

Mimetic Blue® Affinity Adsorbents

Mimetic Blue® 1 P6XL, Mimetic Blue® SA P6XL
Mimetic Blue® SA HL P6XL, Mimetic Blue® ELISA Kit



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 related proteins from a variety of sources, including plasma, serum and recombinant cell cultures to yield highly purified biological products.

They are used commercially for the purification of albumin and albumin related proteins such as albumin fusion proteins, but are also applicable to a variety of other proteins including adenine nucleotide binding proteins. Mimetic Blue® ligands are highly stable, exhibit negligible ligand leakage and maintain a high level chromatographic performance over many process cycles, even after prolonged storage in sodium hydroxide.

Based on PBL's PuraBead® support matrix, Mimetic Blue® P6XL adsorbents offer excellent flow properties, near mono-disperse particle size distribution and high protein binding capacities. Mimetic Blue® products are manufactured in a controlled environment according to an ISO 9001 accredited quality system and are designed for use in process-scale cGMP manufacture. For further quality assurance, a highly sensitive ELISA test kit is available for establishing the absence of ligand leachates in the purified product and also for routine column monitoring.



KEY BENEFITS

- High specificity for albumin and albumin related proteins
- High dynamic binding capacity - in excess of 30 g/L
- Excellent flow properties
- Robust, long life adsorbent
- Sanitizable with NaOH allowing multiple cycles
- High purity, chemically defined ligand structures
- Highly reproducible batch-to-batch manufacture to ISO 9001 standard
- Supported with comprehensive Regulatory Support Files
- Multiple end users in bio-Pharmaceutical manufacturing
- Highly sensitive ELISA assay for leachate analysis and column verification

ADVANTAGES

LIGAND SELECTIVITY

Mimetic Blue® ligands have been developed for their ability to provide maximal binding and purification of albumin and albumin related proteins. Mimetic Blue® ligands are immobilised using a defined and highly stable spacer-arm linkage which promotes optimal interaction with the target protein.

LIGAND PURITY

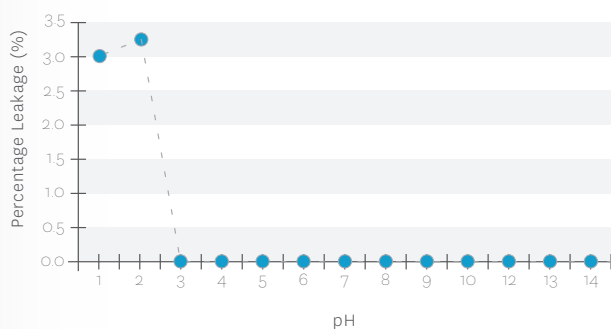
Mimetic Blue® ligands are synthesized by PBL to a very high level of purity and consistency. The ligands are subsequently immobilized onto the PuraBead® base matrix at a precisely controlled ligand density to provide reproducible chromatographic performance.

ADSORBENT STABILITY

Mimetic Blue® adsorbents are stable across a very wide pH range (pH 2.0 – pH 14.0) and are resistant to most chemical treatments including denaturants (urea, guanidine hydrochloride), detergents (Triton, SDS), thiol reducing agents and can be sterilized by autoclaving at neutral pH. The exceptional stability of Mimetic Blue® adsorbents enables routine use of 0.5 M NaOH for column cleaning and sanitisation.

FIGURE 1

Ligand leakage from Mimetic Blue® SA HL P6XL as a function of pH after continuous exposure at ambient room temperature (20° – 25°C) for 3 months

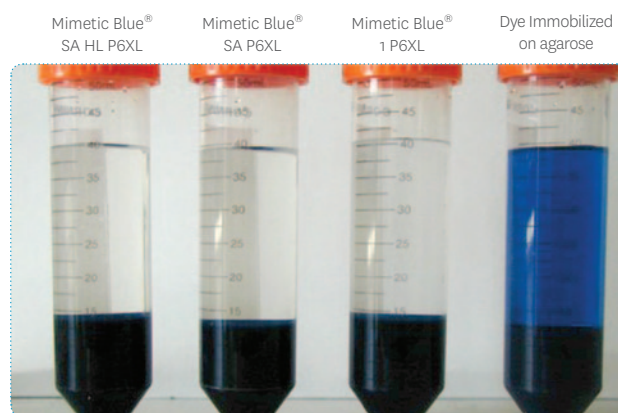


NaOH COMPATIBILITY

Mimetic Blue® adsorbents can withstand prolonged exposure to sodium hydroxide without loss of ligand or impacting on the binding capacity. This is illustrated by incubating Mimetic Blue® SA HL P6XL, Mimetic Blue® SA P6XL and Mimetic Blue® 1 P6XL in 0.5M NaOH for 4 days, compared with C.I. Reactive Blue 2 (Cibacron Blue) immobilised on agarose.

