

## Fabsorbent™ F1P HF (Product Code: 3904)

### Application Note – Optimisation of Fab fragment purification from *E.coli* lysate using Fabsorbent™ F1P HF PuraPlate™

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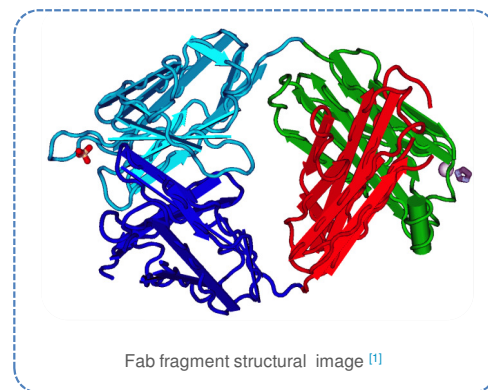
In response to the growth of antibody fragments as research tools and biotherapeutic products, ProMetic BioSciences Ltd (PBL) has developed a new affinity adsorbent for the capture and purification of antibody fragments.

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 antibodies and single domain antibodies.

Fabsorbent™ F1P HF is available in multiple easy to use formats including a variety of slurry pack sizes, pre-packed columns (1 mL) and 96-column block format PuraPlate™ (96 x 0.25 mL). The PuraPlate™ is operated under gravity with buffer/sample loading performed either manually using single or multi-channel pipettes or by automated liquid handling systems.

The Fabsorbent™ F1P HF PuraPlate™ provides a quick and effective method of identifying optimum process conditions whilst minimising the amount of feedstock applied. Optimum conditions identified by the PuraPlate™ can then be scaled up to conventional chromatography columns.

In this application note we describe how the Fabsorbent™ F1P HF PuraPlate™ highlighted a selective elution strategy for Fab fragment purification from an *E.coli* lysate using two successive investigations (Stage 1 and 2). The optimal conditions were then scaled up (Stage 3) onto an automated chromatography workstation using Fabsorbent™ F1P HF to verify the optimal strategy identified by the PuraPlate™.



## STAGE ONE

An initial investigation was performed using the Fabsorbent™ F1P HF PuraPlate™ to identify suitable conditions for the removal of non-target material bound from a null *E.coli* lysate.

The null *E.coli* lysate was loaded onto an equilibrated Fabsorbent™ F1P HF PuraPlate™ using a multi-channel pipette. Non-bound protein was removed by washing with equilibration buffer. A range of buffer conditions were investigated to show the effect of pH on the removal of bound non-target material using three buffers ranging from pH 3.0 to pH 9.0.

The buffer conditions were tested at pH 9.0 and below, as previous experiments using Fabsorbent™ F1P HF showed non-specific elution of the target material at pH 10 and above.

The chromatography conditions for Stage 1 are summarised in Table 1 and the non-reduced SDS-PAGE results for the range of buffer conditions tested (pH 3.0 to pH 9.0) are presented in Figure 1.

**TABLE 1**

Stage 1 PuraPlate™ conditions used to investigate suitable buffers for the removal of non-target material bound from a null *E.coli* lysate using Fabsorbent™ F1P HF.

<b>Platform</b>	Fabsorbent™ F1P HF PuraPlate™
<b>Column parameters</b>	0.25 mL column volume (CV) (0.8 cm diameter, 0.5 cm bed height)
<b>Equilibration buffer</b>	50 mM sodium phosphate, pH 8.0
<b>Load</b>	2 mL null <i>E.coli</i> lysate, pH 8.0
<b>Buffer conditions</b>	(1) McIlvaines <sup>21</sup> buffer (pH 3 to pH 7) (2) 50 mM sodium phosphate buffer (pH 6 to pH 8) (3) 50 mM Tris buffer (pH 7 to pH 9)
<b>Clean in Place (CIP)</b>	0.5 M NaOH

**FIGURE 1**

Non-reduced SDS-PAGE of samples from the Fabsorbent™ F1P HF PuraPlate™ investigation (Stage 1) – Lane 1: Molecular weight marker; Lane 2: null *E.coli* lysate; Lane 3: flow through; Lanes 4 to 8: McIlvaines buffer; Lanes 9 to 11: phosphate buffer; Lanes 12 to 14: Tris buffer.

