PROMETIC BIOSCIENCES

A Synthetic Ligand Affinity Adsorbent for Capture and Purification of Genetically Engineered Antibody Fragment Therapeutics

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Introduction

Protein engineering techniques provide a means for minimizing antibodies into Fab fragments, scFv or even single-domain antibody fragments such as V_H, V_L or V_{HH}. These genetically engineered antibody fragments provide new opportunities for therapeutic and diagnostic applications. However, unlike full-chain monoclonal antibodies where Protein A based affinity adsorbents provide a convenient platform for capture and purification, antibody fragments which lack an Fc region frequently require purification by using a combination of non-affinity based chromatography methods or using the expensive and process inefficient Protein L ligand.

Fabsorbent™ F1P HF is a robust small molecule ligand that captures and purifies antibody fragments directly from biological feedstocks as an alternative to Protein L. The adsorbent is a non-peptidyl triazine ligand that is hydroxide stable and can be sanitised using up to 1 M sodium hydroxide.

The adsorbent can purify fragments from a range of mammalian sources including human, bovine, ovine and murine, allowing purification of monovalent antibody fractions, engineered antibody variants and single domain antibodies.

Fabsorbent F1P HF binds to both the lambda and kappa light chain of IgG allowing a range of different fragments to be purified and to the Fc region of antibody molecules with different binding affinities.

Antibody fragments

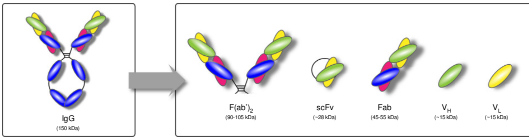

Figure 1: Schematic representation of antibody fragments [1 and 2]

V_L domain antibody purification

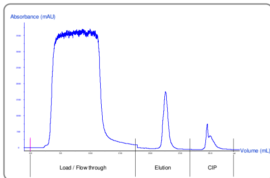

Figure 2: Chromatogram of capture (pH 9.0, 3 mins residence time [RT]), recovery (pH 3.0) and CIP (0.5 M NaOH) of recombinant V_L domain antibody using Fabsorbent F1P HF.

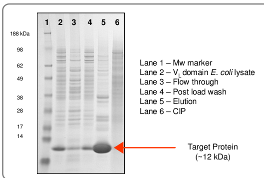

Figure 3: Non-reduced SDS–PAGE analysis of chromatography fractions from the capture of V_L domain antibody using Fabsorbent F1P HF.

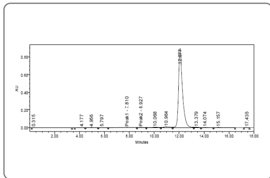

Figure 4: Gel permeation chromatography (GPC) of V_L domain antibody purified from *E. coli* lysate using Fabsorbent F1P HF.

Criterion	Performance
Dynamic Binding Capacity	9 gL^{-1} of adsorbent
Purity	~94% (GPC)
HCP Clearance	98%
DNA Clearance	2 log

Table 1: Summary of column performance for recombinant V_L domain antibody purification using Fabsorbent F1P HF.

scFv fragment purification

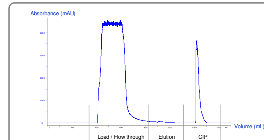

Figure 5: Chromatogram of the capture (pH 8.0, 3 mins RT), recovery (pH 5.0) and CIP (0.5 M NaOH) of scFv fragments using Fabsorbent F1P HF.

	Yield (%)*	Purity (%)**	HCP Clearance (log)	DNA Clearance (log)
Load	100	2	0	0
Flow through	82	-	-	-
Elution	0.6	84	2.0	3.0
CIP	17	-	-	-

*Yield determined by total protein
** Purity determined by GPC

Table 2: Summary of column performance for scFv fragment purification using Fabsorbent F1P HF.

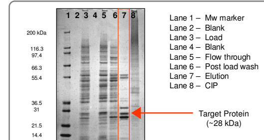

Figure 6: Non-reduced SDS–PAGE analysis of chromatography fractions from the capture of scFv fragments by Fabsorbent F1P HF.

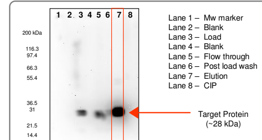

Figure 7: Western blot (using [Fab specific] α-human IgG-HRP) of chromatography fractions from the capture of scFv fragments by Fabsorbent F1P HF.

