

MAbsorbent® A2P HF (Product Code: 3903)

Application Note – Purification of monoclonal antibodies from cell culture supernatant containing Pluronic® F-68 using MAbsorbent® A2P HF

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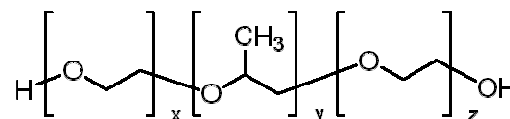
MAbsorbent® A2P HF is a synthetic ligand affinity adsorbent designed and manufactured exclusively by ProMetic BioSciences Ltd (PBL). Our range of MAbsorbent® products provide an innovative alternative to Protein A, maintaining the requirements for binding capacity, yield and purity whilst operating in harsh environments for hundreds of process cycles.

MAbsorbent® A2P HF has a binding capacity in excess of 35 g/L for IgG and greater than 20 g/L for monoclonal antibody directly from cell culture supernatant (CCS); in the absence of Pluronic® F-68.

Pluronic® F-68 is a linear polymer of isopropylene glycol repeating units and has properties similar to those of commonly used detergents such as Triton X-100.

During the production of monoclonal antibodies, in mammalian cell culture supernatant, Pluronic® F-68 is added to prevent shear induced cell damage. However, at a concentration of 0.2 g/L, Pluronic® F-68 significantly reduces the initial binding capacity of MAbsorbent® A2P HF for monoclonal antibody directly from cell culture supernatant by approximately 60% and at a concentration of 0.5 g/L this is further reduced to greater than 90%.

In this application note we describe the use of reverse phase HPLC resin Amberchrom™ CG161^[1] for the removal of Pluronic® F-68 from cell culture supernatant, containing monoclonal antibody, before the capture and purification of the antibody using MAbsorbent® A2P HF.



Pluronic® F-68 structural image

MATERIALS & METHODS

Removal of Pluronic® F-68 using Amberchrom™ CG161

The Amberchrom™ CG161 column was equilibrated with a phosphate buffered saline buffer at pH 7.5.

The required amount of cell culture supernatant (80 mL), containing monoclonal antibody, was loaded onto the column and non-bound material was removed by washing with equilibration buffer.

Both the flow through and wash fraction was collected to generate a non-bound sample for application onto the MAbsorbent® A2P HF column.

The Amberchrom™ CG161 column was cleaned using 0.5 M NaOH and stored in 20% ethanol (preservative).

The conditions are summarised in Table 1.

TABLE 1

Chromatography conditions for the removal of Pluronic® F-68 from CCS containing monoclonal antibody using Amberchrom™ CG161.

Platform	Automated Chromatography Workstation
Column parameters	11.5 mL column volume (CV) (1 cm diameter, 15 cm bed height)
Packing flow rate	600 cm/hr
Loading flow rate	300 cm/hr
Equilibration buffer	Phosphate buffered saline (PBS), pH 7.5
Load	CCS containing monoclonal antibody with the presence of Pluronic® F-68*
Load volume	80 mL
Wash buffer	Phosphate buffered saline (PBS), pH 7.5
Clean in Place (CIP)	0.5 M NaOH

* One column volume of the Amberchrom™ CG161 resin is sufficient for treatment of up to eight to ten volumes of the CCS containing monoclonal antibody with the presence of Pluronic® F-68.

Capture and Purification of Monoclonal Antibodies from pre-treated CCS using MAbsorbent® A2P HF

The non-bound sample collected from the Amberchrom™ CG161 column run was loaded onto equilibrated MAbsorbent® A2P HF.

After loading, non-bound protein was removed by washing with equilibration buffer.

The bound monoclonal antibody was eluted using a low pH buffer (50 mM citric acid, pH 2.5). MAbsorbent® A2P HF is hydroxide stable and was cleaned with 0.5 M NaOH.

The conditions are summarised in Table 2.

TABLE 2

Chromatography conditions for the capture and recovery of monoclonal antibody from pre-treated CCS using MAbsorbent® A2P HF.

Platform	Automated Chromatography Workstation
Column parameters	0.5 mL column volume (CV) (1 cm diameter, 0.7 cm bed height)
Packing flow rate	300 cm/hr
Loading flow rate	100 cm/hr
Equilibration buffer	Phosphate buffered saline (PBS), pH 7.5
Load	Non-bound sample collected from the Amberchrom™ CG161 column run
Load volume	80 mL
Wash buffer	Phosphate buffered saline (PBS), pH 7.5
Elution buffer	50 mM citric acid, pH 2.5
Clean in Place (CIP)	0.5 M NaOH

RESULTS & DISCUSSION

Figure 1 contains a chromatogram for the removal of Pluronic® F-68 from cell culture supernatant, containing monoclonal antibody, using Amberchrom™ CG161.

