ethanol solution. Wash column with another 5 column volumes of 20% ethanol. Remove column to 4 °C refrigerator after capping both ends appropriately. Connect the lines that use to connect to the column. Quit program first and then turn off AKTA unit.