HiTrap Heparin HP

AFFINITY CHROMATOGRAPHY

HiTrap™ Heparin HP is one of a range of prepacked, ready to use columns for affinity chromatography. Fast, simple and easy separations are provided by the prepacked column with a high performance affinity medium. HiTrap Heparin HP is particulary suitable for the isolation and purification of antithrombin III and other coagulation factors, lipoproteins, lipases, protein synthesis factors, hormones, steroid receptors, nucleic acid binding enzymes and interferon.

- Fast and convenient to use
- Packed with Heparin Sepharose™ High Performance
- Simple operation with a syringe, a pump, an ÄKTA™ system, or other chromatography systems

HiTrap Heparin HP columns can easily be operated using a syringe. Alternatively, a laboratory pump, alone or within a chromatography system, can be advantageous, especially when linear gradients are required. 1 ml and 5 ml columns are available.

Column characteristics

The HiTrap column is made of polypropylene, a material which is biocompatible and does not interact with biomolecules. Top and bottom frits are manufactured from porous polyethylene. The column is delivered with a stopper on the inlet and a twist-off end on the outlet.

Medium characteristics

Sepharose High Performance is the base matrix for HiTrap Heparin HP 1 ml and 5 ml. The carbohydrate nature of the agarose base provides a hydrophilic and chemically favorable environment for coupling, while the highly crosslinked structure of the 34 µm spherical beads ensures excellent chromatographic properties. Fast kinetics and high dynamic capacities are properties of all HiTrap affinity columns.

Fig 1. Prepacked with Heparin Sepharose High Performance, HiTrap Heparin HP columns offer fast and simple affinity purifications of proteins such as growth factors, DNA-binding proteins, coagulation factors, lipoproteins and steroid receptors.

The ligand, heparin, is a naturally occurring sulfated glucosaminoglycan that is extracted from the native proteoglycan of porcine intestinal mucosa. It consists of alternating units of uronic acid and D-glucosamine, most of which are substituted with one or two sulfate groups, and is covalently coupled to the agarose beads.

Immobilized heparin has two main modes of interaction with proteins: as an affinity ligand, e.g., in its interaction with growth factors and antithrombin III, and as a cation exchanger due to its high content of anionic sulfate groups, e.g., in its interaction with nucleic acid-binding proteins where it mimics the similarly polyanionic structure of the nucleic acid. Gradient elution with salt is most commonly used in both cases. As individual proteins often bind with a unique combination of affinity and ion exchange, even small differences between bound proteins can result in good purifications.

Figure 2 shows the structure of heparin. Table 1 summarizes the main characteristics of HiTrap Heparin.

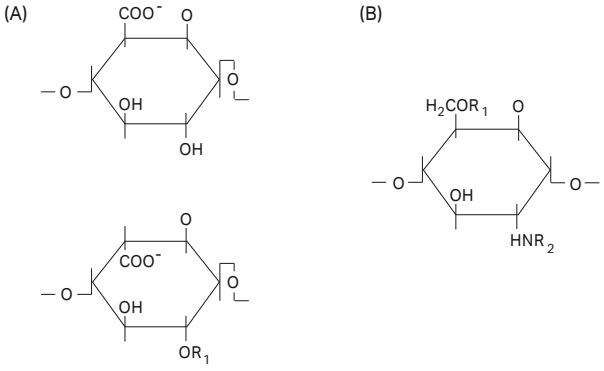


Fig 2. Structure of a heparin polysaccharide, consisting of alternating hexuronic acid (A) and D-glucosamine residues (B). The hexuronic acid can either be D-glucuronic acid (top) or its C-5 epimer, L-iduronic acid.
R_1 = –H or $–SO_3^-$;
R_2 = $–SO_3^-$ or $–COCH_3$

