

## Introduction

Affinity chromatography is ideally suited to the purification of biopharmaceuticals due to the unique specificity of affinity ligands for the target protein. Thus, highly selective separation based on affinity chromatography in packed bed columns incorporating capture, concentration and a high degree of purification provides a simple and effective means of reducing the number of processing steps thereby removing some of the perceived bottlenecks in downstream processing.

Significant improvements in reducing the manufacturing costs are possible and can be achieved by shortening processing times by increasing flow rates through chromatography matrix optimisation with concomitant ability to clean and re-use the chromatography adsorbent.

Albumin-fusion protein technology represents an increasingly important alternative platform for the production of therapeutically significant proteins with extended *in vivo* half-lives. A number of proteins and bioactive peptides fused to albumin are currently being investigated for use in therapeutic applications.

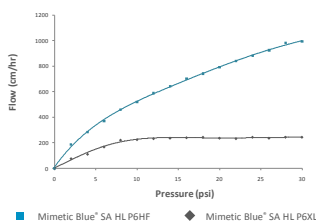
Mimetic Blue® SA ligand is highly selective for albumin and affinity chromatography adsorbents based on this ligand provide a platform technology for the purification of human albumin and genetically engineered albumin-fusion proteins. The existing adsorbents are based on a standard 6% cross-linked agarose. Optimisation of the cross-linking chemistry of the PuraBead® 6 base matrix (a 6% near-monodisperse beaded agarose), with optimal coupling of Mimetic Blue® SA ligand has led to the development of a new adsorbent with improved performance (described below).

The resulting adsorbent Mimetic Blue® SA HL P6HF retains the high binding capacity (~30 g albumin/L) seen for the existing Mimetic Blue® SA products and is characterised by significant (≥3 fold) improvement in the pressure flow properties. One-step purifications of human serum albumin (HSA) from human source plasma and an albumin-fusion protein are described.

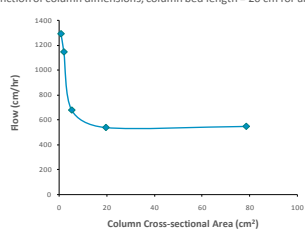
## Flow Properties

**FIGURE 1**

Pressure flow curve using a 10 cm diameter column for Mimetic Blue® SA HL P6HF compared to Mimetic Blue® SA HL P6XL.


**FIGURE 2**

Operating pressure (1 bar) measured for Mimetic Blue® SA HL P6HF as a function of column dimensions, column bed length = 20 cm for all columns.



## Dynamic Binding Capacity & Recovery

### Purified HSA\*

Adsorbent	Binding Capacity (g/L)	Recovery (g/L)
Mimetic Blue® SA HL P6XL	33.1	32.5
Mimetic Blue® SA HL P6HF	31.0	27.0

\*Based on average values obtained for PBL's DBC product release test

### HSA from Human Source Plasma:

Adsorbent	Binding Capacity (g/L)	Recovery (g/L)
Mimetic Blue® SA HL P6XL	>16	17.8
Mimetic Blue® SA HL P6HF	>16	16.9

### Albumin-Fusion Protein:

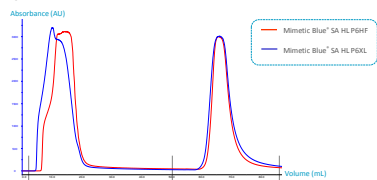
Adsorbent	Binding Capacity (g/L)	Recovery (g/L)
Mimetic Blue® SA HL P6XL	>10.5	9.8
Mimetic Blue® SA HL P6HF	>10.5	9.2

## Human Source Plasma Purification

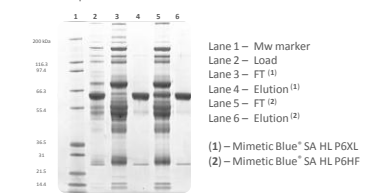
Platform	Automated chromatography workstation
Column parameters	11.6 mL CV (1 cm diameter, 14.8 cm bed height)
Equilibration buffer	50 mM sodium phosphate, pH 6.0
Load	7 mL human source plasma
Elution buffer	50 mM sodium phosphate, 30 mM sodium octanoate (caprylate), 100 mM NaCl, pH 6.0
Clean in Place (CIP)	0.5 M NaOH

**FIGURE 3**

Chromatogram overlays for the capture and recovery of HSA from human source plasma using Mimetic Blue® SA HL P6HF and Mimetic Blue® SA HL P6XL.


**FIGURE 4**

Reduced SDS-PAGE of flow through (FT) and elution chromatography fractions for Mimetic Blue® SA HL P6HF and Mimetic Blue® SA HL P6XL from human source plasma.

