

Application Note

Capture and Purification of Fab fragments from human polyclonal IgG papain digest using Fabsorbent™ F1P HF

Introduction

Fabsorbent™ F1P HF is a synthetic affinity adsorbent derived from ProMetic's Mimetic Ligand™ technology. The Fabsorbent™ F1P ligand binds to antibody light chains and is designed to capture and purify engineered antibodies and antibody related fragments directly from either cell lysates or from culture supernatants.

Papain, a cysteine protease, is used to hydrolyse peptide bonds in the hinge region of an antibody. This results in the generation of two Fab fragments and one Fc fragment from each antibody molecule. This is a commonly used technique to prepare Fab fragments from whole IgG.

This application note describes the use of Fabsorbent™ F1P HF for the capture and purification of Fab fragments from a papain catalysed digest of human polyclonal IgG.

Materials and Methods

Human polyclonal IgG is digested using immobilised papain. The immobilised papain is activated with cysteine and equilibrated with digestion buffer. The papain is mixed with a solution of IgG at 37°C for an appropriate time to produce a digest containing Fab and Fc fragments.

Identifying the elution condition for Fab recovery

Papain digested human polyclonal IgG is pH adjusted to pH 8.0 and loaded onto

equilibrated Fabsorbent™ F1P HF (Table 1). After loading, non-bound protein is removed by washing with equilibration buffer (Table 1). Bound protein is eluted using an elution gradient between pH 7.0 and pH 3.0 and fractions collected and analysed by SDS-PAGE.

Recovery of Fab fragments

The optimal elution pH is identified from the gradient elution profile. Papain digest (~ 60 mg of digest containing residual whole molecule IgG) is loaded onto Fabsorbent™ F1P HF. The non-bound protein is removed by washing with equilibration buffer. The Fab fragments are selectively eluted at pH 4.0.

Fabsorbent™ F1P HF is hydroxide stable and is cleaned with 0.5 M NaOH. The conditions are summarised in Table 1.

Table 1 – Column conditions

| Chromatography conditions for capture and purification of Fab fragments from a papain digest | |
|--|--|
| Column Height | 5.0 cm |
| Column Volume | 4.0 mL |
| Linear Flow Rate | Load – 75 cm/h (4 minute residence time) Operational – 300 cm/h |
| Equilibration Buffer | 25 mM Tris-HCl, pH 8.0 |
| Elution buffer (gradient elution) | 0.1 M citric acid/0.2 M Na ₂ HPO ₄ , pH 7.0-pH 3.0 (20 CV) |
| Elution buffer (isocratic elution) | 0.05 M citric acid/0.1 M Na ₂ HPO ₄ , pH 4.0 and pH 3.0 |
| CIP | 0.5 M NaOH |

Results

In this example both Fab and Fc fragments bind to Fabsorbent™ F1P HF when applied to the column at pH 8.0. An elution gradient from pH 7.0 to pH 3.0 indicates that the Fab fragment can be selectively recovered at ~ pH 3.7 (Figure 1).

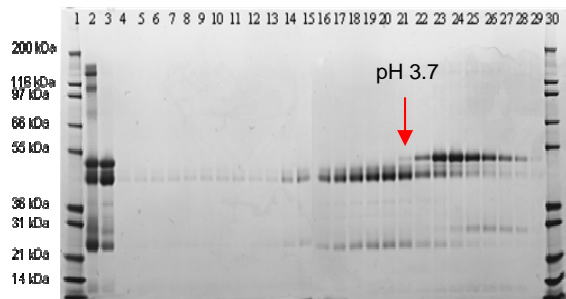


Figure 1: SDS-PAGE of gradient elution of Fab and Fc fragments from Fabsorbent F1P HF; Lane 1 – Molecular weight marker; Lane 2 – Papain digested IgG; Papain digest after SEC to remove whole IgG; Lane 4 – Flow through; Lane 5 – 29 – Fractions of the elution gradient from pH 7.0 to pH 3.0; Lane 30 – Molecular weight marker.

From elution gradient data an isocratic elution regime was identified for recovery of Fab fragments bound to Fabsorbent™ F1P HF. The Fab fragments are separated from Fc fragments using citrate/phosphate buffer at pH 4.0 (Figure 2; Table 1).

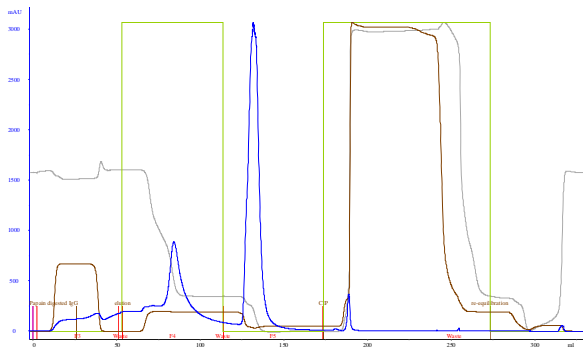


Figure 2: Chromatogram of the capture and recovery of Fab fragments from a papain catalysed IgG digest

The Fab containing peak is identified by SDS-PAGE analysis of the chromatography fractions (Figure 3).

