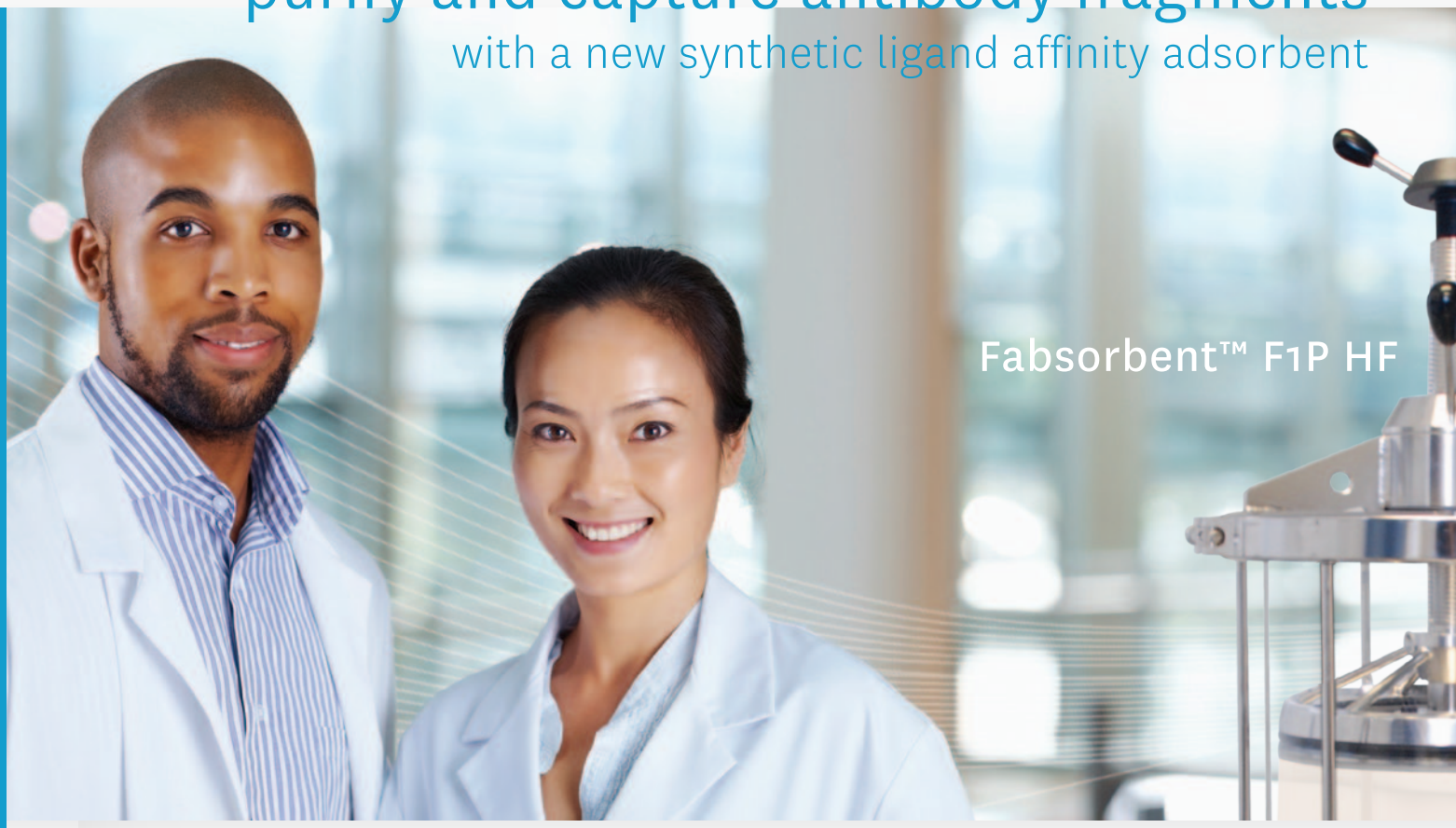
# purify and capture antibody fragments

## with a new synthetic ligand affinity adsorbent




In response to the growth of antibody fragments as research tools and biotherapeutic products, ProMetic BioSciences Ltd (PBL) has developed a new affinity adsorbent for the capture and purification of a wide range of antibody fragments.

Fabsorbent™ F1P HF is an adsorbent with a synthetic ligand 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.



PROMETIC BIOSCIENCES

# KEY BENEFITS

## PROVEN TECHNOLOGY

Sharing the same chemistry as PBL'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 PBL's Chemical Combinatorial Library CCL® technology and extensive computer modelling and ligand screening expertise, PBL have identified the Fabsorbent™ F1P ligand which binds selectively to kappa (**κ**) and lambda (**λ**) light chains of a variety of mammalian antibodies including human, ovine, murine and bovine sources.