Table 1. Main characteristics of HiTrap Heparin HP

Column dimensions	0.7 × 2.5 cm (1 ml) 1.6 × 2.5 cm (5 ml)
Ligand	Heparin
Ligand concentration	approx. 10 mg/ml
Binding capacity	approx. 3 mg antithrombin III/ml medium
Mean particle size	34 µm
Bead structure	Highly cross-linked 6% spherical agarose
Column hardware pressure limit	5 bar (0.5 MPa, 70 psi)
Max. flow rate*	4 ml/min (1 ml), 20 ml/min (5 ml)
Recommended Flow rate	1 ml/min (1 ml), 5 ml/min (5 ml)
pH stability**	
Working	5 to 10
Cleaning	5 to 10
Temperature stability	
Working	4°C to room temp.
Storage	4°C to 30°C
Storage	20% ethanol

* H_2O at room temperature
** The ranges given are estimates based on our knowledge and experience. Please note the following:
i) pH stability, working refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance
ii) pH stability, cleaning refers to the pH interval for regeneration, cleaning in place and sanitization procedures

(A)

(B)

(C)

Fig 3. Using HiTrap Heparin HP with a syringe. (A) Prepare buffers and sample. Remove the column's top cap and twist off the end. Wash and equilibrate. (B) Load the sample and begin collecting fractions. (C) Elute and continue collecting fractions.

Operation

Like all HiTrap columns, HiTrap Heparin HP is quick and easy to use. Instructions and connectors are included with each pack of columns. In general, the separation can be easily achieved with a syringe (using the luer adapter provided). Figure 3 illustrates this technique. Alternatively, the column can be operated using a laboratory pump or a chromatographic system. For easy scaling-up two or more columns can be connected in series by screwing the end of one into the top of the next. Note that the backpressure will increase.

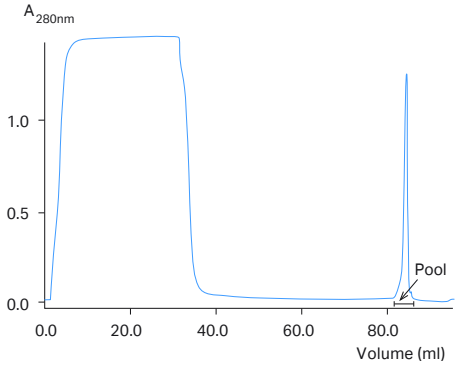
Applications

HiTrap Heparin HP 1 ml and 5 ml can bind a very wide range of biomolecules. These include: enzymes (mast cell proteases, lipoprotein lipase, coagulation enzymes superoxide dismutase); serine protease inhibitors (antithrombin III, protease nexins); growth factors (fibroblast growth factor, Schwann cell growth factor, endothelial cell growth factor); extracellular matrix proteins (fibronectin, vitronectin, laminin, thrombospondin, collagens); nucleic acid-binding proteins (initiation factors, elongation factors, restriction endonucleases, DNA ligase, DNA and RNA polymerases); hormone receptors (oestrogen and androgen receptors) and lipoproteins. Figures 4, 5 and 6 show examples.

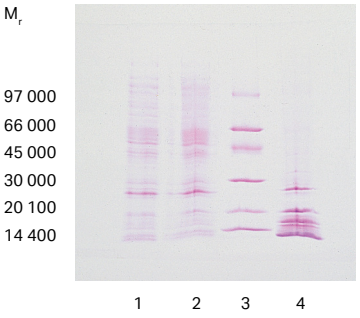
Partial purification of Oct 1

The recombinant DNA-binding protein Oct-1 was bound to HiTrap Heparin HP under conditions using high salt concentrations, indicating that the protein binds in a "non-ionic" but a very specific manner. Oct-1 was eluted from HiTrap Heparin HP in a step gradient. The protein was identified in the eluate using Western blotting as the concentration was too low to be detected by Coomassie™ Blue staining. The protein was not purified further (Fig 6).

