

Fabsorbent™ F1P HF (Product Code: 3904)

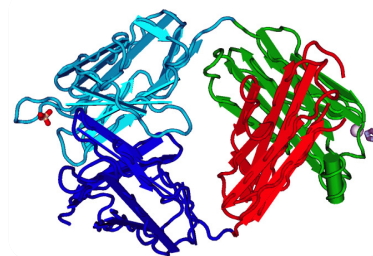
Application Note – Fab and F(ab')₂ fragment purification from CHO cell culture supernatant using Fabsorbent™ F1P HF

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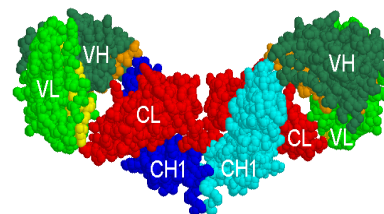
In response to the growth of antibody fragments as research tools and biotherapeutic products, ProMetic BioSciences Ltd (PBL) has developed a new affinity adsorbent for the capture and purification of antibody fragments.

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, F(ab')₂, scFv), engineered antibodies and single domain antibodies.

In this application note we describe the optimisation of both Fab and F(ab')₂ fragment purification from CHO cell culture supernatant (CCS) using Fabsorbent™ F1P HF.



Fab fragment structural image [1]



F(ab')₂ fragment structural image [2]

FAB FRAGMENT PURIFICATION

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

All three columns were equilibrated using 50 mM sodium phosphate (pH 8.0).

Column Run 1 used a generic elution buffer (50 mM sodium citrate, pH 3.0).

Column Run 2 used a sodium acetate elution buffer at pH 4.0, followed by a strip using a generic elution buffer.

Column Run 3 included the addition of a wash step (50 mM glycine, pH 9.0), followed by an acetate elution buffer at pH 4.0.

All three columns used a 0.5 M NaOH CIP (Clean in Place) strategy.

The chromatography conditions for Column Runs 1 to 3 are summarised in Table 1.

TABLE 1

Chromatography conditions for Column Runs 1 to 3 for the purification of Fab fragments from a CHO CCS 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	CHO CCS 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 4.0
Wash buffer (RUN 3)	50 mM glycine-NaOH, pH 9.0
Elution buffer (RUN 3)	50 mM sodium acetate buffer, pH 4.0
Clean in Place (CIP)	0.5 M NaOH

Figure 1 shows the chromatogram of Column Run 2 for the purification of Fab fragments from a CHO CCS using Fabsorbent™ F1P HF.

Figure 2 contains the non-reduced SDS-PAGE for Column Runs 1 to 3, using Fabsorbent™ F1P HF.

FIGURE 1

Column Run 2 chromatogram of the capture and recovery of Fab fragments from a CHO CCS using Fabsorbent™ F1P HF and elution buffer - acetate, pH 4.0 followed by a strip - citrate, pH 3.0.

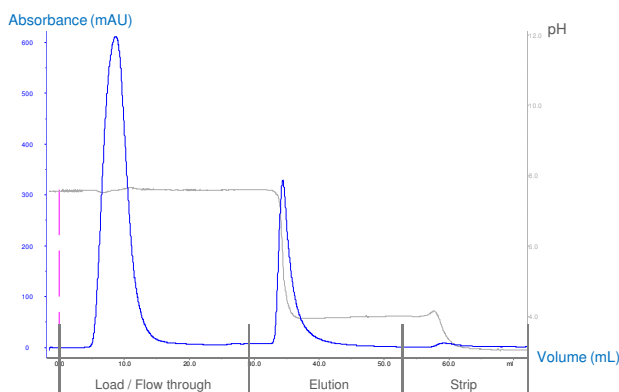
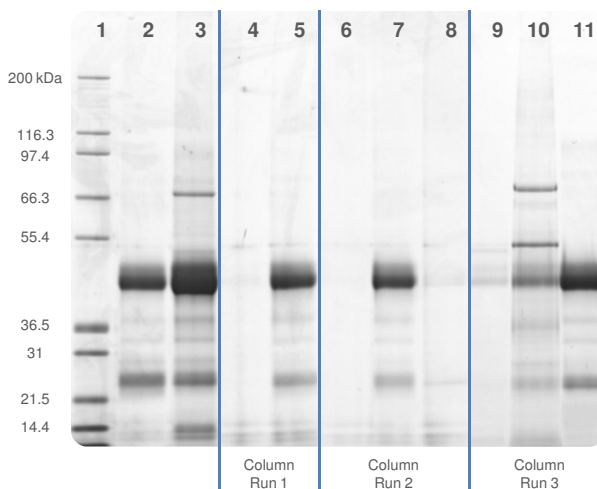


