Affinity chromatography

Heparin Sepharose™ 6 Fast Flow
HiPrep™ Heparin FF 16/10

HiPrep Heparin FF 16/10 is a prepacked, ready to use 20 mL column for affinity chromatography. By coupling heparin to Sepharose 6 Fast Flow, proteins and other biomolecules can be purified based on their affinity for heparin (Fig 1).

Key features include:

- Available as lab packs or prepacked column format for purification of restriction endonucleases, plasma proteins, factors for protein synthesis and other related proteins
- Simple operation and good flow characteristics
- Reliable and reproducible separations
- Compatible with single pump-based configurations as well as ÄKTA™ chromatography systems

Fig 1. Heparin Sepharose 6 Fast Flow is available as process packs, lab packs, and prepacked HiPrep Heparin FF 16/10.

Chromatography resin characteristics

Heparin is a naturally occurring glycosaminoglycan that can be used as an affinity binding and ion exchange ligand for a wide range of biomolecules including: DNA binding proteins, coagulation factors, other plasma proteins, lipoproteins, protein synthesis factors, enzymes that act on nucleic acids, and steroid receptors. Heparin consists of alternating hexuronic acid (D-glucuronic or L-iduronic) and d-glucosamine residues. The polymer is heavily sulfonated, carrying sulfamino (N-sulfate) groups at C-2 of the glucosamine units as well as ester sulfate (O-sulfate) groups in various positions (Fig 2). The heparin ligand used in Heparin Sepharose 6 Fast Flow is isolated from porcine intestinal mucosa and has a molecular weight distribution of M_r 5000 to 30 000. The base matrix, Sepharose 6 Fast Flow, is a 6% crosslinked beaded agarose with good flow properties and high loading capacity. Heparin is linked to the Sepharose matrix by reductive amination, which is stable even under alkaline conditions. The stability of this chromatography resin is dictated by the heparin ligand. The characteristics of Heparin Sepharose 6 Fast Flow are shown in Table 1.

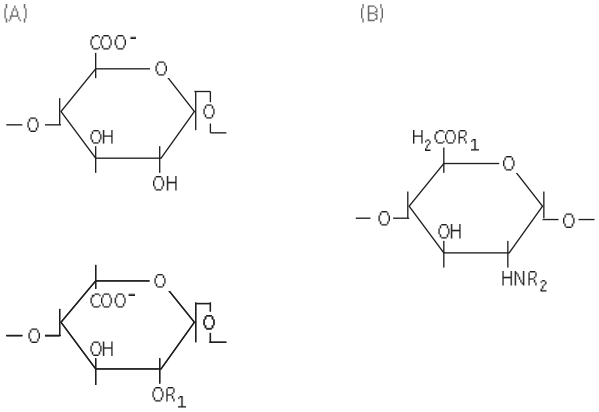


Fig 2. Heparin consists of alternating (A) hexuronic acid and (B) D-glucosamine residues. The hexuronic acid can either be L-glucoronic acid (top) or its C-5 epimer, L-iduronic acid. R_1 = –H or $–SO_3^-$; R_2 = SO_3^- or $–COCH_3$.

Table 1. Heparin Sepharose 6 Fast Flow characteristics

Matrix	Cross-linked agarose, 6%, spherical
Particle size, d_{50v}*	~ 90 µm
Ligand	Porcine heparin
Ligand concentration	~ 2 mg heparin/mL medium
Pressure/flow characteristics:	250 to 400 cm/h at < 0.1 MPa in a XK 50/60 column with 5 cm diameter and 25 cm bed height (at 20°C using buffers with the same viscosity as water)†‡
pH stability, operational**	4–12
pH stability, CIP††	4–13
Chemical stability	Stable to commonly used aqueous buffers, 0.05 sodium acetate pH 4.0, 20% ethanol, 4 M NaCl, 8 M urea, 6 M guanidine hydrochloride, 0.1 M NaOH
Storage	20% ethanol containing 0.05 M sodium acetate, 4°C to 30°C

* Median particle size of the cumulative volume distribution.
† The pressure/flow characteristics describes the relationship between pressure and flow under the set circumstances. The pressure given shall not be taken as the maximum pressure of the resin.
‡ Pressure/flow test performed on the base matrix
** pH range where resin can be operated without significant change in function.
†† pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

Column characteristics

HiPrep 16/10 columns are made of transparent polypropylene that does not interact with biomolecules. HiPrep 16/10 column characteristics are shown in Table 2. The column is not designed to be opened or repacked.

