

AKTA 3D SOP

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| Materials/Reagents/Equipment | Vendor |
|---|----------------|
| 1. 0.2 um filtered 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 Purification Method**

A. Preparing the system for the 3DEXPII purification method. This method will perform affinity – desalt – ion exchange on up to four samples. See B.2 below for details.

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.

- Place inlet 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 for A11, A17, B1 and B2 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 | 225 ml/sample + 100 ml |
| A12 | desalt, IEX A | 450 ml/sample + 150 ml |
| A17 | buffer A11 + 1 M NaCl | 5 ml/sample + 60 ml |
| B1 | aff. elution. A11 + 1M imidazole | 30 ml/sample + 100 ml |
| B2 | IEX elution. A12 + 1M NaCl | 60 ml/sample + 100 ml |
| S 7 | 100% EtOH | 125 ml/sample or fill bottle |
| S8 | same as A11 | 65 ml/sample + 100 ml |

- Change the 20% EtOH pump wash solution. Empty the waste container. The waste container should be able to contain about 3L.
- Check that the pressure limit of the system and sample pumps is set to match the pressure limit of the column to be used (ie HiTrap HP columns have a working pressure limit of 0.3 MPa).
- Click on the instrument control icon. Double click on the Pump A icon in the Flow Scheme window to open the Instruction dialogue box.
- Select Pump - PumpWashExplorer. Select A17 on, A2 off, B1 off, B2 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. This will use ~30 ml of each buffer. Repeat the pumpwashexplorer with A12 on, A2 off, B1 off, B2 on. Repeat the pumpwashexplorer one final time with A11 on, A2 off, B1 on, B2 off.
- Manually flush the sample line(s) to be used. Place the sample line in a container of buffer A. Select: Pump – SampleFlow and input 20 ml/min as flow rate. A one minute flush at 20 ml/min. should be sufficient. In addition, lines S7 and S8 should be flushed if these buffers were changed for this run. Stop the sample pump by inputting 0 for SampleFlow value.
- The affinity 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 position marked on the side of the valve body. Position 1 is used as a bypass and as such no column will be connected there. Position 7 is reserved for the IEX column and position 8 is used for the deslating column. 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 (5 ml/min. for 5 ml HiTrap). Observe the column connections for leakage and tighten fittings as required. After the column has been connected, return the system flow to 0 ml/min. and the column valves to Position 1 Bypass.

10. Fill the fraction collector rack with tubes. Reset the fraction collector by selecting: Frac – Reset_Frac_Position – Execute.
11. Place the sample line(s) into the container of sample. Confirm that the line is free of bubbles as these may prematurely activate the air sensor and stop sample flow.
12. The flow through from affinity column binding will be collected in separate containers. Ensure that the system outlet line to be used for flowthrough collection (outlet valve V4, positions F3 – F6) is connected to a container of sufficient capacity to hold the flow through. This volume is sample volume plus 20 ml.
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. The method **3DEXPII** is programmed on the instrument for automated purification of his tagged proteins. The details of this method are 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

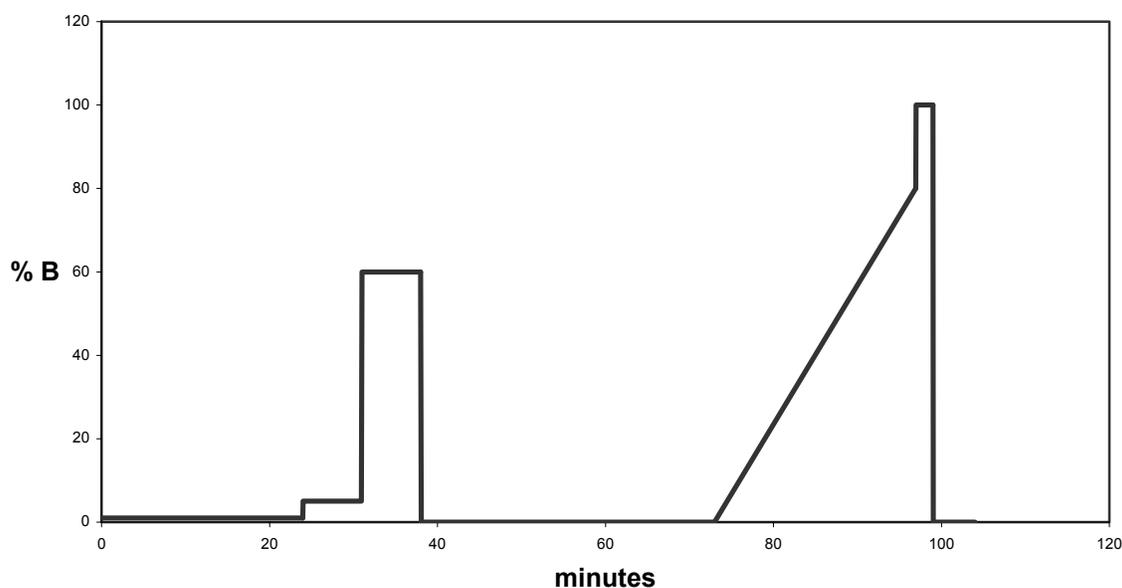
Instruction box this is where changes can be made to the method.

2. The method **3DEXPII** is a convenient method for automated 3 step (affinity – desalt – IEX) purification of his tagged proteins. It uses automated sample injection via the sample pump to individual affinity columns. A common desalting and IEX column are used for all samples. All samples processed during one run should have pIs in the same range because either a cation or an anion exchanger will be used for the final step. Affinity flow through is collected separately in bulk for each sample. The IEX flow through and eluate are collected as fractions. Below is a list of the basic steps.

5 CV equilibration of affinity column with 10 mM imidazole
sample loading and flow through collection
5 CV post sample load wash with 10 mM imidazole
1 CV post sample load wash with 1 M NaCl
7 CV post sample load wash with X mM imidazole
step elution from affinity column with X mM imidazole
collection of eluted peak in a 10 ml loop

equilibration of desalting column
injection of collected affinity peak onto desalting column
collection of desalted sample in a 15 ml loop
5 CV equilibration of the IEX column
injection of collected desalted peak onto IEX column
collection of IEX flow through as fractions
3 CV wash of IEX column collected as fractions
elution of IEX column with 20CV during a defined gradient
collection of IEX eluate as fractions

Gradient of 3DEXPII



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 certain default parameters can be changed to fit the experiment to be performed. If less than 4 runs are to be performed, right click on the run numbers to be excluded. Sample volume should be 200% of actual volume because the air detector will stop sample flow prior to air entering the system. Concentrations of imidazole for affinity column wash and step elution as well as the desired IEX gradient are selected at this time for each run.

Gradient: A graphic view of the %B during the course of the run.

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.8 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.9 above. The desalting or IEX column may be left on the system after equilibration with H₂O, 0.05% NaN₃. This should involve flowing the H₂O + NaN₃ until the conductivity has stabilized.
3. If so desired, the buffers can now be removed from the system and replaced with H₂O, 0.05% NaN₃ for system storage. Flush the water through the buffer lines with pumpwashexplorer as in A.7 above.
4. Click End to stop manual control of the system and end data collection.