FIGURE 1

Chromatogram of the removal of Pluronic® F-68 from CCS containing monoclonal antibody using Amberchrom™ CG161.

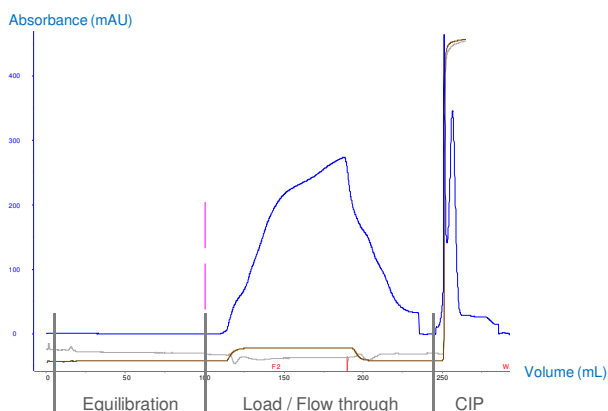


Figure 2 contains the chromatogram for the capture and recovery of monoclonal antibody from pre-treated cell culture supernatant using MAbSorbent® A2P HF.

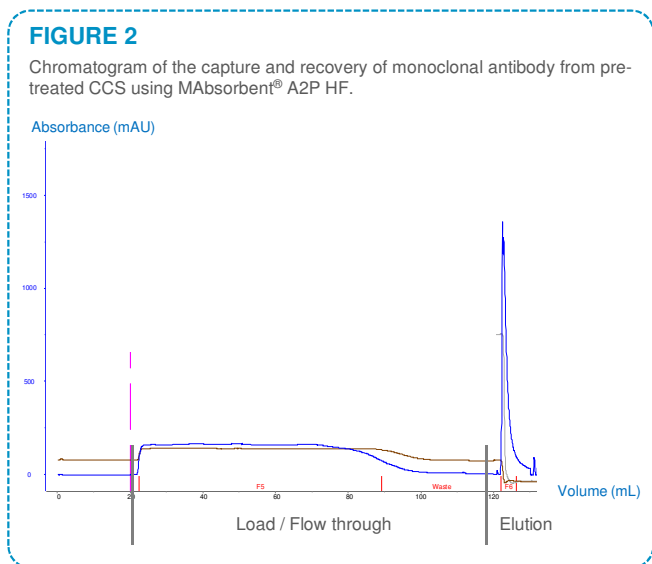
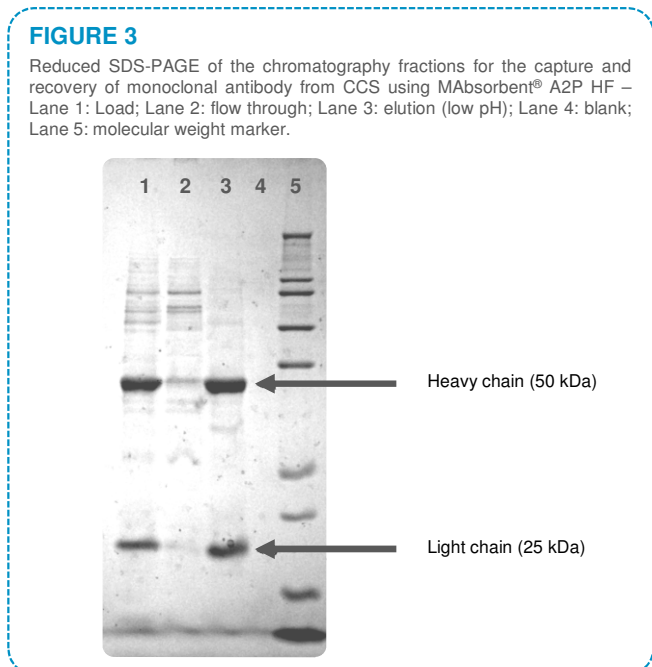


Figure 3 contains the reduced SDS-PAGE of the chromatography fractions from the MAbSorbent® A2P HF column run.



MAbSorbent® A2P HF binds and recovers monoclonal antibody from pre-treated cell culture supernatant with good purity (Lane 3). This indicates that the Amberchrom™ CG161 resin successfully removes Pluronic® F-68 from cell culture supernatants hence, improving adsorbent performance (Figure 3).

CONCLUSIONS

The use of Amberchrom™ CG161 in a flow through mode provides a simple method for the removal of Pluronic® F-68 from cell culture supernatant thereby allowing the capture and purification of the monoclonal antibody using MAbSorbent® A2P HF.

REFERENCES

- [1] Amberchrom™ CG161 technical information can be sourced from –
http://www.dow.com/assets/attachments/business/process_chemicals/amberchrom/amberchrom_cg161c/tds/amberchrom_cg161.pdf

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