USER GUIDE: AM-50 Heparin resin

Introduction:

AM-50 Heparin resin is a well established affinity medium designed for capture or intermediate purification steps. This medium is extensively used for industrial purification of several marketed plasma proteins. Industrial purification of antithrombin III, coagulation factors and other plasma proteins. Coupling chemistry gives chemically-stable ligand attachment. Stability of medium solely dictated by heparin ligand. AM-50 Heparin resin is made with monodispersed polymer beads by coupling the ligand molecules to the matrix using chemically stable and oriented coupling giving high binding capacity. The chemical stability of the media is solely dictated by the heparin ligand. This media supported utilization from lab scale to industrial applications.

AM-50 Heparin allows for fast and reliable separations of biomolecules with an affinity for heparin. These includes but not limited to the following categories:

1. proteins in plasma, such as antithrombin III, coagulation factors and others
2. proteins related to Nucleic acid metabolism, such as DNA and RNA synthetases, ligases
3. DNA binding proteins, lipoproteins, protein synthesis factors
4. steroid receptors.

The proteins including enzymes purified with immobilized heparin have a long list, and some of them are listed below: mast cell proteases, lipoprotein lipase, coagulation enzymes, elastases, extracellular superoxide dismutase, serine protease inhibitors, such as AT3, heparin cofactor 2 and protease nexin, which inactivates serine proteases in the extracellular matrix. Other heparin binding proteins in the extracellular matrix are vitronectin, fibronectin, thrombospondin, laminin, collagen and Growth factors such as fibroblast growth factor (FGF), Schwann cell growth factor, retinal survival factor and homopoietic growth factor.

AM-50 Heparin is linked by reductive amination and the resulting bond is stable even under alkaline conditions. Thus, the chemical stability of AM-50 Heparin resin is limited only by the heparin ligand itself. Because of the oriented coupling of the heparin ligand and the used spacer, the specific binding activity is enhanced. Table 1 lists the basic characteristics of AM-50 Heparin resin.

Specifications:

Particle Size (µm)	50
Pore Size (Å)	800
Ligand Density	about 5 mg/mL heparin of porcine origin
Target Protein Binding Capacity (g/ml)	2 mg antithrombin III
Bead Matrix	Polyacrylate
Recommended Flow Rate (cm/h)	200-400
pH Stability	4-13
Pressure (MPa)	10
Chemical stability	0.1 M NaOH (1 week at 20°C) 0.05 M sodium acetate 4 M NaCl, pH 4.0

	8 M urea 6 M guanidine hydrochloride
Storage buffer	0.05 M sodium acetate containing 20% ethanol
Storage temperature	+4–8 °C

Experimental procedures:

Recommendations and comprehensive instructions for packing and use are included in information supplied with AM-50 Heparin. The following details highlight some key aspects of the operations.

Binding:

Binding normally occurs around physiological pH. A buffer of 10 mM phosphate buffer pH 7.30, 15 M NaCl is suitable for most of the samples. Other buffers could possibly be alternatives in your work.

Washing:

10 column volumes of binding buffer to remove unbound and non-specifically bound material.

Elution:

Normally, proteins elute at concentrations of salt lower than 1.5 M. Suitable elution salts are NaCl or KCl. As individual proteins often bind by a unique combination of specific affinity and/ or ionic interactions, small differences between proteins can result in good purification factors. Substances bound due to ionic interactions may be eluted by using an increasing salt gradient (linear or step).

Regeneration:

Washing the medium with 2-3 bed volumes of alternating high pH (0.1 M Tris-HCl, 0.5 M NaCl, pH 8.5) and low pH (0.1 M sodium acetate, 0.5 M NaCl, pH 5.0) buffers. This cycle should be repeated 3 times followed by re-equilibration in binding buffer. Strongly adsorbed proteins can be removed by including 8 M urea or 6 M guanidine hydrochloride in the regeneration buffer. Re-equilibrate the column with at least 5 bed volumes of binding buffer. An alternative method for regeneration of the medium is to wash with a nonionic detergent, e.g. 0.1% Triton™ X-100 at 37 °C for one minute followed by re-equilibration with at least 5 bed volumes of binding buffer. If necessary, 8 M urea or 6 M guanidine hydrochloride may be added to the detergent solution. Re-equilibrate with at least 5 bed volumes of binding buffer.

Sanitization:

Sanitization reduces microbial contamination of the medium. A recommended sanitization procedure is to treat the packed column with 0.1 M NaOH and 20% ethanol for 1 h or to allow it to stand in 70% ethanol for 12 h. Always wash the packed column with equilibration buffer after sanitization.

Cleaning-in-place (CIP):

Substances such as denatured proteins that do not elute during regeneration can be removed by CIP procedures. AM-50 Heparin resin withstands exposure to 0.1 M NaOH for long periods without significant loss of binding capacity for antithrombin III. When contamination is severe, 0.5 M NaOH can be used. However, a decrease in functionality can be seen over time. Other reagents in which the medium is stable include 8 M urea and 6 M guanidine hydrochloride.

Application Examples:

Purification of antithrombin III

Sample: 45 mL filtered (0.45 μM) human plasma diluted 2:1 in binding buffer
Binding buffer: 0.1 M Tris-HCl, 0.01 M trisodium citrate, 0.225 M NaCl, pH 7.4
Wash buffer: 0.1 M Tris-HCl, 0.01 M trisodium citrate, 0.330 M NaCl, pH 7.4
Elution buffer: 0.1 M Tris-HCl, 0.01 M trisodium citrate, 2.0 M NaCl, pH 7.4
Column: 5/5 column
Chromatographic procedure:
Flow rate: 0.5 mL/min (150 cm/h)
Equilibration: 5 mL binding buffer
Washing step 1: 40 mL binding buffer
Washing step 2: 15 mL wash buffer
Elution: 9 mL elution buffer

Taq DNA Polymerase

Loading Buffer: 100 mM KCl, 50 mM Tris-HCl, pH 7.5, 0.1 mM EDTA, and 0.2% Tween 20
Wash Buffer: 20 mM Tris-HCl, pH 8.0, 100 mM NaCl
Elution Buffer: a linear gradient of 150-650 mM KCl in the same buffer. Collect Fractions between 275 mM and 325 mM KCl.

Pfu DNA Polymerase

Loading & Wash Buffer：150 mM NaCl, 10% glycerol, 20 mM Tris pH7.5
Elution buffer: a NaCl gradient from 0 to 1M, 20 mM Tris pH7.5, 10% glycerol or 3 volumes of 20 mM Tris pH7.5 containing 0.3 M NaCl, 10% glycerol.

DNA Helicase from Calf Thymus

Loading Buffer：20 mM potassium phosphate, pH 7.0, 30% glycerol, 50 mM KCl, 0.5 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 4 mM $Na_2S_20_5$, 1 pM pepstatin
Wash Buffer: the above buffer containing 100mM KCl
Elution Buffer: a gradient of 10 volumes ranging from 100 to 500 mM KCl in the loading buffer.