

Mimetic Blue® SA HL P6XL – Capture and purification of human serum albumin from human plasma

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Introduction

Mimetic Blue affinity chromatography adsorbents selectively bind albumin and related proteins from a variety of sources including plasma, serum and recombinant cell cultures. **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, 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.

Materials and 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 (Table 1).

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.

Platform	Automated Chromatography Workstation
Column Conditions	11.8 mL column volume (1 cm diameter, 15 cm bed height)
Packing Flow Rate	450 cm/hr
Operational Flow Rates	200 cm/hr
Loading Flow Rate	150 cm/hr (6 minute residence time)
Equilibration Buffer	50 mM sodium phosphate, 100 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
CIP (Clean in Place)	0.5 M NaOH

Table 1: Chromatography conditions for the capture and recovery of human serum albumin from human source plasma using **Mimetic Blue** SA HL P6XL.

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 (Figure 1 and Figure 2).

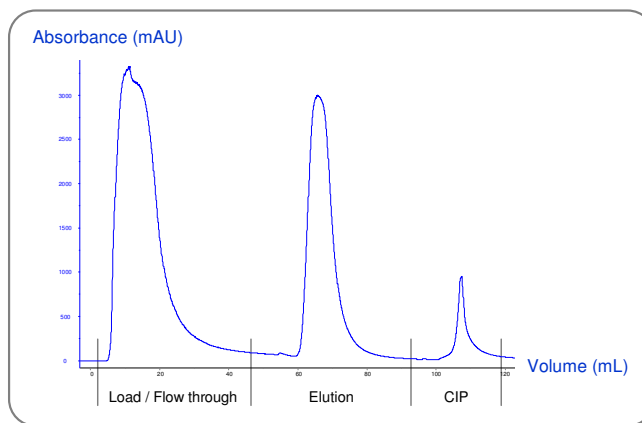


Figure 1: Chromatogram of the capture and purification of human serum albumin from human source plasma using **Mimetic Blue** SA HL P6XL.

APPLICATION NOTE – 3125

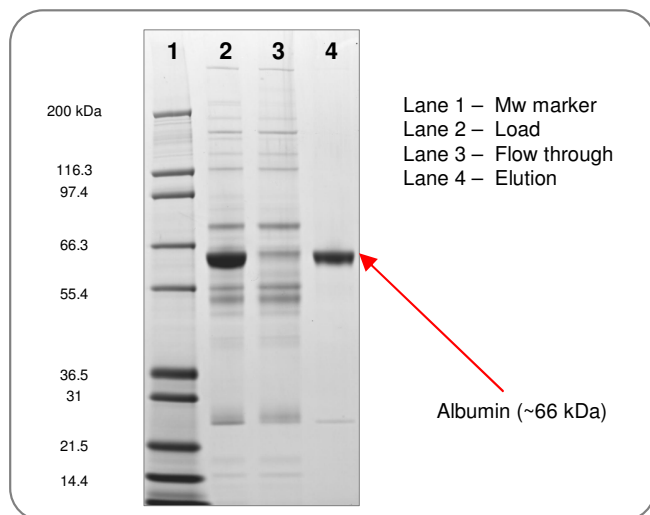


Figure 2: Reduced SDS-PAGE of the chromatography fractions for the capture and purification of human serum albumin from human source plasma.

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

	Volume (mL)	Total albumin in fraction (mg)	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

Table 2: Performance data for **Mimetic Blue** SA HL P6XL determined by Nephelometry and densitometry.

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).

The high specificity of the ligand and flow properties of the adsorbent mean that the dynamic binding capacity can be maintained with less than a 5% drop loading human source plasma at residence times from 6 to 1 minute (Figure 3).

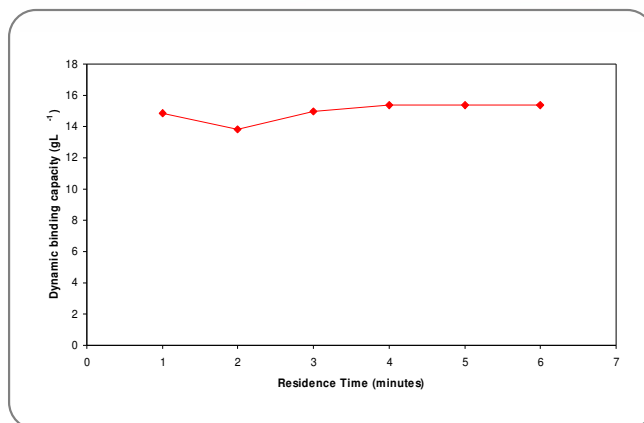


Figure 3: Human serum albumin binding capacity results for **Mimetic Blue** SA HL P6XL loading human source plasma (pH adjusted to pH 6.0) from 6 to 1 minute residence time (100 to 600 cm/hr), using a 2.6 cm diameter column with a 10 cm bed height (53 mL CV).

Conclusions

Mimetic Blue SA HL P6XL can be used to capture and purify albumin from human 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 up to 30 g/L of adsorbent for albumin in other feedstocks.

The dynamic binding capacity of **Mimetic Blue** SA HL P6XL does not significantly reduce when loading at residence times from 6 to 1 minute.

References

[1] Mimetic Blue Affinity Adsorbents Brochure (Issue 240709).

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