

ÄKTA Maintenance

General care during use

- (1) If either of the pumps is in 20% ETHANOL PLEASE WASH BOTH PUMPS WITH WATER BEFORE SWITCHING TO BUFFER. If a pump that has been washed with 20% ethanol is transferred directly to a salt-containing buffer, THE SALT WILL PRECIPITATE. If only one pump is washed with water, some ethanol will still remain in the connecting line and could precipitate salt.
- (2) When finished for the day, please wash pumps THREE times with water to remove salt build-up. If no one plans to use the ÄKTA for a few days, then wash and store both pumps with 20% ethanol.
- (3) Filter and degas all buffers. Use the filter stand by the new ITC bench. Once filtered, the buffers for HiTraps should be fine indefinitely. The gel filtration buffers need to be degassed every time.
- (4) After spinning cell lysate, DO NOT TRY TO GET ALL THE LYSATE INTO THE SUPERLOOP. Leave a few mLs of the last portion, which is mixed with cell debris, in the centrifuge tubes. It is worth the waste.

Gel filtration

- (1) Gel filtration columns should ALWAYS BE PRECEDED BY A PREFILTER. The filter needs to be changed periodically. If the back pressure of a gel filtration column is high but the system is reasonable, then ask Clara to show you how to change the prefilter.
- (2) When finished with gel filtration column, equilibrate with 1.5 column volumes water, then 20% ethanol (the flow rate for 20% ethanol needs to be ≤ 0.5 mL/min). Please leave the prefilter attached to the column and close the end when you are finished with use. Please rinse your loop with water, then 20% ethanol, and store.
- (3) If your protein precipitates on a gel filtration column, please wash the column by injecting 100 mM NaOH/250 mM NaCl in a running buffer of 250 mM NaCl (no NaOH in running buffer). If backpressure is still high, try the same with 500 mM Acetic acid substituted for NaOH.

Regular ÄKTA maintenance

(Note: We have 2 different models: Lemon and Orange="Äkta purifier," Peach and shared Äkta="Äkta pure")

Weekly:

(1) Each ÄKTA has a small bottle or 50-mL tube of 20% EtOH which maintains rinse lines to prevent the pumps from rusting. **These lines must not be allowed to run dry.** Once/week make sure bottles are full of fresh 20% EtOH.

(2) The ÄKTA pumps should be washed with "coffee-hot" water. NANOpure water may be warmed in the microwave, then pump wash as usual.

Once-a-month:

(1A) The titanium filter in each ÄKTA purifier needs to be removed and soaked in 100 mM NaOH and/or 500 mM Acetic Acid overnight to remove build-up. Back pressure increase may indicate treatment is overdue. There should be a clean titanium filter (18-1120-94) available in the ÄKTA supplies drawer that can be swapped out for the one being cleaned. If desperate, the titanium filter can be reversed and pump-washed.

(1B) The ÄKTA pure has a paper filter (18-1027-11) that can be replaced with a new one when the back pressure becomes a problem. They are located in the ÄKTA supplies drawer. (Note that Äkta pure flow restrictor adds ~0.18 MPa back pressure.)

If needed:

(1) Flow restrictor on waste tubing can be removed if necessary to reduce back pressure. However, waste container will need to be moved to top of ÄKTA so that buffer does not siphon dry by gravity.

(2) 500 mM Acetic acid also can be used to wash the pumps if needed. Please do not wash with NaOH since the pH is above the limits of the ÄKTA. Afterwards, immediately wash pumps THREE times with water. Please note: The limits of pH resistance for the ÄKTA are pH 2-12 allowed. 500 mM Acetic Acid is pH 2.5, 100 mM NaOH is pH 13.

(3) The drive sleeve (19-6067-02) on the fraction collector needs to be periodically replaced when it starts to skip tubes. These are in a cardboard box, ÄKTA drawer.

(4) The buffer intakes are covered by replaceable filters (11-0004-14). These can be, but rarely need to be, changed unless unfiltered buffers are used.