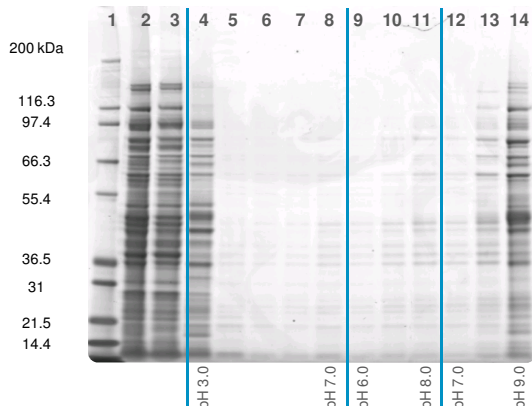


Figure 1 indicates that Fabsorbent™ F1P HF binds non-target material (Lanes 4 and 14). The buffer conditions between pH 4.0 and pH 8.0 (Lanes 5 to 12) indicate minimal removal of bound non-target material although, Tris buffer at pH 8.0 (Lane 13) gives improved protein recovery.

At both pH 3.0 (Lane 4) and pH 9.0 (Lane 14) significant non-target material is recovered.

## STAGE TWO

A further Fabsorbent™ F1P HF PuraPlate™ investigation was performed identifying a more selective range of buffer conditions at pH 3.0 and pH 9.0 highlighted during Stage 1.

An *E.coli* lysate containing Fab fragments was loaded onto an equilibrated Fabsorbent™ F1P HF PuraPlate™ using a multi-channel pipette. Non-bound protein was removed by washing with equilibration buffer.

A range of buffer conditions were investigated to show the effect of pH between pH 3.0 to pH 5.0 (citrate and acetate buffers) and at pH 9.0 (Tris and glycine buffers) on the recovery of bound target material.

The chromatography conditions for Stage 2 are summarised in Table 2.

**TABLE 2**

Stage 2 PuraPlate™ conditions used to investigate the optimal buffer conditions for the purification of Fab fragments from an *E.coli* lysate using Fabsorbent™ F1P HF.

<b>Platform</b>	Fabsorbent™ F1P HF PuraPlate™
<b>Column parameters</b>	0.25 mL column volume (CV) (0.8 cm diameter, 0.5 cm bed height)
<b>Equilibration buffer</b>	50 mM sodium phosphate, pH 8.0
<b>Load</b>	1.5 mL of <i>E.coli</i> lysate containing Fab fragments, pH 8.0
<b>Buffer conditions</b>	(1) 50 mM sodium acetate (pH 3.5, pH 4.0, pH 4.5 and pH 5.0) (2) 50 mM sodium citrate (pH 3.0, pH 3.5, pH 4.0 and pH 4.5) (3) 50 mM Tris-acetate (pH 4.5) (4) 50 mM glycine-NaOH (pH 9.0) (5) 50 mM Tris (pH 9.0)
<b>Clean in Place (CIP)</b>	0.5 M NaOH

Figure 2 contains the non-reduced SDS-PAGE for the range of buffer conditions investigated, using Fabsorbent™ F1P HF PuraPlate™, for the purification of Fab fragments from an *E.coli* lysate.

**FIGURE 2**

Non-reduced SDS-PAGE of samples from the Fabsorbent™ F1P HF PuraPlate™ investigation (Stage 2) – Lane 1: Molecular weight marker; Lane 2: Load; Lane 3: Purified Fab; Lanes 4 to 7: acetate buffer (pH 3.5 to pH 5.0); Lanes 8 to 11: citrate buffer (pH 3.0 to pH 4.5); Lane 12: Tris-acetate buffer (pH 4.5); Lane 13: glycine buffer (pH 9.0); Lane 14: Tris buffer (pH 9.0).