FIGURE 2

Mimetic Blue® products and a commercially available agarose-immobilised dye product following storage at room temperature for 4 days in 0.5 M NaOH



PURIFICATION PERFORMANCE

Mimetic Blue® adsorbents can be used for direct capture and purification of albumin and albumin related proteins from a variety of sources. The selection of the most appropriate Mimetic Blue® adsorbent for a given application is determined by adsorbent screening in scale down experiments. For these applications convenient pre-packed column kits and 96-column block format PuraPlate™ adsorbent screening kits are available from ProMetic.

Mimetic Blue® 1 P6XL often provides the highest protein purity and is particularly well suited to intermediate polishing steps or the removal of albumin impurities from other proteins.

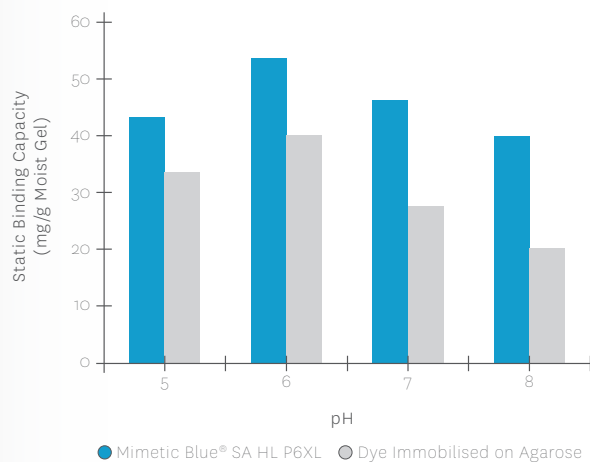
Mimetic Blue® SA P6XL provides enhanced binding capacities for albumin and is often used in initial capture applications. The Mimetic Blue® SA HL (“high ligand”) P6XL variant provides outstanding binding capacities (in excess of 30 g/L dynamic binding capacity) and is often the product of choice for large-scale capture applications.

PROTEIN BINDING CAPACITIES

Binding capacities of Mimetic Blue® adsorbents are dependent on both the pH and conductivity of the sample and equilibration buffer. Maximum binding is at pH 6.0, although the adsorbents can bind the target protein across a broad pH range (pH 5.0 – pH 8.0). Inclusion of 150 mM NaCl in the equilibration and wash buffers is useful for improved binding and maximal purity of the eluted protein.

FIGURE 3

Static binding capacity of Mimetic Blue® SA HL P6XL for human serum albumin as a function of pH compared with a commercially available immobilized textile dye.

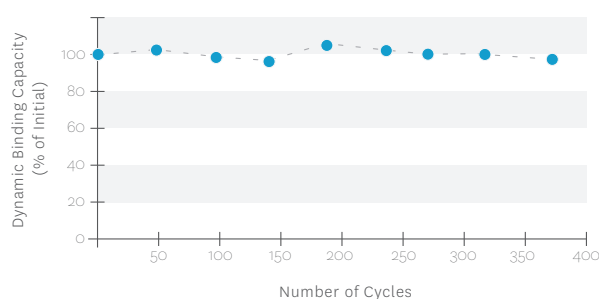


ADSORBENT RE-USE

Mimetic Blue® adsorbents are specifically designed for extensive re-use. In the case of Mimetic Blue® SA HL P6XL, over 350 process cycles have been achieved using industry derived feedstock without any impact on column performance.

FIGURE 4

Albumin binding capacity (% initial protein binding capacity) as a function of Mimetic Blue® SA HL P6XL re-use.



PRODUCT PURITY

The high selectivity of Mimetic Blue® ligands for albumin and related proteins provides high product purities for most applications. Purification of human serum albumin directly from human plasma provides an example of the high selectivity and low non-specific binding properties of Mimetic Blue® adsorbents:

| | |
|-----------------------------|---------------------------------------------------------------------|
| Column | 1 cm dia x 15 cm bed height (11.8 mL) |
| Adsorbent | Mimetic Blue® SA HL P6XL |
| Loading Flow Rate | 150 cm/hr (6 min residence time) |
| Equilibration & Wash 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 caprylate, pH 6.0 |
| Clean In Place (CIP) | 0.5 M NaOH |

HIGH PROTEIN RECOVERIES

Since both adsorption and desorption steps are performed at physiological pH, without the requirement for extreme conditions, high recoveries of active protein are obtained. Elution of the bound protein can be performed using specific elution agents such as sodium caprylate or fatty acids, or performed non-specifically by using sodium chloride at concentrations in the range 0.2 to 2.0 M. Polarity reducing agents such as ethylene glycol may also be used to assist protein elution. Combinations of sodium caprylate (25 – 30 mM) and sodium chloride are often very effective for the elution of bound albumin and enable sharp elution peaks and minimal elution volumes.