Sample:	Extract containing Oct-1, filtered through a 0.45 µm Millex™ HA filter
Sample preparation:	Buffer exchange to binding buffer on a PD-10 column
Sample volume:	30 ml
Column:	HiTrap Heparin HP, 5 ml
Binding buffer:	20 mM Tris-HCl, 5% (v/v) glycerol, 0.1 mM EDTA, 10 mM 2-mercaptoethanol, 0.1 mM Pefabloc™ PC, 0.5 M NaCl, pH 8
Elution buffer:	20 mM Tris-HCl, 5% (v/v) glycerol, 0.1 mM EDTA, 10 mM 2-mercaptoethanol, 0.1 mM Pefabloc, 2 M NaCl, pH 8
Flow rate:	1 ml/min (30 cm/h)
Electrophoresis:	SDS-PAGE, PhastSystem, PhastGel 10–15, 1 µl sample, Coomassie Blue stained



Rabbit anti-Oct human-1 was from Santa Cruz Biotechnology, Inc. Anti-rabbit IgG (g-chain specific), Alkaline Phosphatase Conjugate and SIGMAFAST™ BCIP™. NBT Tablets (insoluble Alkaline Phosphatase Substrate) were from Sigma.



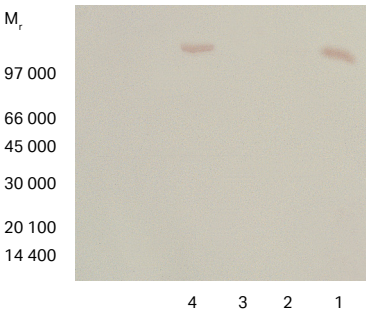
SDS electrophoresis using PhastGel 10–15, Coomassie Blue Staining

Lane 1: Starting material, *E. coli* extract, dil. 4×

Lane 2: Flow-through

Lane 3: Low Molecular Markers

Lane 4: Eluate pool



Western blotting of the electro-phoresed gel

Lane 1: Starting material

Lane 2: Flow-through

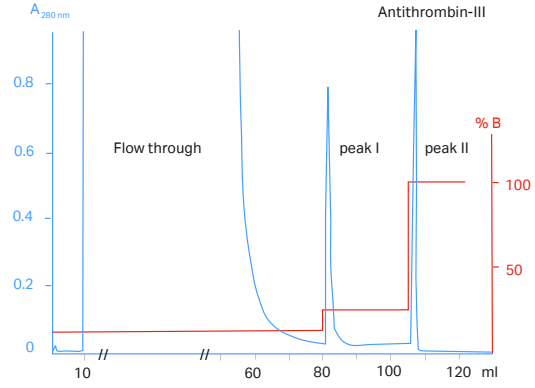
Lane 3: Low Molecular Markers

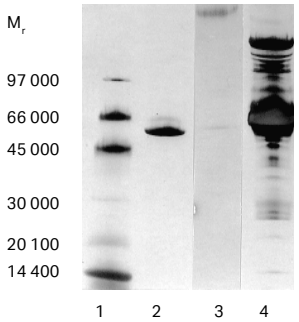
Lane 4: Eluate pool

Fig 4. Partial purification of the recombinant DNA binding protein Oct-1. The *E. coli* strain BL21 pLysS expressing the recombinant Oct-1 protein was kindly provided by Dr. Gunnar Westin, University Hospital, Uppsala, Sweden.

Purification of antithrombin-III from bovine plasma

Sample:	30 ml bovine plasma diluted with 15 ml 0.1 M Tris, 0.01 M citrate, 0.225 M NaCl, pH 7.4
Column:	HiTrap Heparin HP, 1 ml
Flow rate:	1.0 ml/min
Binding buffer (A):	0.1 M Tris, 0.01 M citric acid, 0.225 NaCl, pH 7.4
Elution buffer (B):	0.1 M Tris, 0.01 M citric acid, 2 M NaCl, pH 7.4
Chromatographic procedure:	8 ml 12.5% buffer B, 45 ml sample, 27 ml 12.5% buffer B, 26 ml 25% buffer B, 26 ml 100% buffer B. 2.9 mg antithrombin-III was eluted in peak II
Electrophoresis:	SDS-PAGE, PhastSystem™, PhastGel™ Gradient 8–25, 1 µl sample, silver stained