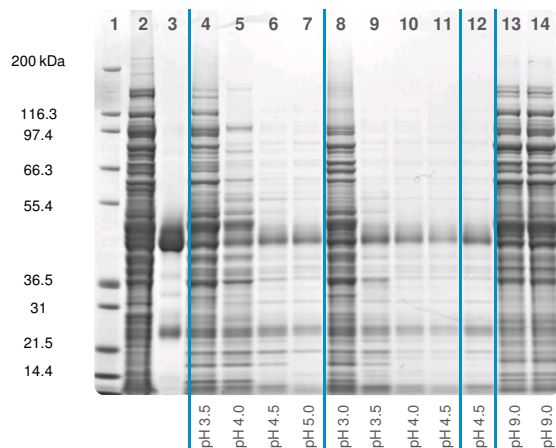


Figure 2 shows the buffer conditions in lanes 4 to 14 give a range of Fab fragment recovery and purity. Buffer conditions - acetate, pH 3.5 (Lane 4), citrate, pH 3.0 (Lane 8), glycine, pH 9.0 (Lane 13) and Tris, pH 9.0 (Lane 14) all show high recovery of non-target and target material and are not suitable elution strategies for optimum purity.

As a result, buffer conditions - acetate, pH 4.5 (Lane 6) and citrate, pH 4.0 (Lane 10) are the most suitable conditions for use as selective elution strategies for Fab fragment recovery from an *E.coli* lysate (Figure 2).

## STAGE THREE

Two column runs were performed using Fabsorbent™ F1P HF on an automated chromatography workstation.

Column Run 1 used a generic elution buffer (sodium citrate, pH 3.0) for target recovery. Column Run 2 used one of the optimum selective elution buffers highlighted by the PuraPlate™ investigation in Stage 2 followed by a strip using a generic elution buffer.

The chromatography conditions for both Column Runs 1 and 2 are summarised in Table 3.

Figure 3 is the chromatogram of Column Run 2 for the purification of Fab fragments from an *E.coli* lysate using Fabsorbent™ F1P HF. The Fab Fragments are selectively eluted at pH 4.5 using an acetate buffer followed by a strip (sodium citrate, pH 3.0).

Figure 4 contains the non-reduced SDS-PAGE for both chromatography Column Runs 1 and 2 using Fabsorbent™ F1P HF for purification of Fab fragments from an *E.coli* lysate.

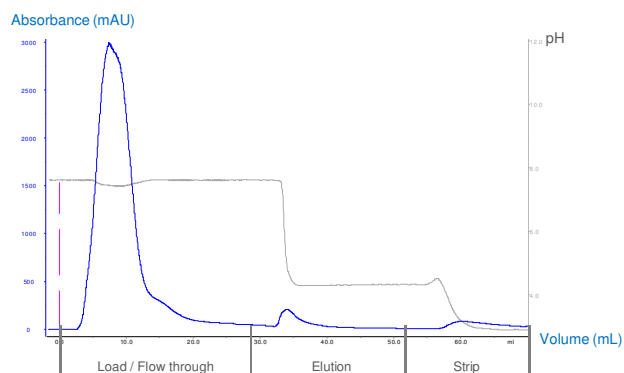
**TABLE 3**

Stage 3 chromatography conditions for Column Runs 1 and 2 for the purification of Fab fragments from an *E.coli* lysate using Fabsorbent™ F1P HF adsorbent.