FIGURE 5

Purification of human serum albumin from human source plasma using Mimetic Blue® SA HL P6XL. Dynamic binding capacity and recovery in excess of 20 g/L with a purity of approximately 96%.

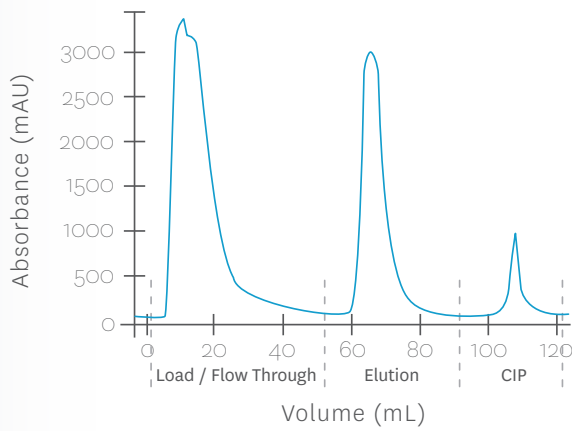
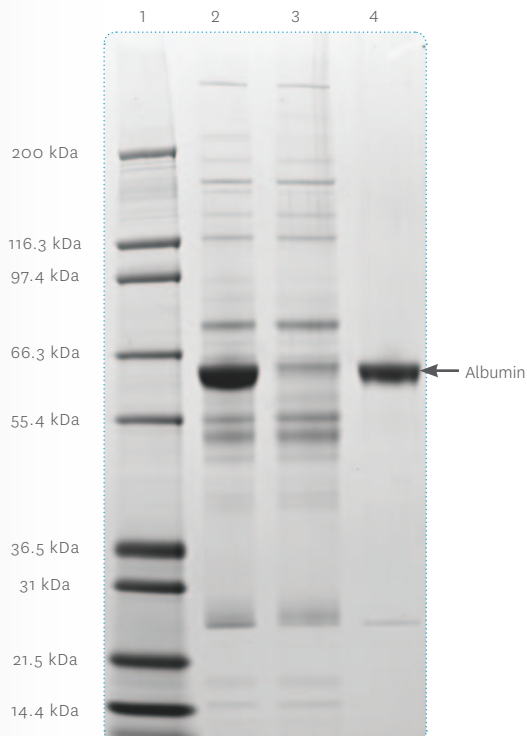


FIGURE 6

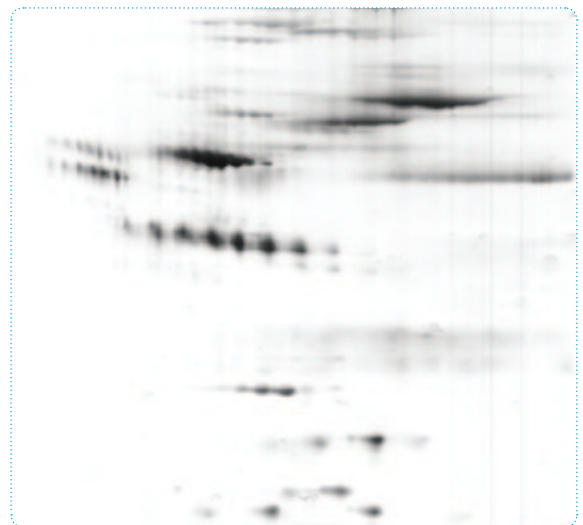
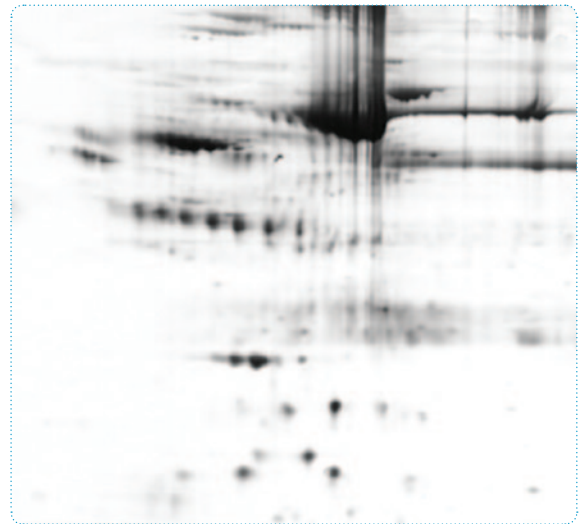
Reduced SDS-PAGE of Mimetic Blue® SA HL P6XL column fractions. Lane 1: Molecular weight marker; Lane 2: Load; Lane 3: Flow through; Lane 4: Elution.



