

Fabsorbent™ F1P HF

A new synthetic ligand affinity adsorbent for antibody fragment purification

In response to the growth of antibody fragments as research tools and bio therapeutic products, ProMetic has developed a new affinity adsorbent for the capture and purification of a wide range of antibody fragments. Fabsorbent™ F1P HF is a synthetic-ligand light-chain binding adsorbent which can be used as a superior alternative to Protein L for the capture and purification of antibody fragments including monovalent antibody fragments (e.g. Fab, scFv), engineered antibody variants and single domain antibodies.

The robustness and broad applicability of Fabsorbent™ F1P HF makes this adsorbent the ideal choice for both early stage research and development applications and also for larger-scale manufacturing uses where the full benefit of the high-flow/low pressure Purabead® HF support matrix can be utilised. Given its broad selectivity, Fabsorbent™ F1P HF provides a “platform” technology for the purification of antibody fragments.

■ Proven technology:

Sharing the same chemistry as ProMetic's proven Mimetic Ligand™ adsorbents which are used extensively in commercial manufacturing processes, Fabsorbent™ F1P HF has been developed in close collaboration with antibody fragment producer companies. Using ProMetic's Chemical Combinatorial Library™ technology and extensive computer modelling and ligand screening know-how, ProMetic have identified the Fabsorbent™ F1P ligand which binds selectively to kappa and lambda light chains of a variety of mammalian antibodies including human, sheep, mouse and cow.

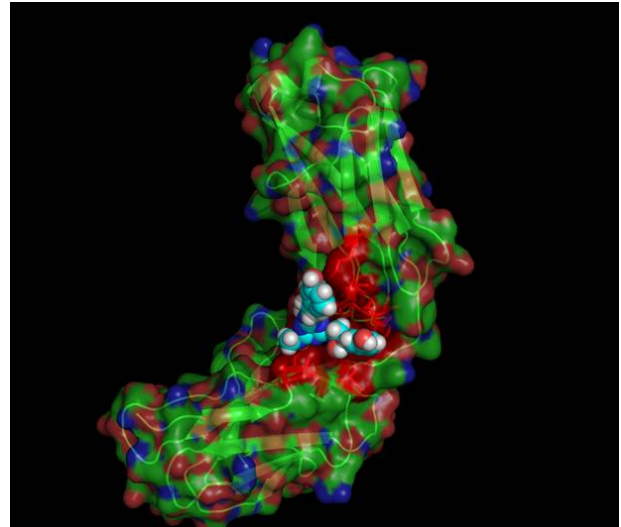


Figure 1: Fabsorbent™ F1P ligand interaction with antibody light chain

■ Universally applicable:

Binds and purifies most antibody species (antibody fragments and whole antibodies) that contain kappa and/or lambda light chains.

■ Robust:

Fabsorbent™ F1P HF is fully resistant to concentrated sodium hydroxide (up to 1 M) enabling effective column cleaning and sanitisation thereby prolonging column lifetime.

■ Safe:

Synthetic ligand not derived from cell culture. No components of animal origin are used in the manufacture of Fabsorbent™ F1P HF. This product is manufactured in ProMetic's ISO 9001:2000 certified facility and is supported by a comprehensive Regulatory Support File.

■ Operational flexibility:

Purabead® HF base matrix enables the operation of large diameter columns at high linear flow rates (≥ 500 cm/h).

■ Multiple formats for ease-of-use:

Fabsorbent™ F1P HF is available in a variety of easy to use formats including 96 microcolumn Puraplates™, PIKSI scouting columns, and a variety of slurry pack sizes.

Antibody fragments purified using Fabsorbent™ F1P HF

- Fab' expressed in *E.coli*.
- Mammalian Fab fragments from papain catalysed antibody digests.
- Mammalian F(ab')₂ fragments from pepsin catalysed antibody digests.
- Human antibody λ and κ light chains.
- ScFv expressed in *E.coli*.
- Single domain antibody fragments expressed in *E.coli*.
- Full length monoclonal antibodies.
- Human, bovine, ovine and murine antibodies and antibody fragments.

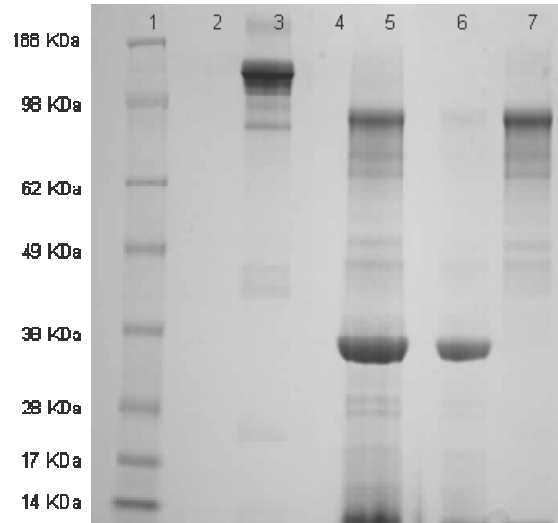


Figure 3: Non-reduced SDS-PAGE of F(ab')₂ from pepsin digest. Lane 1: molecular weight markers; Lane 3: IgG standard; Lane 5: pepsin digest; Lane 6: flow through and post load wash; Lane 7: elution fraction.