Lane 1: Low Molecular Markers, reduced

Lane 2: Peak II, reduced, diluted 1:2

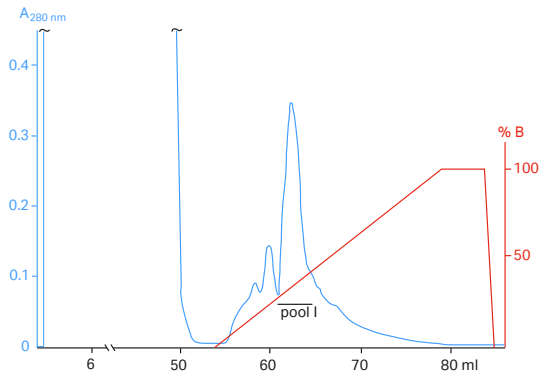
Lane 3: Peak I, reduced, diluted 1:2

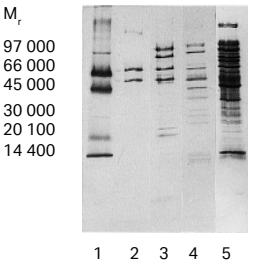
Lane 4: Unbound material, reduced, diluted 1:15

Fig 5. Purification of antithrombin III from bovine plasma. Antithrombin-III is eluted in the second peak.

Partial purification of recombinant HIV-reverse transcriptase

Sample:	49 ml *E. coli* lysate (= 1 g cells) after passage through a 5 ml DEAE Sepharose Fast Flow column
Column:	HiTrap Heparin HP, 1 ml
Flow rate:	1.0 ml/min
Binding buffer (A):	20 mM Tris-HCl, 1 mM EDTA, 1 mM 2-mercaptoethanol, 2% glycerol, pH 8.0
Elution buffer (B):	Buffer A + 1.0 M NaCl, pH 8.0
Gradient:	Linear gradient 0% to 100% buffer B, 25 ml
Electrophoresis:	SDS-PAGE, PhastSystem, PhastGel Gradient 8–25, 1 µl sample, silver stained





Lane 1: Low Molecular Markers, reduced

Lane 2: Reverse transcriptase, reduced

Lane 3: Pool I from HiTrap Heparin HP 1 ml, reduced

Lane 4: Unbound material from DEAE Sepharose Fast Flow, reduced

Lane 5: Cell lysate, reduced

Fig 6. Partial purification of recombinant HIV-reverse transcriptase.

Ordering information

Products	Quantity	Code number
HiTrap Heparin HP	5 × 1 ml	17-0406-01
HiTrap Heparin HP	1 × 5 ml	17-0407-01
HiTrap Heparin HP	5 × 5 ml	17-0407-03

Related products	Quantity	Code number
HiPrep™ 16/10 Heparin FF	1 (20 ml)	17-5189-01
Heparin Sepharose 6 Fast Flow	50 ml	17-0998-01
HiTrap Desalting	1 × 5 ml	29-0486-84
HiTrap Desalting	5 × 5 ml	17-1408-01
HiPrep 26/10 Desalting	1 (53 ml)	17-5087-01
HiPrep 26/10 Desalting	4 (53 ml)	17-5087-02

Accessories	Quantity	Code number
1/16" male/Luer female*	2	18-1112-51
Tubing connector flangeless/M6 female	2	18-1003-68
Tubing connector flangeless/M6 male	2	18-1017-98
Union 1/16" female/M6 male	6	18-1112-57
Union M6 female/1/16" male	5	18-3858-01
Union luerlock female/M6 female	2	18-1027-12
HiTrap/HiPrep, 1/16" male connector for ÄKTAdesign	8	28-4010-81
Stop plug female, 1/16"†	5	11-0004-64
Fingertight stop plug, 1/16"‡	5	11-0003-55

* One connector included in each HiTrap package

† Two, five, or seven stop plugs female included in HiTrap packages depending on products

‡ One fingertight stop plug is connected to the top of each HiTrap column at delivery

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