Heparin Superflow 4

Protein binding to Heparin Superflow 4 is generally most efficient at neutral pH and low ionic strength. Elution usually requires a high ionic strength buffer. Heparin Superflow 4 is manufactured to withstand flow rates of 680 cm/hr. Batch processing is an option that can lead to improved product recovery as well as reduced processing time. As with all chromatography, purification conditions may need optimization.

Protocol:

(Note: buffers for heparin chromatography vary greatly depending on the application; these conditions are recommended for AT3 purification and are not to be considered generic).

1. Measure the appropriate amount of resin to fill the column.
2. Wash the resin with 5 bed volumes (BV) of deionized (DI) water.
3. Equilibrate the resin by washing it thoroughly with 3 BV of Binding Buffer. The recommended flow rate for the washing and equilibrations steps is 200cm/hr.
4. Load protein sample. The recommended flow rate for this step is 100cm/hr.
5. Wash to remove unbound material with excess Binding Buffer. The recommended flow rate for washing is 200cm/hr. The optical density of the wash should approach baseline.
6. Elute bound protein with Elution Buffer until the OD (A280) shows that most of the bound material has been eluted. The recommended flow rate for this step is 100cm/hr.

Reagents:

Binding Buffer: 10mM Citrate, 50mM NaCl, 20% Ethanol, pH 6.8
Elution Buffer: 10mM Imidazole, 4M NaCl, pH 6.5
Storage Buffer: 20% Ethanol

Sanitization and regeneration:

1. Sanitize the resin with 1 BV of 1M NaOH for 1 hour. The maximum recommended flow rate for steps 1-3 is 200cm/hr.
2. Wash the resin with DI water until neutral.
3. Equilibrate the resin by washing with 3 BV of Binding Buffer.
4. Column is now ready for reuse.

Storage:
Store the cleaned beads in 0.1M NaOH at 2-8 °C for up to six months or as a 70% slurry in 20% ethanol for extended storage.