Table 2. HiPrep 16/10 column characteristics

Column dimension	1.6 × 10.0 cm
Bed volume	20 mL
Maximum operating flow rate*	10 mL/min
Recommended operating flow rate*	2 to 10 mL/min
Maximum operating pressure	0.15 MPa (1.5 bar, 22 psi)
Column hardware pressure limit	5 bar (0.5 MPa, 73 psi)

* At room temperature using buffers with the same viscosity as water.

Applications

Improved purification of scCro8 from bacteriophage lambda using HiPrep Heparin FF 16/10

The bacteriophage lambda Cro repressor is a DNA binding protein that functions as an oligomer (Fig 3). It binds to the OR (operating region) on the lambda genome, and together with the cI-repressor, detemines whether the phage will undergo lysis or survive as a lysogenic strain.

The affinity of the bacteriophage lambda Cro repressor for its OR is limited by dimer dissociation at submicromolar concentrations. Since the formation of dimers at nanomolar levels is low, the binding affinity between the Cro dimer and DNA must be high. One way to confirm this high binding affinity is to study DNA binding to dimers whose dissociation is hampered. By introducing a linker between the subunits, the dissociation is prevented.

In order to gain a solid knowledge of the physical principles behind protein-DNA binding and to study this particular dimer-DNA interaction, an improved purification protocol for scCro8 (single-chain Cro with an 8 amino acid long linker between the two subunits) is presented. The main difference between typically used protocols is the introduction of a prepacked HiPrep Heparin FF 16/10 column.

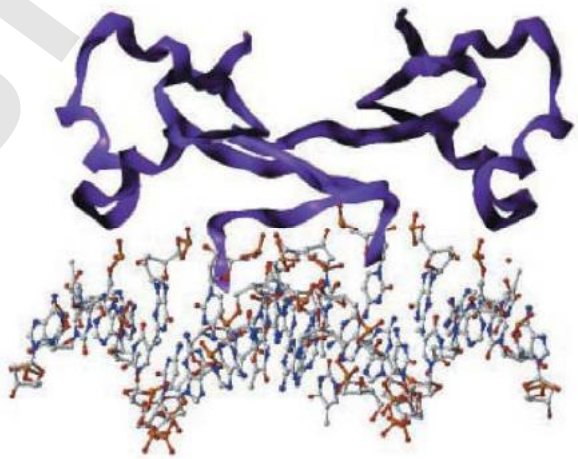


Fig 3. A model of the Cro repressor dimer (purple) interacting with DNA.

The scCro8 oligomer was expressed in *E. coli* JM109(DE3)/pscCro8 cells under the control of a T7 promoter. For protein expression, cells were grown in 2YT culture medium to an OD_{600} of 1 at 37°C. IPTG was added to a final concentration of 1 mM. The cultures were allowed to grow for 3 h at 37°C after induction. The cells were harvested by centrifugation at 3000 × g, and the bacterial pellet was frozen at –80°C.

Cells were resuspended in twice the pellet volume of buffer and lysed by sonication, 6 × 20 s burst, and 60 s cooling between each burst.

The supernatant was cleared by centrifugation, 20 000 × g for 15 min. The cleared supernatant was passed over a HiPrep 26/10 Desalting column equilibrated with 10 mM sodium phosphate, pH 7.0. The pooled G-25 fraction was applied to DEAE Sepharose CL 4B packed in an XK 16/10 column and equilibrated with 10 mM sodium phosphate pH 7.0. During this step, scCro8 remained unbound and was retrieved in the flowthrough. The flowthrough was cleared by centrifugation at 110 000 × g for 1 h before applying it to an equilibrated HiPrep Heparin FF 16/10 column. After sample loading, the column was washed and the protein eluted (Fig 4).