## UNIVERSALLY APPLICABLE

Binds and purifies most antibody species (antibody fragments and whole antibodies) that contain **κ** and/or **λ** 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 PBL's ISO 9001 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-column block format PuraPlate™ adsorbent screening kits, pre-packed columns and a variety of slurry pack sizes.

# ANTIBODY FRAGMENTS

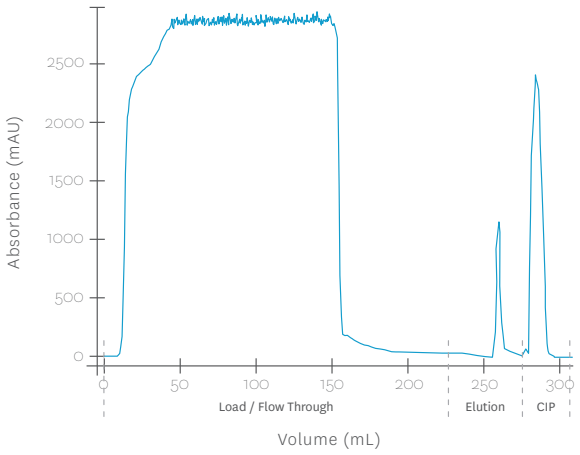
- Fab' expressed in *E.coli*.
- Mammalian Fab fragments from papain catalysed antibody digests.
- Mammalian F(ab')$_2$ fragments from pepsin catalysed antibody digests.
- Human antibody **λ** and **κ** light chains.
- scFv expressed in *E.coli*.
- Single domain antibody fragments expressed in *E.coli*.
- Full length monoclonal antibodies.
- Human, bovine, ovine and murine antibodies and antibody fragments.

# 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 (Figures 1 and 2).

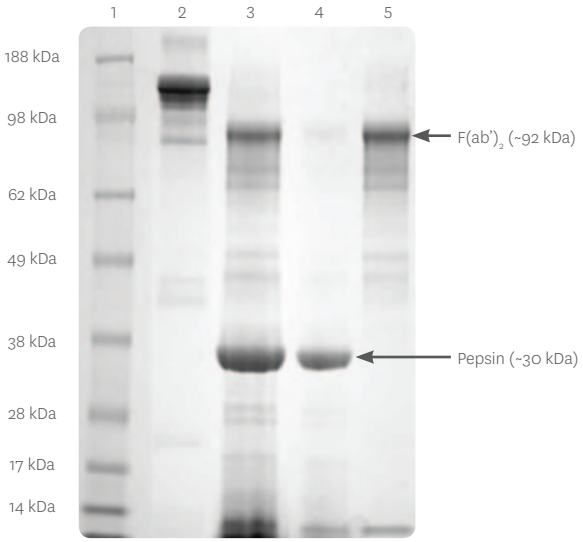
## FIGURE 1

Capture and elution of F(ab')$_2$ from pepsin digest.
Column volume: 10 mL; flow rate: 250 cm/h; equilibration buffer: 25 mM Tris, pH 8.0; elution buffer: 50 mM sodium citrate, pH 3.0; CIP: 0.5 M NaOH.




## FIGURE 2

Non-reduced SDS-PAGE of F(ab')$_2$ from pepsin digest. Lane 1: molecular weight marker; Lane 2: IgG standard; Lane 3: pepsin digest; Lane 4: flow through and post load wash; Lane 5: elution.

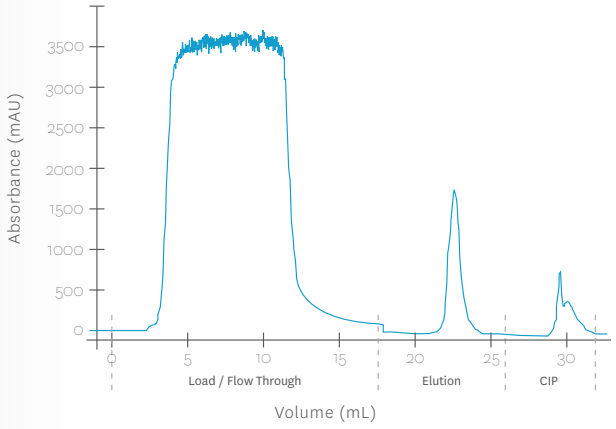


## DOMAIN ANTIBODY FROM *E.COLI* LYSATE

$V_L$ 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 3). Non-reduced SDS-PAGE of the fractions from Fabsorbent™ F1P HF (Figure 4) demonstrates the capture and purification of $V_L$ domain antibody from *E.coli* lysate.

