AKTA METAL CHELATING SOP

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Materials/Reagents/Equipment	Vendor
1. Water + 0.05% sodium azide	
2. 0.2 um filtered buffers required for method to be run.	
3. ~300 mL extra buffer A to wash, prime lines	
4. 100% EtOH	
5. 20% EtOH	
6. Columns required for method to be run	
7. collection tubes 12 x 75 mm (up to 4.5 ml)	VWR #60818-383
18 mm (up to 12.5 ml)	Sarstedt tubes

Procedure

I   Performing a HTP Metal Chelation Purification Method

A. Preparing the system for the HTP MC purification method

1. If computer is not on, start it. Log on as Administrator with **bsgcakta** password. Start Unicorn software by double clicking on the Unicorn 4.11 icon on the desktop. Log in as yourself. The instrument should always be on in the cold box. Unicorn will communicate with the instrument and acknowledge when the instrument is ready to operate. There are four windows available in Unicorn. They can be accessed from tabs at the bottom of the screen.

The **Main menu** will appear on the screen. From here purification methods can be selected to run or edit and result files can be selected to view or edit.
The **Method editor** window which allows for the writing or editing of existing methods
The **Evaluation** window allows for analyzing previous runs.
The **System Control** window is where manual operations are controlled and all methods are monitored.

Click on the System Control tab. This screen has 4 windows.

Run Data   is where current values are observed for any selected parameters
Curves is where selected data is graphed vs. time
Flow Scheme   is where manual control of the system is initiated
Logbook   is where actions are recorded

2. It is important to know how many samples of what volume will be processed for proper set up of the system.

3. Place instrument lines into the respective buffer. Make sure buffer amount is sufficient for method to be run. Below is a table of the recommended amounts of the various buffers required for the method. The volumes listed/ sample are for a 5 ml column. The values should be adjusted for a column of a different CV. The second volume is fixed for all column volumes.

inlet position	buffer	volume
A11	affinity binding, washing	130 ml/sample + 100 ml
A17	A11 with 1M NaCl	5 ml/sample + 60 ml
B1	elution buffer, A11 + 1M imidazole	45 ml/sample + 100 ml
S7	100% ETOH for sample pump wash	125 ml/sample
S8	buffer A11 for sample pump wash	80 ml/sample + 100 ml
S1-S6	buffer A11	~200 ml

4. Change out 20% EtOH pump wash solution. Empty the waste container.

5. Check that the pressure limit of the system and sample pumps is set to match the pressure limit of the column(s) to be used (ie 5 ml HiTrap MC columns have a working pressure limit of 0.3 MPa).

6. Select Pump - PumpWashExplorer. Select A17 on, the others off. Select Execute to start the macro. If air bubbles are seen in the buffer lines, they can be purged at the pump head with a syringe. Pumps are located behind the instrument door. Pump A on the left and pump B on the right. When the A17 wash is done, repeat the PumpWashExplorer with A11 on, A2 off, B1 on, B2 off. This will use ~30 ml of each buffer.

7. Manually flush each of the sample lines to be used. Place each sample line in a container of buffer A. Sample line 8 should remain in it's own bottle of buffer A as it will be used for washing the sample pump in between runs. Select: Pump – SampleFlow and input 20 ml/min as flow rate. Each of the separate sample lines can be rinsed by selecting: Flowpath – SampleValve and selecting the appropriate sample line. A 30 second flush at 30 ml/min. should be sufficient. Stop the sample pump by inputting 0 for SampleFlow value.

8. Up to six columns can be used for each run. The columns will be connected to a pair of rotary valves. Column valve V2 is the column inlet valve and column valve V3 is the column outlet valve. Each of the column valves has the valve positions marked on the side of the valve body. Position 1 is used as a bypass and as such no column will be connected there. With the system pump running at 0.5 ml/min. (Pump – Flow – FlowRate = 0.5), direct the system flow to the column position desired (Flowpath – ColumnPosition – position X). Connect the inlet of the desired column to the appropriate tubing from column valve V2. The columns' outlet will connect to the parallel port on column valve V3. After a column has been connected, increase the flow rate to the maximum used for the method (4 ml/min. for 5 ml HiTrap MC). Observe the column connections for leakage and tighten fittings as required. After all columns have been connected, return the system flow to 0 ml/min. and the column valves to Position1Bypass.

9. Fill the fraction collector rack with tubes. Reset the fraction collector by selecting Frac – Reset_Frac_Position – Execute.

10. Place the sample lines into the container(s) of filtered clear lysate. Confirm that the lines are free of bubbles as these may prematurely activate the air sensor and stop sample flow.

11. Ensure that each of the system outlet lines to be used (outlet valve V4, positions F3 – F6, F7 = loop1, F8 = loop 2) is connected to a container of sufficient capacity to hold the flow through. Container should be sized for ~200% of load volume.

