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Akta Pure General Protocol V.2

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We use this protocol and it's working

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Abstract

A general protocol for running and cleaning the Akta Pure. Always refer to manuals for specific columns before a run for specific details regarding a column's pressure limits, capatability with specific buffers and reagents, equilibration procedures, and cleaning procedures.

Troubleshooting

Connect buffers and prime pumps

1 Complete a pump wash with water on all pumps (A and B). To do this, put all buffer lines to be used (i.e. A1, B1) into filtered water, then in Unicorn, select a buffer inlet line (i.e. A1), and click "Start pump wash."

2 If the system is in ethanol, do a system wash with water before running any buffer through the system (especially buffers with high salt concentrations)!

3 Connect up to four buffer bottles to buffer lines A1, A2, B1, and B2. The end of the buffer line should be submerged fully in the buffer. Buffers should be filtered through a 0.22 µm filter before connecting to the buffer lines.

4 Prime pump(s), especially if the buffer line(s) have a lot of air.

In Unicorn, select the buffer inlet line you want to prime (i.e. A1) and start a pump flow (5-10 mL/min).

Pump flow is not necessary when priming, but can help to get rid of air bubbles.

Attach the purge syringe to the pump by connecting the purge tubing to the purge valve of the pump you are priming. Open the pump valve at least half a turn and gently draw back on the syringe. Continue until all air bubbles are removed. Close the pump valve, remove the syringe, and repeat on the other purge valve.

Always purge both valves on the pump!

Pump wash

5 Perform a pump wash (and/or system wash) with buffers in reverse order of how they will be used. For example, if you intend to run a sequence of 1) A1-buffer1, 2) B1-buffer2, you will want to run a pump wash (and/or system wash) for B1, then A1.

Connect column

6 In Unicorn, go to "Manual Instructions" > "Alarms" > "Alarm pre column pressure." Set the high alarm pressure limit that is appropriate for the column.

7 Set a low flow rate (i.e. 0.5 mL/min) and change the column setting from "bypass" to "column down flow."

8 Remove the stop plugs from both ends of the column. Connect the column outlet tubing (from port 1B of the column valve V9-Cs) to the bottom of the column.

9 With the flow running, take the column inlet tubing (from port 1A of the column valve V9-Cs) and let the buffer flow drop-by-drop into the connection port on the column. When the connection port is filled with buffer, connect the column inlet tubing to the column.

Connecting the column "drop-to-drop" ensures that no bubbles are introduced into the column!

Equilibrate column

10 Remove storage buffer from the column by running at least one column volume of water. Equilibrate the column with at least 1.5 column volumes of starting buffer. Refer to specific column manuals for details of how columns should be equilibrated before a run.

Prime and fill sample loop

11 Fill a syringe with at least 2x the amount of water as the size of the sample loop. With the injection valve set to "inject," connect the syringe to the syringe port in the injection valve. Set the injection valve to "manual load" and start a pump flow with buffer. With the flow running, plunge the buffer in the syringe into the syringe port. Water should flow out from the waste line.

12 Repeat with your starting buffer.

13 Set the injection valve to "inject." Remove the syringe and connect a syringe with your sample to the syringe port. Set the injection valve to "manual load," then plunge the sample in the syringe into the syringe port.

To conserve sample so that sample is not lost due to laminar flow effects, do a partial loop fill where less than 1/2 of the loop volume is filled into the loop. To prevent sample dilution, do a total loop fill where the sample loaded into the loop is 2x the loop volume.

Fill fraction collector

14 Fill the fraction collector with tubes. The tube sensor on the arm of the fraction collector should be placed against Tube 1.

Inject sample into column

15 Set the desired flow rate with the starting buffer. The column should be equilibrated, and all pressure, UV, and conductivity reading should be stable. Set the injection valve to "inject" to load the sample onto the column. To fractionate the column "flow through," set the fraction collector to collect fractions by volume or UV.

Slower flow rates tend to increase resolution!

Run column

16 Run the column as desired by manipulating the flow rate and buffer concentrations (%B).

Elution

17 Elute samples off of the column as desired by manipulating the flow rate and buffer concentrations (%B).

Collecting smaller fractions can increase the resolution!

Cleaning the column and system

18 Refer to specific column manuals for details on how columns should be cleaned. Attach up to two cleaning solutions (one will likely be 0.1 - 1M NaOH), water, and 20% ethanol to the buffer lines and purge pumps as necessary. Perform system wash methods for the buffer inlet line for the solution you intend to use first.

19 Run the first cleaning solution through the column until UV levels stabilize at 0, then run NaOH through the column. When running NaOH, flow the buffer through to all flowpaths by setting the injection valve to "inject" and sending the column outflow to the fraction collector, then to the waste outlet. Run NaOH until UV levels stabilize at 0.

20 Flush the column and the system with water. Again, flush all flowpaths with water by setting the injection valve to "inject" and sending the column outflow to the fraction collector, then to the waste outlet.

21 Run 20% ethanol through the column and system. Fill the entire flowpath with ethanol by setting the injection valve to "inject" and sending the column outflow to the fraction collector, then to the waste outlet.