

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

Application Note – Capture and purification of human serum albumin from human source plasma 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.

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.

Human plasma contains approximately 30-35 g/L of albumin in a complex mixture of proteins including IgG, fibrinogen and alpha 1-antitrypsin. Mimetic Blue[®] SA HL P6XL captures and purifies albumin from human plasma with high binding capacities and high purity.

In this application note we describe how human serum albumin is captured and purified from human source plasma using Mimetic Blue[®] SA HL P6XL.

MATERIALS & METHODS

Human source plasma (untreated) was loaded onto equilibrated Mimetic Blue® SA HL P6XL packed to a bed height of 15 cm in a 1 cm diameter column. After loading, non-bound protein was removed by washing with equilibration buffer. The bound albumin 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 recovery of human serum albumin from human source plasma using Mimetic Blue® SA HL P6XL.

Platform	Automated Chromatography Workstation
Column parameters	11.8 mL column volume (1 cm diameter, 15 cm bed height)
Packing flow rate	450 cm/hr
Operational flow rate	200 cm/hr
Loading flow rate	150 cm/hr (6 minute residence time)
Equilibration buffer	50 mM sodium phosphate, 150 mM NaCl, pH 7.5
Load	7 mL human source plasma, pH 7.5
Elution buffer	25 mM sodium phosphate, 150 mM NaCl, 30 mM sodium octanoate, pH 6.0
Clean in Place (CIP)	0.5 M NaOH

RESULTS & DISCUSSION

Mimetic Blue® SA HL P6XL selectively binds to the albumin in plasma, while other plasma proteins are not retained by the adsorbent and are present in the flow through fraction (Figures 1 and 2).

FIGURE 1

Chromatogram of the capture and purification of human serum albumin from human source plasma using Mimetic Blue® SA HL P6XL.

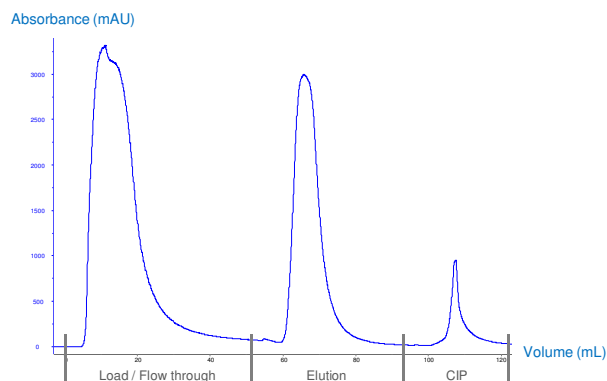
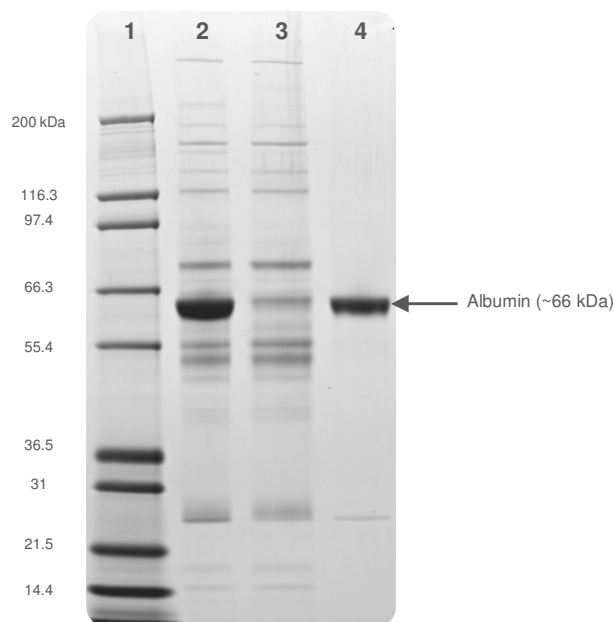


FIGURE 2

Reduced SDS-PAGE of the chromatography fractions for the capture and purification of human serum albumin from human source plasma using Mimetic Blue® SA HL P6XL – Lane 1: Molecular weight marker; Lane 2: Load; Lane 3: Flow through; Lane 4: Elution.



The resulting eluate has a purity of 96.1%, as determined by densitometry (Figure 2 and Table 2).

TABLE 2

Performance data for Mimetic Blue® SA HL P6XL determined by Nephelometry and densitometry.

	Volume (mL)	Total albumin (mg/fraction)	Yield (%)	Purity (%)
Load	7.0	261.1	100	
Flow through	54.1	<18.9	<7.2*	
Elution	35.4	235.8	90.3	96.1

*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.

Mimetic Blue® SA HL P6XL has a dynamic binding capacity in excess of 20 g/L of adsorbent and high recovery, as determined by Nephelometry (Table 2).

CONCLUSIONS

Mimetic Blue® SA HL P6XL can be used to capture and purify human serum albumin from human source plasma with a dynamic binding capacity and recovery in excess of 20 g/L of adsorbent with a purity of ~96%.

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

REFERENCES

- [1] Mimetic Blue® Affinity Adsorbents Brochure (Issue: 060510).

SALES & TECHNICAL SUPPORT

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