Purification of F(ab')₂

F(ab')₂ antibody fragments are prepared by pepsin cleavage of full-chain IgG which cleaves the Fc region to yield two Fab' fragments linked together. Fabsorbent™ F1P HF may be used to capture and purify F(ab')₂ fragments and, since pepsin is not bound by Fabsorbent™ F1P HF, this adsorbent may be used to simultaneously purify F(ab')₂ fragments and remove free pepsin (Figure 2 and 3).

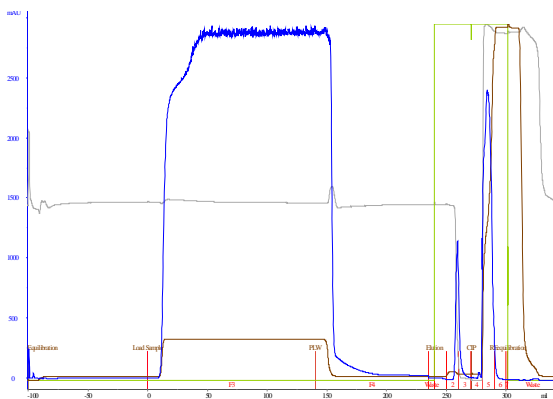


Figure 2: Capture and elution of F(ab')₂ from pepsin digest. Column volume: 10 mL; flow rate: 250 cm/h; equilibration buffer: 25 mM Tris-HCl, pH 8.0; elution buffer: 50 mM sodium citrate, pH 3.0.

Capture and purification of domain antibody from *E.coli* lysate

V_L domain antibodies are the smallest antigen binding antibody fragments. Recombinant V_L antibodies are commonly produced in microbes such as *E.coli* with the resulting fragment being purified from cell lysate. Fabsorbent™ F1P HF has been used to successfully capture and purify V_L domain antibodies (Figure 4). Non-reduced SDS-PAGE of the fractions from Fabsorbent F1P HF (Figure 5) demonstrates the capture and purification of V_L domain antibody from *E.coli* lysate.

IgG₁ directly from CHO cell culture supernatant (Figure 6).

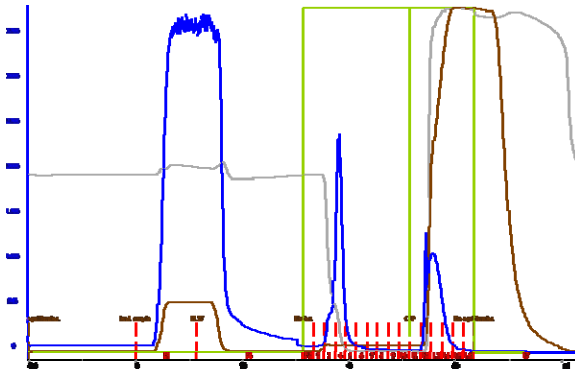


Figure 4: Capture and elution of a domain antibody from *E.coli* lysate. Column volume: 1 mL; 3 minute residence time; equilibration buffer: 25 Tris-HCl, pH 8.0; elution buffer 50 mM sodium citrate, pH 3.0; CIP 0.5 M NaOH.

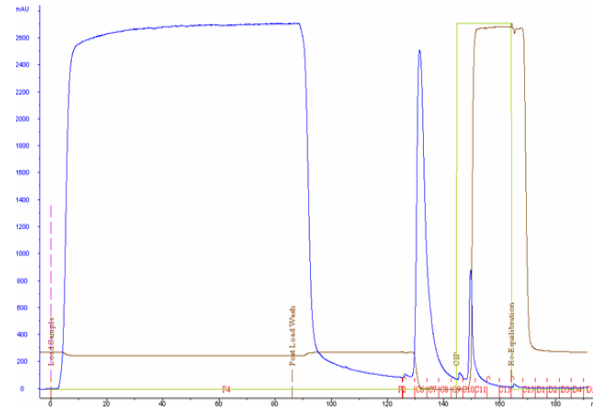


Figure 6: Chromatogram of the capture and elution of IgG₁ from CHO cell culture supernatant. Column volume 2 mL; 3 minute residence time; equilibration buffer: PBS, pH 7.4; elution buffer: 50 mM sodium citrate, pH 3.0; CIP: 0.5 M NaOH.

