

# AKTA PURIFIER PROCEDURE

## START UP EACH DAY

1. **Select Unicorn Version** 4,0 main menu. Unicorn screen comes up. Press Okay
2. Four windows are automatically opened:
  - a) Unicorn Main menu
  - b) Method editor
  - c) System Control Panel
  - d) Evaluation
3. **Select System Control Panel**
4. Click Manual on the Toolbar
5. Pump Instructions Window opens

The following parameters need to be changed if you want to rinse/clean a column in place or at the end when you want to store the column

6. **Select pump option:**
  - a) Flow-can set the flow rate from 0,1 ml/min (always begin this low)
  - b) Gradient-can set up a buffer gradient or to wash with 20% ethanol (this is always processed through inlet B1 and B2)
  - c) Buffer Prep pH (only used during runs when system sets up a buffer/pH gradient)
  - d) Pump Wash Purifier-can select the inlets to be used to wash the pumps
  - e) Pump Wash Base-do not use yet-only use when
7. **Select Flow Path option:**
  - a) Injection Valve-can select load or inject
  - b) Pump inlet-can select A1, A2, B1 or B2
8. **Select Alarm option:** Most of the options are preprogrammed but you can change the alarm pressure value for each column. Never set it higher than 1,8mPa. Use this option to switch the UV light off when rinsing the column.
9. **Select Frac option:** most of these values are preprogrammed
10. **Select Other option:** when rinsing/washing a column you can select the run to end after a specified volume has flushed the column (acquired volume) or after a specified time (acquired time). The former option is always easier.
11. Once the variables are set up you can start rinsing the column as follows
12. **Switch on the Akta** at toggle switch
13. Wait for pump LCD to display END at the completion of its synchronization cycle
14. **Select Flow path.** Tell system to rinse with solvent connected to inlet A2
15. **Press Execute** (check for bubbles in line-if present remove with syringe as described below)
16. Purge the pump manually with a syringe to remove any bubbles (attach syringe to outlet, unscrew black stopper, draw solvent through the pump by withdrawing the syringe until no bubbles appear in the tubing)
17. **Select pump option.**
18. **Select pump wash/purifier**
19. **Press Execute** (will wash pump at max 10ml/min with water/buffer for 4 minutes. Water/buffer goes to waste)
20. Slowly increase the flow rate until the pressure stabilizes at 1,2mPa. If pressure alarm is activated (level goes beyond 1,8mPa set), chose the Acknowledge option on the window that pops up. Drop the flow rate. Select Execute followed by Continue on the toolbar.

21. Check flow rate into waste bottle. If regular then no bubbles are in the system. If irregular then remove bubbles with a syringe by purging the correct pump.
22. Check volumes in solvent bottles (you need calculate to have enough volume to prevent the pump running dry under the pressure selected - this is very bad as piston can be damaged)

## **SOLVENT PREPARATION**

1. Use only MilliQ water for buffer and all solvent preparation
2. Do not store buffers longer than a week
3. Sterile filter all buffers through a 0,45µm Millipore filter system
4. Sonicate buffer to get rid of air bubbles for 10-15 minutes. Not longer otherwise buffer heats up too much
5. When using pure MilliQ water, there is no need to filter sterilize, but it needs to be sonicated for 30 minutes to get rid of bubbles
6. Prepare 20% ethanol in MilliQ water to keep the pumps lubricated by recirculation at end of every run. Small bubbles in storage system not a problem
7. Remember always to flush with water after storage in ethanol. Do not go directly from ethanol to a buffer solution containing salts as precipitation of salts will occur and may damage pump/column