14. When all preparations are complete, click the END button on the menu bar. This will stop the manual operations.

B. Selection of Method and Variables

1. There are several methods already programmed on the instrument for automated MC purification. The details of these can be viewed in the Method Editor window. Open the User file and select the method of interest. There will be three panes to view.
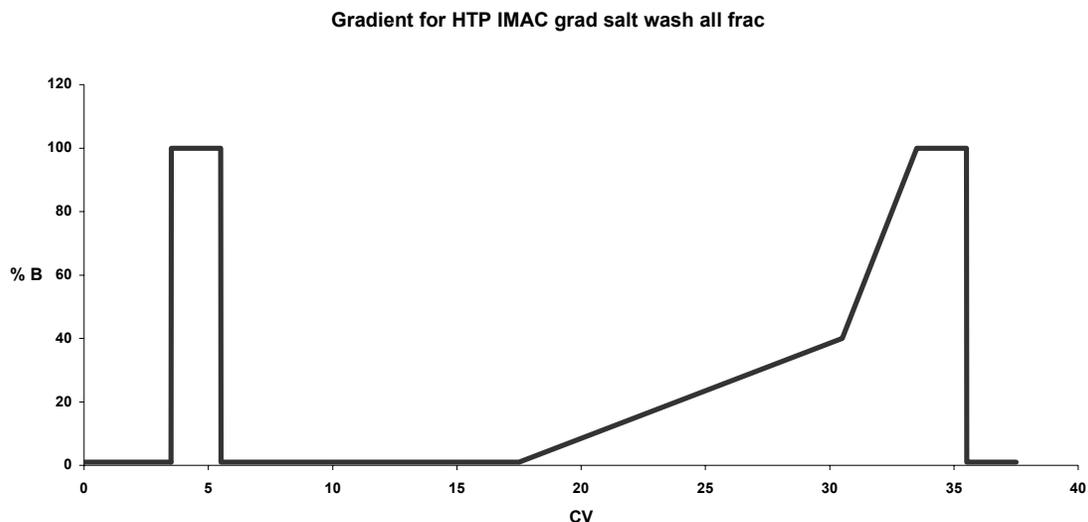
Gradient   shows %B buffer over the course of the method

Text   shows the instruction blocks for the method

Instruction box   this is where changes can be made to the method.

2. The method   *HTP IMAC grad salt wash all frac* is a convenient method. Is has a scout function selected that allows for running 1-6 separate purifications with the same method. 3 ml fractions are collected. Sample loading and elution is done at 2 ml/ min. and washes are at 4 ml/min. This method includes:

1 CV 100% B blank wash
5 CV column equilibration with 1% B (10 mM imidazole)
discrete sample loading and flow through collection
5 CV post sample load wash with 1% B
1 CV 1M salt wash with 1% B (start of fractionation)
2 CV 1% B wash
13 CV 1 – 40% B (10 – 400 mM imidazole) gradient
3 CV 40 – 100% B (400 – 1000 mM imidazole) gradient
2 CV 100% B (1000 mM imidazole) wash
2 CV 1% B (10 mM imidazole) re-equilibration
end of fractionation

Gradient for HTP IMAC grad salt wash all frac

Note: Please consult an experienced AKTA user if a modified method is desired.

3. To start a method, go to the System Control window and click the Run button. Select the method to be run. A series of instruction windows will come up that will require the operator to acknowledge and/or input parameters. They are:

Scouting: this is the page where the number of columns can be selected. Right click the run# of the column for a run that is to be excluded. Column, sample and flow through position, are selected. Sample size is indicated. Usually this volume is 200% of the actual sample volume. The AKTA uses an air sensor on the sample line to stop the sample pump and begin pump and column washing, etc. Fraction size can be selected. 3 ml is the default if 6 x 5 ml columns are to be run with this method.

Method information: various information about the method to be run including duration and buffer requirements.

Result name: the location and name of the results file is defined and the method started.

C. Analysis of results

1. Results of completed runs can be analyzed from the Evaluation window. Files may be selected from the Results pane of the main window or by opening the desired file directly from the Evaluation window.

2. When the file is opened, a chromatogram of the run is displayed. By selecting the **Chromatogram Layout** icon from the menu bar various attributes of the chromatogram can be selected; which curves to be viewed, X and Y axis attributes etc. The desired chromatogram can then be printed for reference with fraction analysis. De-select **thick lines** and select **landscape** for the best printed image.

D. Shutting down the system

1. When the runs are complete, Manual control will have to be initiated again to clean the system. Any of the soiled sample and outlet lines will be rinsed with H2O, 0.05% NaN3. This is accomplished by placing the sample lines to be cleaned in a container of H2O, 0.05% NaN3. Place the outlets lines in a waste container. Check that the pressure limit of the sample pump is set to 0.3 MPa. Have the column valve set to the Bypass position and the Injection valve set to the inject position. Follow the instructions as in A.9 to activate the sample pump and sample valve. After the final sample line has been rinsed, switch the outlet valve from waste, to F3, F4 etc. until all outlet lines have been rinsed.

2. Columns will be disconnected from the system for storage. With the injection valve in the inject position, H2O, 0.05% NaN3 can be pumped through the columns at 0.5 ml/min. with the sample pump to maintain a liquid stream while the columns are being disconnected. The details of column connection are found in A.8 above.

3. If so desired, the buffers can now be removed from the system and replaced with H2O, 0.05% NaN3 for system storage.

4. Click End to stop manual control of the system and end data collection.