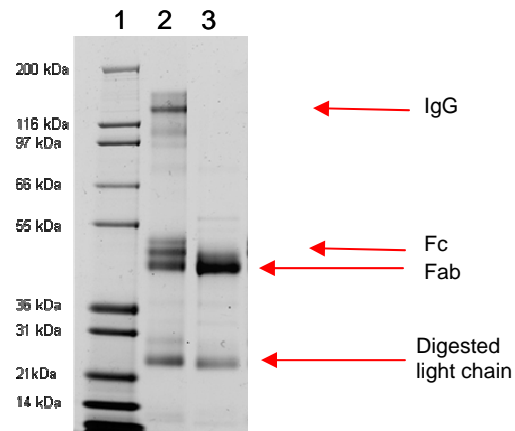


Figure 3: SDS-PAGE of the chromatography fractions for the capture and purification of Fab fragments using Fabsorbent F1P HF; Lane 1 – Molecular weight marker; Lane 2 – Papain digested IgG; Lane 3 – pH 4.0 elution.

The results indicate that at pH 4.0 the Fab fragments are selectively eluted. Fc and whole molecule IgG co-elute at pH 3.0.

The elution of Fab fragments is both pH and buffer dependent. Different buffer systems will elute Fab and Fc fragments at different pH values. Sodium acetate, Tris-succinate and sodium citrate buffers at pH 4.0 were investigated for elution of Fab fragments from Fabsorbent™ F1P HF (Figure 4).

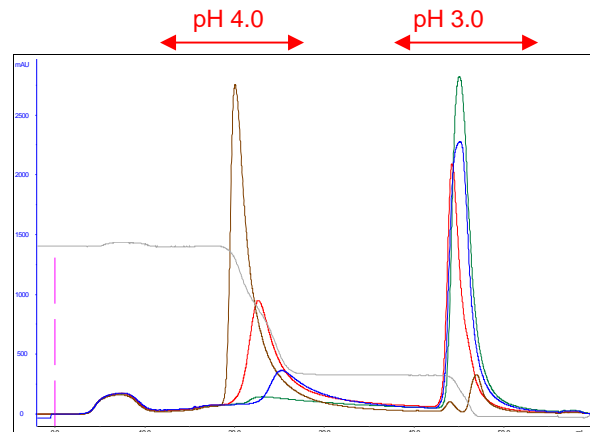


Figure 4: Chromatograms of the capture and recovery of Fab fragments using citrate/phosphate (blue), sodium acetate (brown), sodium citrate (green) and Tris-succinate (red) buffers at pH 4.0

Figure 5 shows the SDS-PAGE profiles for the various elution buffers tested.

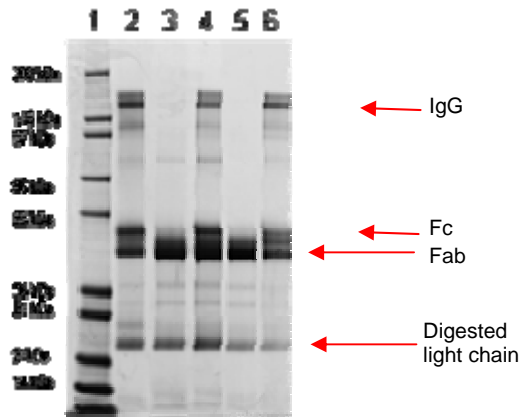


Figure 5: SDS-PAGE of the chromatography fractions for the elution of Fab fragments from Fabsorbent F1P HF using; Lane 1 – Molecular weight marker; Lane 2 – Papain digested IgG; Lane 3 – Citrate/phosphate pH 4.0 elution; Lane 4 – Tris succinate pH 4.0 elution; Lane 5 – sodium citrate pH 4.0 elution; Lane 6 – Sodium acetate pH 4.0 elution.

The results indicate that the optimal buffer for elution of Fab fragments in this instance was 0.05 M citric acid/0.1 M Na₂HPO₄, pH 4.0. In the case of Tris-succinate buffer at pH 4.0 both Fab and Fc fragments are eluted (Figure 5). For sodium citrate elution buffer, Fab fragments are eluted at pH 4.0, but recovery of Fab fragment is reduced. Sodium acetate elution at pH 4.0 removes both Fab and Fc fragments from Fabsorbent™ F1P HF. Some free light chain is visible in all elution fractions as Fabsorbent™ F1P HF binds to both kappa and lambda light chain.

Conclusions and Summary

Fab fragments from a papain catalysed digest of IgG can be effectively captured and purified using Fabsorbent™ F1P HF. In this example both Fab and Fc fragments were found to bind to Fabsorbent™ F1P HF.

The fragments could be separated by selective elution at the appropriate pH.

The best elution buffer conditions for a particular Fab digest can be determined by performance of a pH elution gradient from pH 7.0 to pH 3.0 over 20 column volumes.

However, on the basis of the data presented here, 0.05 M citric acid/0.1 M Na₂HPO₄, pH 4.0 is a good starting point for elution of Fab fragment as this buffer provides good purity and high recovery of Fab fragment and good resolution from Fc and whole molecule IgG.

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