

FPLC method for P69

Clark Lab

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We need to ask to use the Baker Lab FPLC. Check with Renee and Becky and Ana before planning a run. We have found it is best to re-launch the software to make sure every part of the FPLC is communicating well.

Our Column is on valve #8 on the Baker Lab FPLC system. We share this valve with other columns, so re-connect it to the former column when finished. All runs have been done as Manual runs from the 'Kay' directory. If you do a run from another directory, we can move your files later.

Presently we are using a Source 15Q 10/10 column. (We also have a Mono Q.)

Buffer A = 50 mM Tris pH 8.8

Buffer B = 1M NaCl in 50 mM Tris pH 8.8

Buffers are filtered, degassed, and stored at 4°C.

WASH OUT LINES:

Initially start up the Source 15Q column by washing out the lines at 4 ml/min, 50%B, injector set on load, valve set on Bypass, 5 minutes end time, with water in both A & B lines.

PRIME LINES:

Place lines for Buffers A&B into reservoirs. Prime lines with 50% B, injector set on load, Valve set on bypass, 2 minutes end time should be enough at 4 ml/min.

CONDITION COLUMN:

Run at least 10 ml of buffer 100%B on the column. 4 ml/min, injector set at load, valve #8, 3 min.

Then, wash out salt with 100% A, 4 ml/min, injector set on load, Valve set #8, 10 minutes end time.

INJECT SAMPLE:

Loops of various sizes are available: 500ul, 1ml, 2ml, and the loops can be connected in series to add up to 5.5 ml. To inject with loops and syringe:

Stop flow, put injector setting on LOAD, using syringe, wash loop with buffer (5x loop vol.)

Set injection valve to INJECT and remove syringe. This prevents air entering the loop and/or buffer draining out.

Fill syringe with sample: up to or equal 1/2 total loop volume.

Insert syringe in port on injection valve, set valve to position LOAD.

Load sample into loop, leave syringe in position.

Sample gets onto column when INJECT is selected as position in method, or manually selected.

To load larger samples: Samples can be pumped onto the column using the A buffer line, if the pressure is watched and any overflow is captured in case of errors in valve position or overloading. A buffer line is rinsed with A buffer and this is pumped on the column as well: 4ml/min, valve position #8, set end time to volume.

Once sample is on the column, wash with A buffer to baseline (2X col vol = 20ml) and then start the gradient. We have been doing 0%B to 30%B over 1hr. 4ml/min, injection valve #8, fractions of 8ml to start then down to 2ml when we see our peaks start to appear. Just change the fraction settings (do not restart the gradient.)

We have stopped before the run gets to the 1 hr, after peaks elute and absorbance returns to baseline. Be sure to watch that the fractions are being collected!

Print report!

To clean up the column, run the gradient up to 100% B to clean resin bed. Leave here until next run.

Wash lines out on 50% B, with water in A & B reservoirs, 4 ml/min, valve position set on bypass, then pump and store in 20% EtOH if no one is waiting for the FPLC.

To store column if finished for over 1 week: equilibrate column in 20% EtOH/H₂O.