FIGURE 2

Non-reduced SDS-PAGE of samples from Column Runs 1 to 3 using Fabsorbent™ F1P HF – Lane 1: Molecular weight marker; Lane 2: Purified Fab; Lane 3: Load; Lane 4: flow through; Lane 5: elution (citrate, pH 3.0); Lane 6: flow through; Lane 7: elution (acetate, pH 4.0); Lane 8: strip (citrate, pH 3.0); Lane 9: flow through; Lane 10: wash (glycine, pH 9.0); Lane 11: elution (acetate, pH 4.0).



The SDS-PAGE results (Figure 2) shows high purity (>90%) for all elution strategies evaluated, however an improvement in the purity of the target material without significant reduction in recovery is observed for the sodium acetate elution buffer at pH 4.0 (Lanes 7 and 8).

The wash step (50 mM glycine, pH 9.0) used during Column Run 3 did not show improved purity of the target material in the elution (Lane 11) in this example.

However, the wash step (Lane 10) does show significant recovery of non-target material and minor loss of target protein. As a result, this wash strategy could be used as a suitable application for alternative feedstocks.

F(AB')₂ FRAGMENT PURIFICATION

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

All four columns were equilibrated using 50 mM sodium phosphate (pH 8.0).

Column Run 1 used a generic elution buffer (citrate, pH 3.0).

Column Run 2 investigated an acetate elution buffer at pH 4.0.

Column Run 3 investigated an acetate elution buffer at pH 4.5.

Column Run 4 included the addition of a wash step (glycine, pH 9.0) followed by a generic elution buffer step.

All four columns used a 0.5 M NaOH CIP (Clean in Place) strategy.

The chromatography conditions for Column Runs 1 to 4 are summarised in Table 2.

TABLE 2

Chromatography conditions for Column Runs 1 to 4 for the purification of F(ab')₂ fragments from a CHO CCS 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	CHO CCS containing F(ab') ₂ 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 4.0
Elution buffer (RUN 3)	50 mM sodium acetate buffer, pH 4.5
Wash buffer (RUN 4)	50 mM glycine-NaOH, pH 9.0
Clean in Place (CIP)	0.5 M NaOH

Figure 3 shows the chromatogram of Column Run 2 (acetate, pH 4.0) for the purification of F(ab')₂ fragments from a CHO CCS using Fabsorbent™ F1P HF.

Figure 4 contains the non-reduced SDS-PAGE for Column Runs 1 to 4 using Fabsorbent™ F1P HF.

FIGURE 3

Column Run 2 chromatogram of the capture and recovery of F(ab')₂ fragments from a CHO CCS using Fabsorbent™ F1P HF and elution buffer - acetate, pH 4.0.

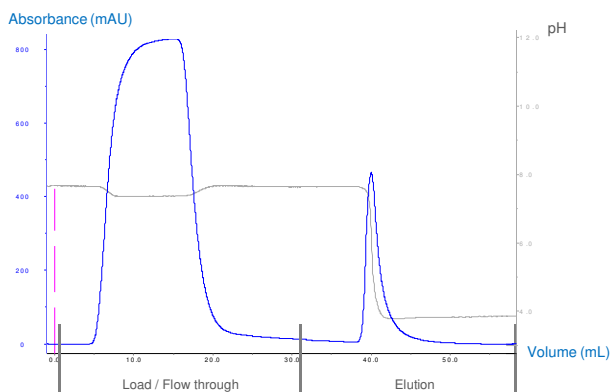
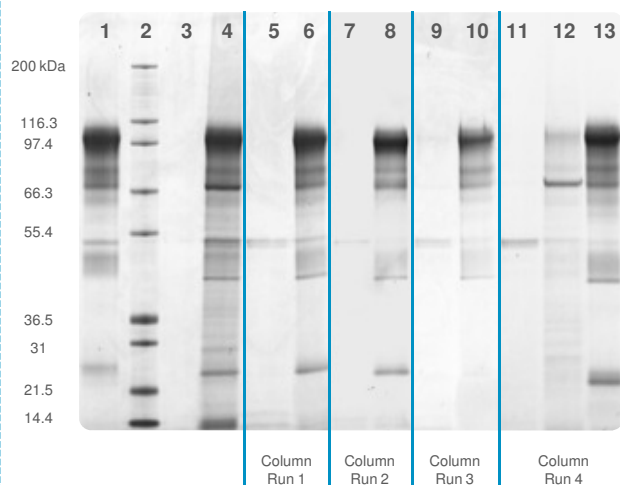