## **SAMPLE PREPARATION**

1. Remove approximately 2 liters of the supernatant from a cell culture by centrifugation at 15 000rpm for 10 minutes. This supernatant contains all the extra-cellular proteins or enzymes or secreted proteins and enzymes
2. Check for the presence of protein using a dipstick.
3. If protein is present, concentrate the supernatant from 2 liters to about 40 ml using a Minitan or Amicon system
4. Centrifuge the concentrate again at 15 000rpm for 10 minutes
5. If running an ion exchange column, dialyse the sample with the start buffer to get the pH the same as the start buffer. This usually involves 20 ml sample in 2 000 ml start buffer overnight in cold room.
6. If running a hydrophobic interaction column, check whether salt dialysis is necessary to equilibrate the sample with the start buffer's salt concentration
7. Put the dialysed sample through a 0,45µm filter
8. Check the pH of the sample for ion exchange columns
9. Put this sample through a 0,22µm filter
10. Do an accurate protein determination on the concentrate using the Bradford method
11. If doing a run for the first time with unknown protein profile, run an SDS page gel of the crude concentrate to see how many different proteins to expect.
12. Add sample to AKTA purifier.
13. The volume to be used depends on the column. The protein concentration to be used also depends on the column

## **PREPARING FOR A RUN:**

Between each samples the following steps need to be carried out:

1. Pump Wash Purifier for 5 minutes with correct buffer
2. Wash the column with 1 column volume of buffer (about 25ml for Superdex 75)
3. Pump wash purifier with the same buffer for 5 minutes

## LOADING SAMPLE

1. Select or create method to be run. In the file entitled BEN the following programmes exist:
  - a) superdex 75 (gel filtration)
  - b) mark (anion exchange)
2. For commercially bought columns check that all parameters are within column specifications
3. For home packed column, check resin specifications and column parameters
4. Before loading the sample wash the sample loop with starter buffer 5X the sample size as described below.
5. Various sample loops exist
  - blue hold 10 $\mu$ l
  - Orange holds 100 $\mu$ l
  - Green holds 1ml
  - Super loop holds 10ml
6. Select flow-path option. Select on LOAD position (default position)
7. Using a syringe containing about 5ml buffer, attach it to the port and load 5ml onto the sample loop
8. Select on INJECT position and press EXECUTE
9. Remove the large syringe and add about 0,3ml sample to a smaller syringe.
10. Attach smaller syringe to port. Select on LOAD position and load 0,3 ml to the sample loop.
11. Select INJECT and press END. The programme selected now controls the run further.
12. Each time you switch between LOAD and INJECT you can hear the valve changing position.
13. Set the arm of the fraction collector over eppendorff tube number 1
14. Do not fiddle with the System Control Menu during a run except to change the flow rate when alarm conditions are reached (e.g. pressure reaches 1,8mPa)

## POINTS TO REMEMBER

1. To clean the On-line filter:

Remove for cleaning when pump pressure goes up

Add to 50 ml 100% ethanol

Put in sonicator bath for 10 minutes

Remove ethanol

Replace with fresh ethanol

Repeat

Rinse with distilled water and replace in line

2. Use of the Flow Restrictor:

This is added to the system when large column are installed to enable the pressure to remain constant over the entire length of the column. It creates a back-pressure of 0,25mPa. When using a short column with low pressure limits, remove the flow restrictor from the line.

## SERVICING INFORMATION:

1. Supplier is Separations Scientific Tel no: 021 671 6748
2. Contact person: Rikus Lotter
3. Amersham Pharmacia Biotech supplies all the columns
4. Spares available: In lab A341
5. Columns available:
  - a) Superdex™ (Code: 17-104-01)
  - b) HiTrap™ HIC Selection kit
  - c) HiTrap™ PhenylFF
  - d) HiLoad™ 16/10 SP Sepharose (Code: 17-1137-01)
  - e) QFF-5ml self pack column
  - f) Resource Q (1ml) (Code: XK16 040985)
  - g) MonoQ HR5/5 prepacked with Monobeads (Code: 17-0546-01)
6. Resins available:
  - a) CM Sepharose™ Fast Flow 25ml (Code: 17-0719-10)
  - b) Resource™ S (Code: 17-1178-01)
  - c) Resource™ Q (Code: 17-1177-01)