Fab fragment purification

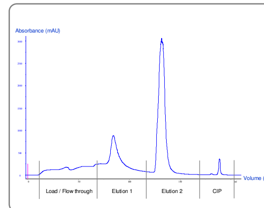

Figure 8: Chromatogram of the capture and recovery of Fab fragments from a papain catalysed IgG digest using Fabsorbent F1P HF, elution 1 – pH 4.0 and elution 2 – pH 3.0. [3]

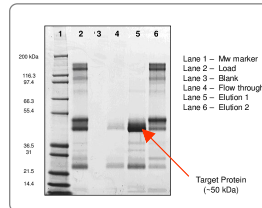

Figure 9: Non-reduced SDS-PAGE of the chromatography fractions from the capture of Fab fragments (papain digest) using Fabsorbent F1P HF [3].

Fabsorbent F1P HF performance

Protein	Origin	Feedstock	Strength of Binding*
Fab	Human	Purified	+++
Fab	Ovine	Purified	+++
Fab	Bovine	Purified	+++
Fab'	Human	*E.coli* lysate	+++
$F(ab')_2$	Human	Purified	++++
$F(ab')_2$	Ovine	Purified	++++
$F(ab')_2$	Bovine	Purified	++++
scFv	Human	*E.coli* lysate	++
IgG_1	Human	CHO cell culture supernatant	++++
IgG_1	Murine	Hybridoma cell culture supernatant	++++
IgG_4	Human	CHO cell culture supernatant	+++
Kappa light chain	Human	Purified	+++
Lambda light chain	Human	Purified	+++

*Relative strength of binding (+), (++), (+++), (++++), no binding (-)

$F(ab')_2$ fragment binding - κ and λ light chains

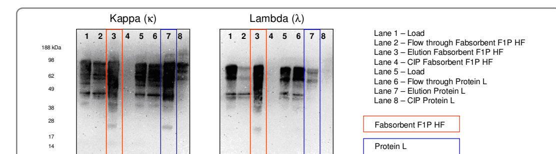

Figure 10: Western blots of chromatography fractions from the capture of kappa (α-human κ light chain-HRP [bound and free]) and lambda (α-human λ light chain-HRP [bound and free]) light chains for $F(ab')_2$ fragments using Fabsorbent F1P HF and Protein L.

IgG, Fab and $F(ab')_2$ fragment binding isotherms

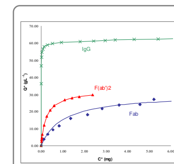

Figure 11: Fabsorbent F1P HF binding isotherms for IgG, $F(ab')_2$ and Fab fragments.

	Kd
Fab fragments	2.7×10^{-8} M
$F(ab')_2$ fragments	5.2×10^{-9} M
Whole molecule IgG	8.6×10^{-9} M

	Q_{max} (gL^{-1} of adsorbent)
Fab fragments	32.1
$F(ab')_2$ fragments	34.1
Whole molecule IgG	65.8

Table 3: Fabsorbent F1P HF Q_{max} and Kd results for IgG, $F(ab')_2$ and Fab fragments.

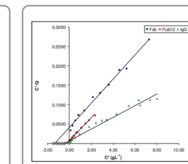

Figure 12: Fabsorbent F1P HF linear isotherms for IgG, $F(ab')_2$ and Fab fragments.

Conclusions

- Fabsorbent™ F1P HF is a robust small molecule ligand that successfully captures and purifies antibody fragments directly from mammalian sources including human, bovine, ovine and murine.
- Fabsorbent F1P HF is a superior alternative to Protein L, binding both kappa and lambda light chains of antibody fragments.
- Fabsorbent F1P HF has a broad utility for the purification of a wide variety of antibody fragments separating whole molecule IgG from Fab, $F(ab')_2$ and Fc fragments by selective elution.

References

[1] Modified from Holliger & Hudson, Nature Biotechnology, 2005, 23(9), 1126-1136.

[2] Antibodies: A Laboratory Manual, Harlow, E. and Lane, D., eds., Cold Spring Harbor Laboratory, pp628-629.

[3] Capture and Purification of Fab fragments from human polyclonal IgG papain digest using Fabsorbent F1P HF, Application Note, 2009, ProMetic BioSciences Ltd.

Acknowledgements

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