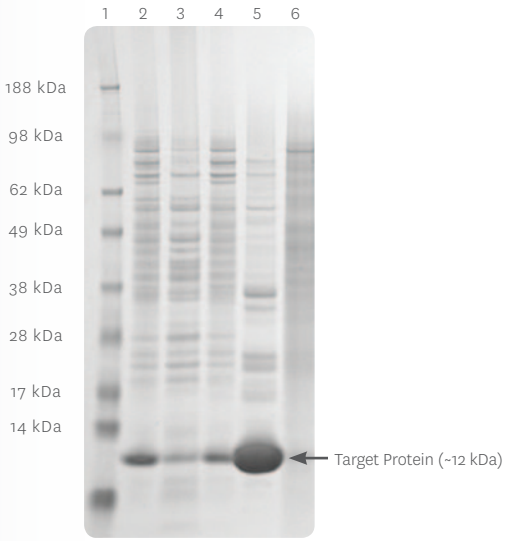
### FIGURE 3

Capture and elution of a domain antibody from *E.coli* lysate. Column volume: 1 mL; 3 minute residence time; equilibration buffer: 25 mM Tris, pH 9.0; elution buffer: 50 mM sodium citrate, pH 3.0; CIP: 0.5 M NaOH.




### FIGURE 4

Non-reduced SDS-PAGE. Lane 1: molecular weight marker; Lane 2: $V_L$ domain *E.coli* lysate; Lane 3: flow through; Lane 4: post load wash; Lane 5: elution; Lane 6: CIP.

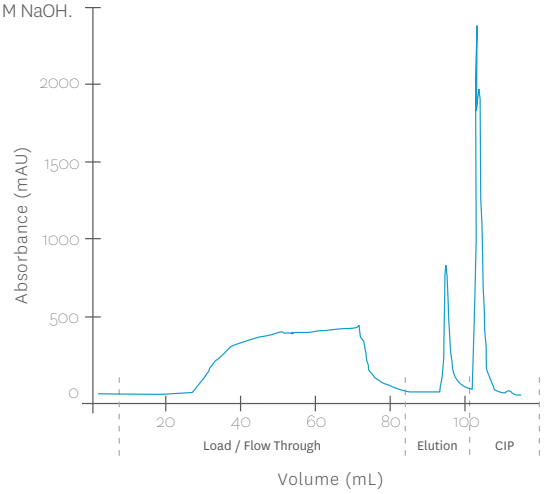



## MONOCLONAL $IgG_1$

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 $IgG_1$ directly from CHO cell culture supernatant (Figure 5 and 6). Fabsorbent™ F1P HF is designed for the capture of light chain containing fragments. All antibody related proteins in CHO cell culture supernatant will be captured and eluted by Fabsorbent™ F1P HF.

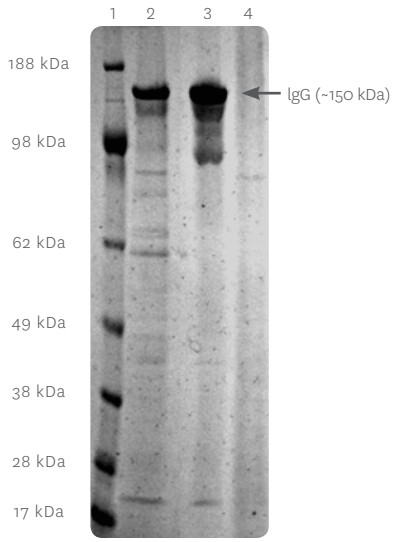
### FIGURE 5

Chromatogram of the capture and elution of $IgG_1$ from CHO cell culture supernatant. Column volume: 2 mL; 3 minute residence time; equilibration buffer: PBS, pH 6.0; elution buffer: 50 mM sodium citrate, pH 3.0; CIP: 0.5 M NaOH.




### FIGURE 6

Non-reduced SDS-PAGE; Lane 1: molecular weight marker; Lane 2: load; Lane 3: elution; Lane 4: CIP.



## PROPERTIES

| | |
|---|---|
| Ligand | F1P synthetic triazine ligand |
| Matrix | PuraBead® P6HF (6% near monodisperse highly cross-llinked 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 - pH 14.0 (Intermittent) |
| pH stability | Long term (3 months)<br>pH 3 – pH 13 |
| Chemical stability | All commonly used aqueous buffers and co-solvents. |
| Sanitisation | 0.5 – 1.0 M sodium hydroxide, 25°C |
| Storage | 20% ethanol |

## ORDER

### 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 |

PBL 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 Pre-Packed Columns (5 x 1 mL) | 4904-00001 |

### FABSORBENT™ F1P HF PURAPLATE™

(96 column plate for rapid antibody screening and purification method development)

| | |
|---|---|
| F1P HF PuraPlate™ (96 column plate) | 2233 |

## CONTACT US

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