FIGURE 4

Non-reduced SDS-PAGE of samples from Column Runs 1 to 4 using Fabsorbent™ F1P HF – Lane 1: Purified F(ab')₂; Lane 2: Molecular weight marker; Lane 3: blank; Lane 4: Load; Lane 5: flow through; Lane 6: elution (citrate, pH 3.0); Lane 7: flow through; Lane 8: elution (acetate, pH 4.0); Lane 9: flow through; Lane 10: elution (acetate, pH 4.5); Lane 11: flow through; Lane 12: wash (glycine, pH 9.0); Lane 13: elution (citrate, pH 3.0).



The SDS-PAGE results in Figure 4 shows that the generic elution buffer (citrate, pH 3.0) used in Column Run 1 (Lane 6) gives good qualitative purity. However, the acetate elution buffer at pH 4.0 used in Column Run 2 (Lane 8) gives optimal selective recovery of F(ab')₂ fragments, from a CHO CCS using Fabsorbent™ F1P HF, with a purity of >95% determined by densitometry.

Column Run 3 has lower recovery of target protein for the acetate buffer at pH 4.5 (Lane 10) compared to the acetate buffer at pH 4.0 used in Column Run 2. However, the acetate buffer at pH 4.5 provides a separation of light chain.

The additional wash step (glycine, pH 9.0) used during Column Run 4 did not provide improved purification on elution (Lane 13) for this example. However, the wash step (Lane 12) showed significant recovery of non-target material, minimal loss of target protein and could be used as a suitable application for alternative feedstocks.

CONCLUSIONS

Overall, both Fab and F(ab')₂ fragments from a CHO CCS can be effectively captured and purified using Fabsorbent™ F1P HF with high purity and recovery in a single step without the need for complex buffers.

Fab fragments from a CHO CCS can be selectively eluted from Fabsorbent™ F1P HF with purities of >90% using:

- 50 mM sodium citrate, pH 3.0
- 50 mM sodium acetate, pH 4.0

F(ab')₂ fragments from a CHO CCS can be selectively eluted from Fabsorbent™ F1P HF using:

- 50 mM sodium citrate, pH 3.0
- 50 mM sodium acetate, pH 4.0
- 50 mM sodium acetate, pH 4.5

The optimal elution buffer highlighted was sodium acetate at pH 4.0 with a purity of >95% as determined by densitometry.

If required, a glycine buffer at pH 9.0 could be used as a suitable wash condition for Fabsorbent™ F1P HF, to remove non-target material bound from a CHO CCS, with minimal loss of either Fab or F(ab')₂ fragments.

For further process optimisation Fabsorbent™ F1P HF is available in multiple easy to use formats including pre-packed columns (1 mL) and 96-column block format PuraPlate™ (96 x 0.25 mL).

For examples of Fabsorbent™ F1P HF PuraPlate™ investigations for the purification of both Fab and F(ab')₂ fragments from *E.coli* lysate please view the following two application notes available on the ProMetic BioSciences website:

- 1) Optimisation of **Fab** fragment purification from *E.coli* lysate using Fabsorbent™ F1P HF PuraPlate™ (Issue - 041110).

- 2) Optimisation of **F(ab')₂** fragment purification from *E.coli* lysate using Fabsorbent™ F1P HF PuraPlate™ (Issue - 041110).

REFERENCES

- [1] Fab fragment image sourced from –
http://commons.wikimedia.org/wiki/File:1MRC_Igg_Jel_103_Fab_Fragment.png
- [2] F(ab')₂ fragment image sourced from –
http://imgt.cines.fr/textes/IMGTEducation/Tutorials/GandBcells/_UK/3Dstructure/Figure3.html

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