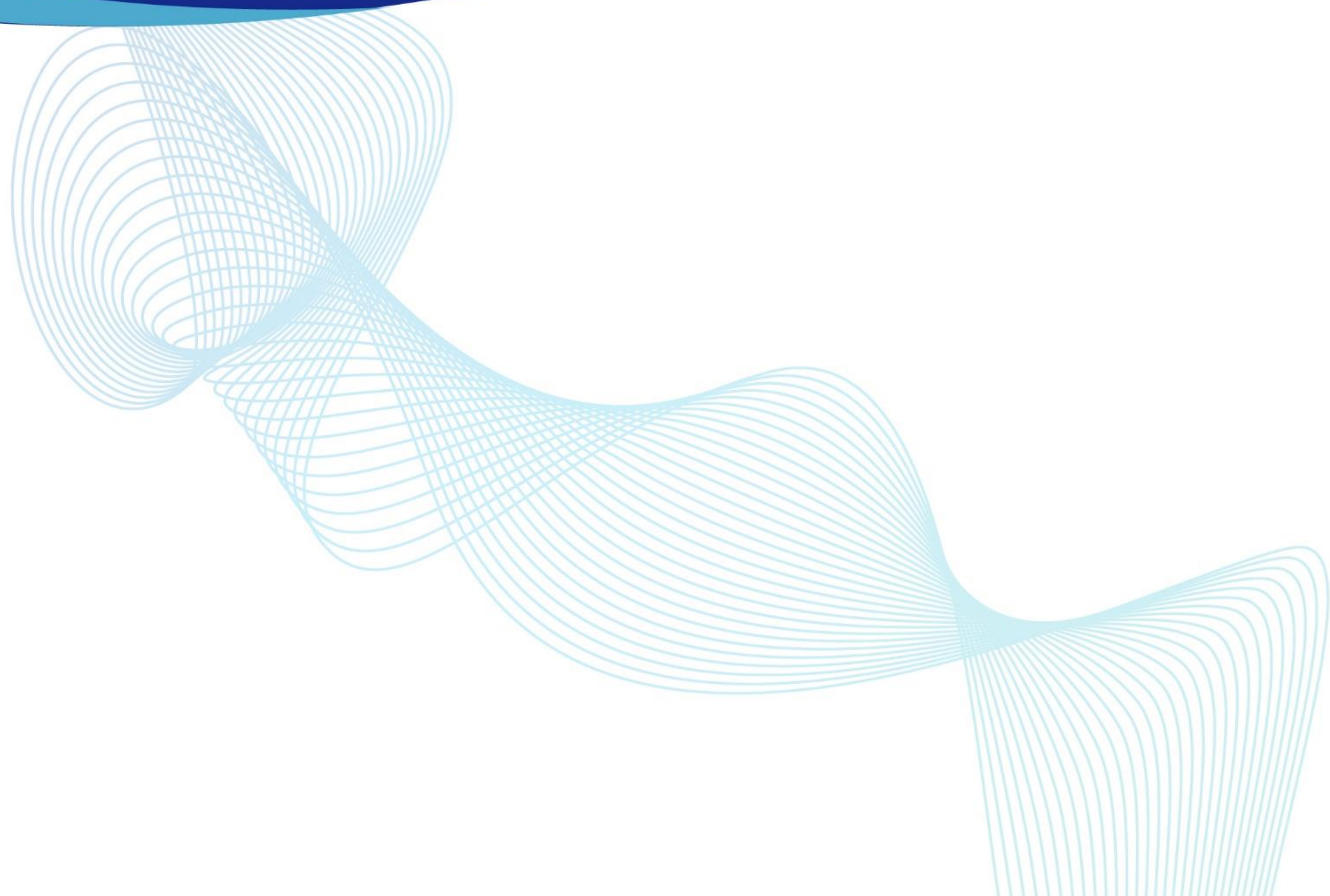
# HP- Heparin Affinity Chromatography Resin Product Manual



## 1. Product Introduction

Heparin is a sulfate-containing acidic polysaccharide that can interact with anticoagulant factor III, thrombin, thrombin-like, and human coagulation factors. It can also interact with biological macromolecules expressed by *Escherichia coli*, including human interleukin, human prostate growth factor, recombinant human coagulation factor, vascular endothelial growth factor, cartilage growth factor, basic fibroblast growth factor, recombinant human acidic fibroblast growth factor, recombinant hepatocyte growth factor, recombinant mouse heparin cofactor II, recombinant human platelet factor IV, recombinant human endothelial inhibitor, recombinant human keratinocyte growth factor. Heparin affinity chromatography resin (HP-heparin) uses polyacrylate microspheres as the matrix and heparin as the ligand. It is suitable for the purification of the above-mentioned coagulation factors and features good pressure resistance.

## 2. Product Properties

| Parameter | Technical Specification |
|---|---|
| Average particle size | 70±20 μm |
| Pore size | 30 nm |
| Matrix beads | Polyacrylate microspheres |
| Ligand | Heparin |
| Dynamic binding capacity | ≥15 mg LF/ml wet gel |
| Pressure upper limit | 1 MPa |
| pH stability | 4 - 12 (long term); 4 - 13 (short term) |
| Storage | 2 - 10 ℃ (0.05 M sodium acetate + 20% ethanol) |

## 3. Operation Steps

Heparin affinity chromatography resin (HP-heparin) is designed for the separation and purification of biological macromolecules such as anticoagulation factor III, thrombin, thrombin-like, human coagulation factors IX, XI and VIII. Chromatography operations usually include column packing, equilibration, loading, washing, elution, regeneration and other steps.

**Equilibration:** Equilibrate the chromatography column with 5-10 CVs of equilibration buffer (20-50 mM PB or Tris-HCl, pH 7.4-8.0, 0.15 M NaCl can be added to inhibit non-specific adsorption) until the conductivity and pH of the effluent remain stable (consistent with the equilibration buffer).

**Loading:** The buffer of sample should be as consistent as possible with the equilibration solution. Solid samples can be prepared by dissolving in equilibration solution; low-concentration sample solutions can be dialyzed with equilibration solution; high-concentration sample solutions can be diluted with equilibration solution. To avoid clogging the column, samples should be centrifuged or micro-filtrated. The loading amount is calculated based on the loading capacity of the resin and the

content of the target protein in the feed.

**Wash:** After loading the sample, continue to wash to the baseline with equilibration buffer.

**Elution:** Elute with elution buffer (20-50 mM PB or Tris-HCl + 1-2M NaCl, pH 7.4-8.0, the NaCl concentration needs to be adjusted appropriately according to the binding force of the target protein) and collect the effluent. Linear gradient or step gradient elution can be used.

**Cleaning in place**: To avoid cross-contamination between different samples, or the resin is seriously contaminated (back pressure increases), the resin needs to be cleaned in place.

(1) For proteins bound by ion bonds, clean with >2-3 CVs of 2 M NaCl and rinsed with >3Cvs of pure water.
(2) For precipitated or denatured proteins, clean with 0. 1 M NaOH (1-2 h) and rinsed with 3-10 CVs equilibration solution and >3 CVs pure water. You can also use 6 M guanidine hydrochloride or 8 M urea for cleaning (0.5~1h).
(3) For hydrophobically bound proteins, clean with 0.1-0.5% non-ionic detergent (1-2h) and rinsed with 3~10 CVs pure water.

**Other precautions**: During packing, operating and storing the column, avoid the column from drying out and air bubbles from entering.

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Product information is subject to change without prior notice. Duoning reserves the right of final interpretation.