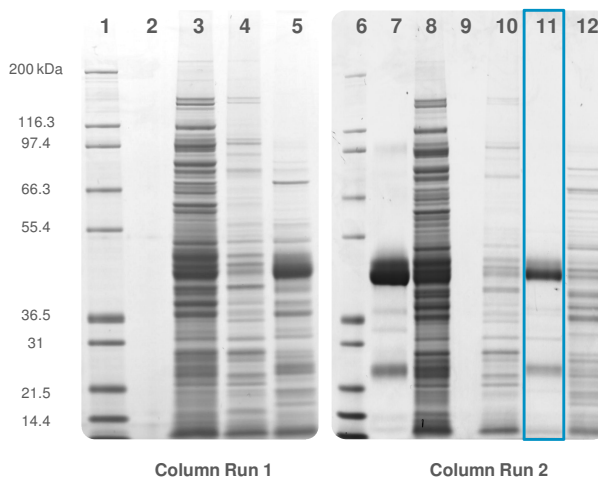
Platform	Automated Chromatography Workstation
Column parameters	4 mL column volume (CV) (1 cm diameter, 5 cm bed height)
Packing flow rate	600 cm/hr
Operational flow rate	300 cm/hr
Loading flow rate	50 cm/hr (6 minute residence time)
Equilibration buffer	50 mM sodium phosphate, pH 8.0
Load	<i>E.coli</i> lysate containing Fab fragments, pH 8.0
Elution buffer (RUN 1)	50 mM sodium citrate buffer, pH 3.0
Elution buffer (RUN 2)	50 mM sodium acetate buffer, pH 4.5
Clean in Place (CIP)	0.5 M NaOH

**FIGURE 3**

Column Run 2 chromatogram of the capture and selective elution (acetate, pH 4.5) of Fab fragments from an *E.coli* lysate using Fabsorbent™ F1P HF.

**FIGURE 4**

Non-reduced SDS-PAGE of samples from Columns Runs 1 and 2 using Fabsorbent™ F1P HF – Lane 1: Molecular weight marker; Lane 2: blank; Lane 3: Load; Lane 4: flow through; Lane 5: elution (citrate, pH 3.0); Lane 6: Molecular weight marker; Lane 7: Purified Fab; Lane 8: Load; Lane 9: blank; Lane 10: flow through; Lane 11: elution (acetate, pH 4.5); Lane 12: strip (citrate, pH 3.0).



The SDS-PAGE results in Figure 4 indicate that the generic elution buffer (50 mM sodium citrate, pH 3.0) from Column Run 1 (Lane 5) recovers all of the Fab fragments but also elutes a significant proportion of non-target material.

The sodium acetate elution buffer at pH 4.5 from Column Run 2 (Lane 11), identified by Stage 2, shows selective recovery of the Fab fragments. The remainder of the non-target material is recovered in the generic elution buffer strip (Lane 12) or is removed during the clean-in-place (not shown).

By densitometry, the purity of both elution fractions was determined. The purity of the Fab fragments in the generic elution buffer (citrate, pH 3.0) from Column Run 1 was calculated at ~59%. Whilst the purity of the optimum identified elution strategy (acetate, pH 4.5) used in Column Run 2 was significantly higher at >95%.

Overall, the results show that the elution of Fab fragments from an *E.coli* lysate is both pH and buffer dependent, as different buffer systems will elute Fab fragments at different pH values.

## CONCLUSIONS

Fab fragments from an *E.coli* lysate can be effectively captured and purified using Fabsorbent™ F1P HF.

The PuraPlate™ provided an easy to use gravity fed platform for bench top applications which required no specialised equipment. As a result, investigating Fabsorbent™ F1P HF in PuraPlate™ format provided an excellent tool to quickly identify optimal buffer conditions whilst minimising feedstock usage.

In this example, Fab fragments bound to Fabsorbent™ F1P HF as well as a host of non-target material from the *E.coli* lysate.

However, an optimum selective elution buffer was identified using the Fabsorbent™ F1P HF PuraPlate™ and verified using a conventional chromatography platform.

As a result, the Fab fragments were separated from the bound non-target material from the *E.coli* lysate and selectively eluted using a 50 mM sodium acetate buffer at pH 4.5.

This buffer gave good Fab fragment recovery and purity of >95% in a single step without the need for complex buffers.

## REFERENCES

- [1] Fab fragment image sourced from – [http://commons.wikimedia.org/wiki/File:1MRC\\_Igg\\_Jel\\_103\\_Fab\\_Fragment.png](http://commons.wikimedia.org/wiki/File:1MRC_Igg_Jel_103_Fab_Fragment.png)
- [2] McIlvaines buffer is a buffer consisting of 0.1 M citric acid and 0.2 M dibasic sodium phosphate and has a buffering capacity between pH 2.6 and pH 7.6.

## SALES & TECHNICAL SUPPORT

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