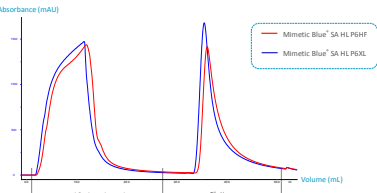


## Albumin-Fusion Protein Purification

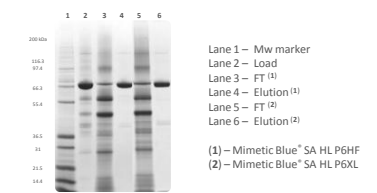
Platform	Automated chromatography workstation
Column parameters	4 mL CV (1 cm diameter, 5.0 cm bed height)
Equilibration buffer	50 mM sodium phosphate, 25 mM NaCl, pH 7.0
Load	10 mL yeast cell culture containing albumin-fusion protein
Elution buffer	50 mM sodium phosphate, 30 mM sodium octanoate (caprylate), 25 mM NaCl, pH 7.0
Clean in Place (CIP)	0.5 M NaOH

**FIGURE 5**

Chromatogram overlays for the capture and recovery of albumin-fusion protein using Mimetic Blue® SA HL P6HF and Mimetic Blue® SA HL P6XL.


**FIGURE 6**

Non-reduced SDS-PAGE of flow through (FT) and elution chromatography fractions for albumin-fusion protein using Mimetic Blue® SA HL P6HF and Mimetic Blue® SA HL P6XL.

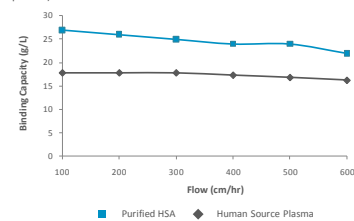


## Improved Performance

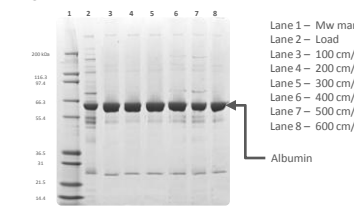
The high selectivity and superior flow properties of Mimetic Blue® SA HL P6HF provides rapid processing of albumin without significant loss of performance. The Mimetic Blue® SA HL P6HF adsorbent retains >90% of the dynamic binding capacity loading human source plasma at residence times from 6 to 1 minute (Figures 7 & 8).

**FIGURE 7**

Binding capacity versus flow using a 2.6 cm diameter column for Mimetic Blue® SA HL P6HF at increasing flow rates (purified HSA and human source plasma).


**FIGURE 8**

Reduced SDS-PAGE of the chromatography elution fractions for the capture and purification of HSA from human source plasma at increasing flow rates using Mimetic Blue® SA HL P6HF.



A comparison purification of HSA from human source plasma at two different scales is shown in the table below for both Mimetic Blue® SA HL P6XL and Mimetic Blue® SA HL P6HF.

Adsorbent	Binding Capacity (g/L) at 600 cm/hr	
	2.6 cm diameter column	10 cm diameter column
Mimetic Blue® SA HL P6XL	14.8	-
Mimetic Blue® SA HL P6HF	16.2	>14.2

At a flow rate of 600 cm/hr both Mimetic Blue® SA HL P6XL and Mimetic Blue® SA HL P6HF show comparable binding capacities for HSA in 2.6 cm diameter columns. When scaled-up from a 2.6 cm to a 10 cm diameter column Mimetic Blue® SA HL P6HF provides similar performance however, a flow rate of 600 cm/hr was unattainable for the Mimetic Blue® SA HL P6XL adsorbent in the larger scale column.

## Conclusions

- Mimetic Blue® SA HL P6HF enables a linear flow rate of up to 1000 cm/hr at 30 psi using a 10 cm diameter column.
- Mimetic Blue® SA HL P6HF and Mimetic Blue® SA HL P6XL show comparable dynamic binding capacity, recovery and purity results for the purification of both HSA from human source plasma and albumin-fusion protein.
- Mimetic Blue® SA HL P6HF shows no significant decrease in dynamic binding capacity loading from 100 to 600 cm/hr (6 to 1 minute residence time), using both purified HSA and human source plasma feedstocks in bench top columns.
- Mimetic Blue® SA HL P6HF adsorbent can be scaled-up to large scale columns without affecting performance even at a flow rate of 600 cm/hr.
- Mimetic Blue® SA HL P6HF provides comparable binding and purity to Mimetic Blue® SA HL P6XL however, with superior flow properties (≥3 fold) providing reduced process times.

## Acknowledgements

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