Column:	HiPrep Heparin FF 16/10*
Sample:	2000 mL flowthrough from DEAE Sepharose CL 4B, pH 7.0
Binding/washing buffer:	50 mM sodium phosphate, pH 7.5
Elution buffer:	50 mM sodium phosphate, 1 M sodium chloride, pH 7.5
Flow rate:	1.5 mL/min (45 cm/h)
Operation:	1. Equilibrate with 80 mL binding buffer 2. Load sample 3. Wash with 100 mL washing buffer 4. Elute using linear gradient, 0 to 100% elution buffer (0 to 1 M sodium chloride) in 300 mL

* Note: Data was obtained using first-generation HiPrep 16/10 column

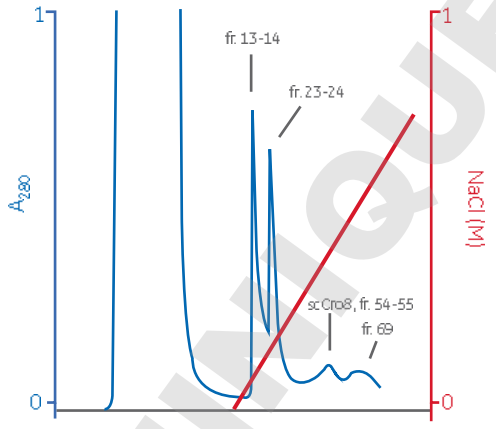
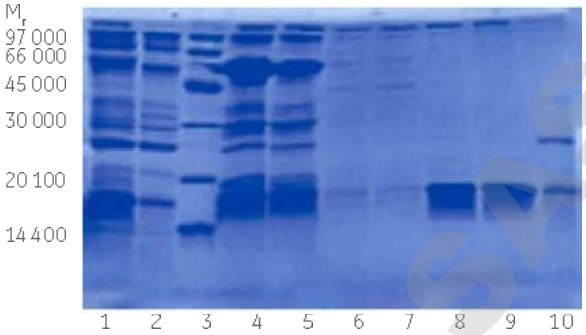


Fig 4. scCro8 purification using HiPrep Heparin FF 16/10.



Lanes
1. Pool from HiPrep 26/10 Desalting
2. Flowthrough pool from DEAE Sepharose CL 4B
3. LMW markers (LMW-SDS Marker Kit)
4-10. Eluted fractions from HiPrep Heparin FF 16/10
4. Fraction 13
5. Fraction 14
6. Fraction 23
7. Fraction 24
8. Fraction 54
9. Fraction 55
10. Fraction 69

Fig 5. Coomassie™ staining of SDS polyacrylamide gel (12%).

Analysis by mass spectrometry and SDS-PAGE

The scCro8 peak eluted from HiPrep Heparin FF 16/10 was lyophilized and dissolved in 70% acetonitrile. The sample was run on a linear MALDI–TOF calibrated using hACTH 7–38. The matrix was saturated sinapinic acid in 50% acetonitrile and 0.5% TFA.

The mass-to-charge ratio (m/z) was 15 128, which corresponded well with the result from the SDS-PAGE (Fig 5). The SDS-PAGE showed a purity of > 95%.

Summary

The prepacked HiPrep Heparin FF 16/10 column made it possible to purify 35 mg scCro8 from 2 l cell culture with an improved purity compared to previous protocols. The recovery in this two-step protocol was 95% with > 95% purity.

Storage

Heparin Sepharose 6 Fast Flow and HiPrep Heparin FF 16/10 should be stored at 4°C to 30°C in 20% ethanol containing 0.05 M sodium acetate.

Reference

1. Jana R. *et al.*, Single-Chain Lambda Cro Repressors Confirm High Intrinsic Dimer-DNA Affinity. Biochemistry 37, 6446–6455 (1998).

Ordering information

Product	Quantity	Code number
HiPrep Heparin FF 16/10	1 × 20 mL	28936549
Heparin Sepharose 6 Fast Flow	50 mL 250 mL 1 L 5 L	17099801 17099825 17099803 17099804

Accessories

HiTrap/HiPrep 1/16" male connector for ÄKTA systems	8	28401081
To connect columns with 1/16"connections to FPLC System: Union M6 female 1/16" male	5	18385801

Related products

HiTrap™ Heparin HP	5 × 1 mL 1 × 5 mL 5 × 5 mL	17040601 17040701 17040703
HiPrep 26/10 Desalting	1 × 53 mL 4 × 53 mL	17508701 17508702

Related literature

Affinity Chromatography Handbook, Principles and Methods	18102229
Affinity Chromatography Columns and Media, Selection Guide	18112186
Prepacked chromatography columns for ÄKTA systems, Selection guide	28931778

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