

### **Introduction**

Adar's Heparin Beads are made by coupling 20-35 KD size Heparin extracted from Porcine intestinal mucosa (Type I) to Sepharose beads. These beads are useful for isolating heparin-binding protein such as Antithrombin III, lipoproteins, various enzymes and DNA binding proteins.

### **Heparin Beads Specifications**

Matrix: Sepharose™ CL-4B

Coupling method: TargetLock.

Heparin density: 0.7-1.2 mg/ml of 20-35 KD Heparin

Thrombin binding capacity minimum 0.7 mg/ml of beads

Mean bead size: 40 -165 µm

Range of pH values allowed: 3.0-11.0

Bead structure: Highly cross-linked spherical agarose, 4%

Max back pressure: 0.3 MPa, 3 bar

Max. flow rates: 4 ml/min/cm<sup>2</sup>

Recommended flow rate: 1-3 ml/min/cm<sup>2</sup>

Storage: 4°C in PBS pH 7.4, 0.3N NaCl added with 0.01% (w/v) Thimerosal as a preservative.

### **Protocol: Salt purification of Heparin-binding proteins**

1. Invert bottle several times to mix beads in storage buffer.
2. Pack column with 2-4ml beads
3. Equilibrate column with 10mM Tris-HCl pH 7.5. Matrix is robust -You may modify buffer to include reducing agents, urea, guanidine, sodium thiocyanate and detergents as needed.
4. Load you protein sample on column at 0.5-1.0 ml/min.
5. Wash column with approximately 30 volumes of the equilibration buffer.
6. Elute bound proteins by applying high salt buffer (10mM Tris-HCl pH 7.5 added with NaCl to 1.5N or other commonly used salts such as KCl and Ammonium sulfate).
7. Wash column with 20 volumes of ddH<sub>2</sub>O to regenerate it.
8. Equilibrate column with 10mM Tris-HCl pH 7.5.
9. Store column at 4°C in PBS pH 7.4, 0.3N NaCl added with 0.01% (w/v) Thimerosal as a preservative