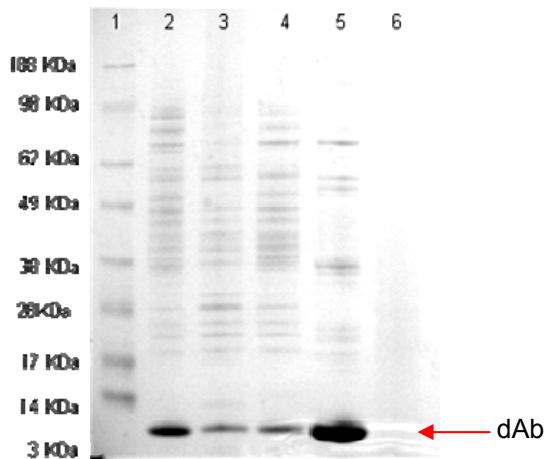


Figure 5: Non-reduced SDS/PAGE. Lane 1: molecular weight markers; Lane 2: domain antibody containing *E.coli* lysate; Lane 3: flow through fraction; Lane 4: post load wash; Lane 5: elution fraction; Lane 6: 0.5 M NaOH CIP

Fabsorbent™ F1P HF is designed for the capture of light chain containing fragments therefore the excess light chain in the CHO cell culture supernatant is also captured and eluted by Fabsorbent™ F1P HF (Figure 7).

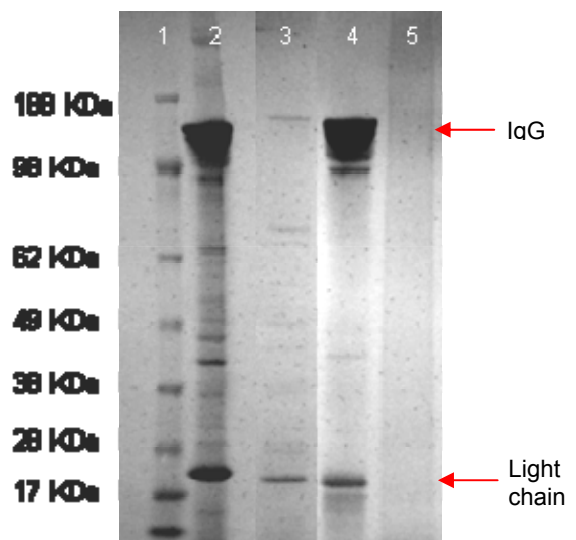


Figure 7: Non-reduced SDS-PAGE; Lane 1: Molecular weight markers; Lane 2: CHO cell culture supernatant; Lane 3: flow through fraction; Lane 4: Elution fraction; Lane 5: CIP.

Purification of monoclonal IgG₁ from CHO cell culture supernatant

Fabsorbent™ F1P HF was designed for the capture of antibody fragments and can therefore be used for the capture and purification of whole molecule IgG. Fabsorbent™ F1P HF can capture and purify

Properties: Fabsorbent™ F1P HF

Ligand: F1P Synthetic triazine ligand

Matrix: PuraBead® P6HF (6% near-monodisperse highly cross-linked agarose).

Particle size: 90 ± 10 µm

Binding capacity: Up to 60 mg IgG/mL and 20 mg Fab/mL resin (dependant on antibody/fragment type and source).

Operational Flow rate: Up to 500 cm/h

Operating pressure: Up to 3 bar (45 psi)

Operating pH: .pH 1.5 – 14

pH stability: . . Long term (3 months)
pH 3 – 13

Chemical stability: All commonly used aqueous buffers and co-solvents.

Sanitisation: . . 0.5 – 1.0 M sodium hydroxide, 25°C

Storage: 20% ethanol

International Sales and Technical Support:

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ORDERING INFORMATION

Fabsorbent™ F1P HF slurry for column packing

Fabsorbent™ F1P HF (25 mL): 3904-00025

Fabsorbent™ F1P HF (100 mL): 3904-00100

Fabsorbent™ F1P HF (500 mL): 3904-00500

Fabsorbent™ F1P HF (1000 mL): 3904-01000

ProMetic offers a range of larger pack sizes for supply of bulk resins into development and regulated cGMP manufacturing processes. For more information on Fabsorbent™ F1P HF and supply related matters please do not hesitate to contact us at: sales@prometicbiosciences.com.

Fabsorbent™ F1P HF pre-packed columns (for attachment to chromatography workstations)

F1P HF Screening Columns (5 x 1 mL) tba

Fabsorbent™ F1P HF PuraPlate™ (96 column plate for rapid antibody screening and purification method development)

F1P HF Puraplate™ (96 column plate). tba