

Mimetic Blue[®] SA HL P6XL (Product Code: 3125)

Application Note – Capture and purification of recombinant albumin-fusion protein using Mimetic Blue[®] SA HL P6XL

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Mimetic Blue[®] affinity chromatography adsorbents developed and manufactured exclusively by ProMetic BioSciences Ltd (PBL) comprise Mimetic Blue[®] ligands attached to beaded agarose supports. These adsorbents selectively bind albumin and albumin related proteins from a variety of sources including plasma, serum and recombinant cell cultures to yield highly purified biological products.

Mimetic Blue[®] SA HL P6XL has a high binding capacity for albumin and is intended for use as the initial capture and purification step of albumin-fusion proteins.

Mimetic Blue[®] SA HL P6XL is stable across a very wide pH range (pH 2.0 to pH 14.0), exhibits negligible ligand leakage by ELISA^[1] and maintains chromatographic performance over 350 process cycles^[1]. This adsorbent is used in regulated large scale capture applications.

Recombinant albumin-fusion proteins are becoming established biopharmaceuticals due to the extended half-life in the body. This and other characteristics of albumin have led to the development of an albumin-fusion technology platform^[2].

In this application note we describe how recombinant albumin-fusion protein is captured and purified using Mimetic Blue[®] SA HL P6XL.

MATERIALS & METHODS

Cell culture supernatant (CCS) containing albumin-fusion protein was loaded onto equilibrated Mimetic Blue® SA HL P6XL. Non-bound protein was removed by washing with equilibration buffer. The bound albumin-fusion protein was eluted using a buffer containing sodium octanoate. Mimetic Blue® SA HL P6XL is hydroxide stable and was cleaned with 0.5 M NaOH. The conditions are summarised in Table 1.

TABLE 1

Chromatography conditions for the capture and purification of albumin-fusion protein from a CCS using Mimetic Blue® SA HL P6XL.

Platform	Automated Chromatography Workstation
Column parameters	4 mL column volume (1 cm diameter, 5 cm bed height)
Packing flow rate	450 cm/hr
Operational flow rate	200 cm/hr
Loading flow rate	75 cm/hr (4 minute residence time)
Equilibration buffer	50 mM sodium phosphate, 25 mM NaCl, pH 7.0
Load	10 mL cell culture supernatant containing albumin-fusion protein
Elution buffer	50 mM sodium phosphate, 25 mM NaCl, 30 mM sodium octanoate, pH 7.0
Clean in Place (CIP)	0.5 M NaOH

RESULTS & DISCUSSION

Mimetic Blue® SA HL P6XL can be used to capture and purify albumin-fusion protein from a CCS. The albumin-fusion protein is selectively captured by the adsorbent with the non-target proteins present in the column flow through (Figures 1 and 2).

FIGURE 1

Chromatogram of the capture and purification of albumin-fusion protein from a CCS using Mimetic Blue® SA HL P6XL.

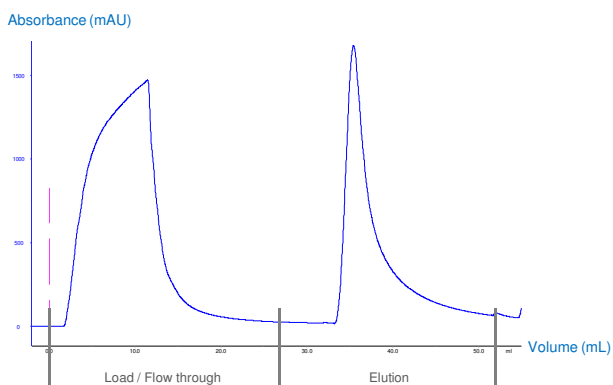


FIGURE 2

Non-reduced SDS-PAGE of the chromatography fractions for the capture and purification of albumin-fusion protein from a CCS using Mimetic Blue® SA HL P6XL – Lane 1: Molecular weight marker; Lane 2: Load; Lane 3: Flow through; Lane 4: Elution.

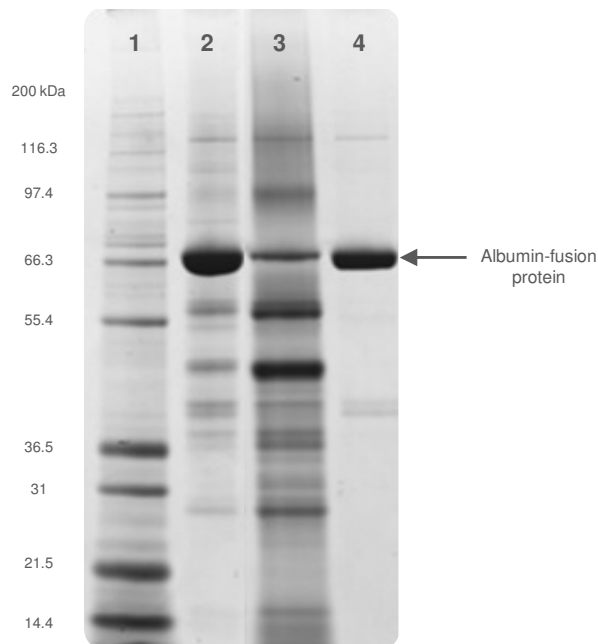


Figure 2 shows high albumin-fusion protein binding (Lane 3), recovery and purity (Lane 4). Table 2 shows the performance data for the Mimetic Blue® SA HL P6XL adsorbent determined by Nephelometry.

TABLE 2

Performance data of the chromatography fractions for the capture and purification of albumin-fusion protein from a CCS using Mimetic Blue® SA HL P6XL.

	Volume (mL)	Total albumin (mg/fraction)	Yield (%)
Load	10.0	52.2	100
Flow through	29.5	<10	<19*
Elution	20	39	75

*The flow through sample, measured by Nephelometry, was below the limit of detection. As a result, the Yield (%) result is presented as a less than value.

Table 2 shows that Mimetic Blue® SA HL P6XL has a dynamic binding capacity of >10 g/L of adsorbent and recovery of 9.75 g/L of adsorbent, as determined by Nephelometry.

CONCLUSIONS

Mimetic Blue® SA HL P6XL can be used to capture and purify albumin-fusion protein from a CCS with a dynamic binding capacity of >10 g/L of adsorbent with >90% recovery and high purity.

Mimetic Blue® SA HL P6XL has a dynamic binding capacity of up to 30 g/L of adsorbent for albumin in other feedstocks.

REFERENCES

- [1] Mimetic Blue® Affinity Adsorbents Brochure (Issue 060510).
- [2] Subramanian *et al*, Albinterferon α -2b: a genetic fusion protein for the treatment of chronic hepatitis C, Nature Biotechnology, December 2007, Volume 25 (Number 12), pp 1411-1419.

SALES & TECHNICAL SUPPORT

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