

Design of Synthetic Ligands and their Application in Therapeutic Antibody Purification

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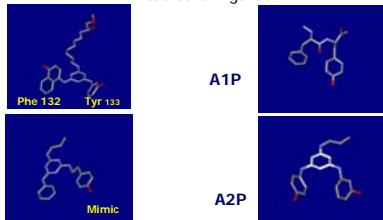


Introduction

Purification processes for therapeutic antibodies use Protein A based adsorbents for initial capture and concentration of the target molecule from clarified cell culture. The advantages of Protein A include effective removal of bulk impurities coupled with viral clearance by partitioning and viral inactivation resulting from low pH used for elution. However the cost, ligand compatibility with robust column cleaning procedures and ligand leakage are of ongoing concern and frequently necessitates the inclusion of two or more additional column steps. To address these issues ProMetic BioSciences have developed synthetic chemical ligands for IgG purification. Ligand concentration and spacer arm length has been optimised and the ligands attached to a new agarose matrix (PuraBead). These adsorbents have a different IgG sub-class specificity to Protein A as demonstrated by binding of IgG subclasses from plasma.

Binding conditions are at neutral pH (not affected by NaCl concentrations up to 1.0M) and IgG elution is effected using a pH drop to pH 3 - 3.5. High pH elution (at up to pH 7) in the presence of low molecular weight polyethylene glycol is a valuable alternative. The ligands contain hydrophobic moieties and the ligand-target interaction is weakened in the presence of competitive hydrophobic agents. However, these can be removed in an initial cation exchange capture and a standard purification sequence has been developed using Fractogel® EMD SO₃⁻ followed by MABsorbent A1P or A2P without any adjustment of the intermediate fraction. Data on product purity, clearance of host cell proteins and DNA using this generic two column process for genetically engineered antibodies is presented opposite.

MABsorbent® ligands



REFERENCES

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 Teng, *et al.*, (1999), *J. Mol. Recognit.*, **12**: 67-75
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 Behizad, M. and Curling, J., (2000), *BioPharm*, (September 2000), 42-45
 MABsorbent® and PuraBead® are registered trademarks of Prometic Biosciences Inc.

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PURIFICATION SEQUENCE

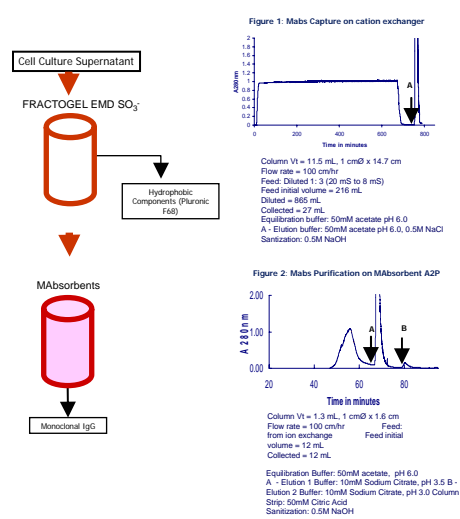


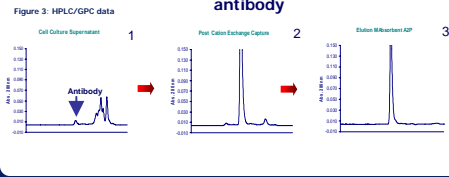
Table 1. Purification of Antibody from CHO cell culture

Fraction	Volume (ml)	Total protein (mg)	Total IgG (mg)
IEX load	865	718	209
IEX elution	247	215	239
MABsorbent A1P load	12	99	106
MABsorbent A1P elution	12	64	69
MABsorbent A2P load	12	99	106
MABsorbent A2P elution	12	65	61

* Split and run on A1P and A2P

Volume ion exchange column = 12 ml Capacity ion exchange column = 21 mg/ml
 Volume MABsorbent A1P column = 1.7 ml Capacity MABsorbent A1P column = 41 mg/ml
 Volume MABsorbent A2P column = 1.3 ml Capacity MABsorbent A2P column = 47 mg/ml

Purification of monoclonal antibody



Purification of monoclonal antibody

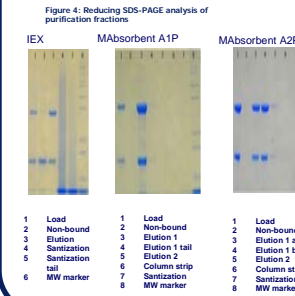
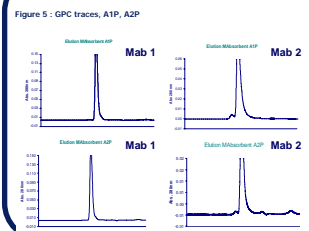


Table 2: CHOPs and DNA Clearance

Step	CHO (log ₁₀)	CHO (cpm)	DNA (log ₁₀)	DNA (cpm)
Preion exchange	3.9	16,075	1.56	6,500
Post ion exchange	30.5	3,449	1.85	210
Post A1P	0.129	22	Not detected	Not detected
Post A2P	0.118	23	Not detected	Not detected

Comparison of two antibody samples



Virus Clearance on MABsorbent

- ▶▶ 4.9 log₁₀ removal of MVM
 - ▶▶ >3.7 log₁₀ removal of X-MuLV
 - ▶▶ 2.0 log₁₀ inactivation of X-MuLV
 - ▶▶ Total clearance of X-MuLV >5.7 log₁₀
- Data courtesy of Biogen

Figure 6: Effect of PEG Molecular Weight on elution of human polyclonal IgG

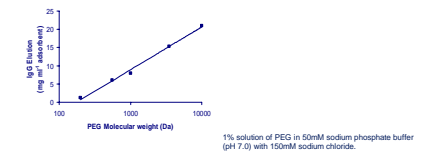


Table 3: Relative ratio of polyclonal IgG subclasses determined by nephelometry.

Subclass	IgG ₁	IgG ₂	IgG ₃
IgG ₁			
II + III Paste extract	64.1%	29.3%	2.9%
MABsorbent A2P elution	62.8%	29.2%	3.1%

CONCLUSIONS

- A purification scheme has been developed comprising initial capture on Fractogel EMD SO₃⁻ followed by purification with MABsorbent A1P or A2P which avoids the need for intermediate buffer changes.
- The binding of antibodies to both MABsorbents occurs at neutral pH and is insensitive to high concentrations of salt (1.0M NaCl).
- The ligands contain hydrophobic moieties and the ligand target interaction is weakened by the presence of hydrophobic additives in the cell culture supernatant feedstock.
- High yields and effective clearance of host cell proteins and DNA has been demonstrated.
- A choice of elution pH is available when PEG is used in the elution system.
- MABsorbents can be used for purification of all subclasses of human antibodies.

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