Mimetic Blue® adsorbents may also be used for the capture and removal of albumin from plasma and serum samples enabling the analysis of less abundant proteins. Mimetic Blue® P6XL PuraPlate™ products are particularly useful for sample processing in relation to high-throughput proteomic applications.

FIGURE 7

2-D gel electrophoresis of human plasma before (top) and after (bottom) albumin depletion using Mimetic Blue® 1 P6XL.

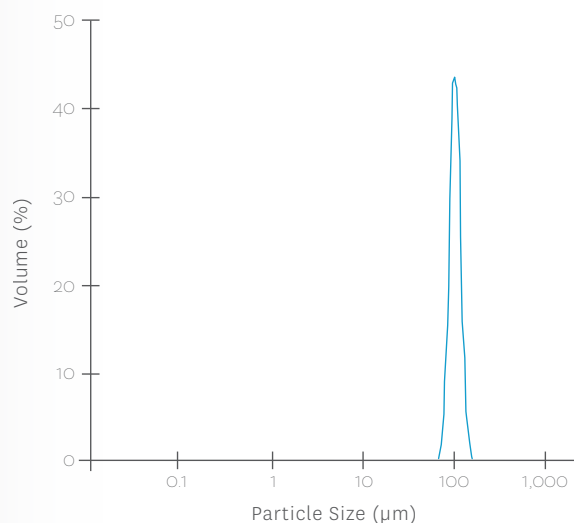


PARTICLE SIZE DISTRIBUTION

Mimetic Blue® ligands are coupled to PuraBead® P6XL - a 6% near monodisperse agarose bead with a mean particle size of 100 µm. This ensures excellent column packing and reliable performance of these adsorbents, even in very large diameter process columns (>1.0 m dia.).

FIGURE 8

Particle size distribution of Mimetic Blue® SA HL P6XL

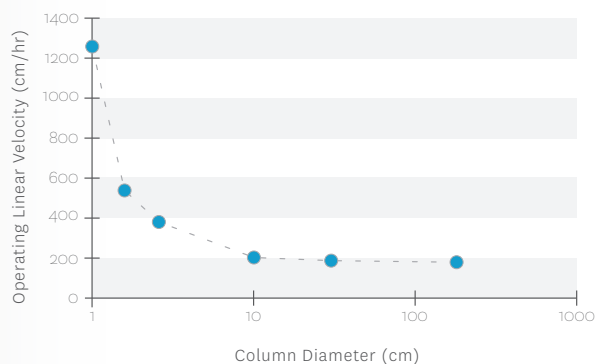


FLOW PROPERTIES

Mimetic Blue® adsorbents can be operated at high linear flow rates with low column back-pressures. The excellent flow properties of these adsorbents enable their use in very large diameter process columns.

FIGURE 9

Maximum operating flow rates measured for Mimetic Blue® SA HL P6XL as a function of column diameter; column bed length = 25 cm for all columns.

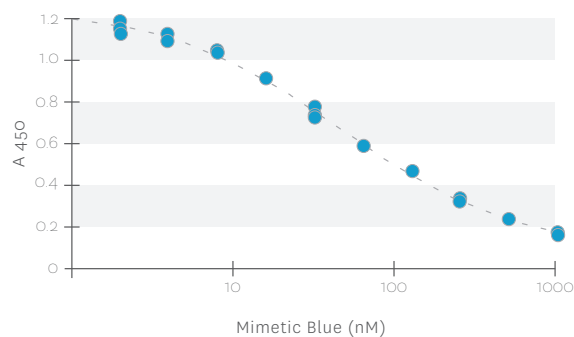


REGULATORY SUPPORT

Mimetic Blue® adsorbents are supported by regulatory support files and Drug Master Files (DMF's). A sensitive ELISA assay is also available for leakage analysis and verification of column performance. The ELISA assay is capable of detecting the presence of Mimetic Blue® 1 and Mimetic Blue® SA ligands with a detection limit below 10 nM concentration.

FIGURE 10

ELISA standard curve for Mimetic Blue®



BULK MANUFACTURE

PBL can provide Mimetic Blue® adsorbents in volumes sufficient to meet the requirements of the largest biomanufacturing processes. Single batches of >250 litres are produced in PBL's controlled environment (cGMP) manufacturing facility under an ISO 9001 quality system.

TECHNICAL SUPPORT

PBL has comprehensive knowledge of the properties and applications of Mimetic Blue® adsorbents. We can provide full support for the development of new applications and their implementation for biopharmaceutical manufacture.

PROPERTIES

| | |
|-----------------------|-------------------------------------------------------------|
| Ligand | Synthetic anthraquinone |
| Matrix | PuraBead® P6XL (6% near-monodisperse cross-linked agarose). |
| Particle size | 100 ± 10 µm |
| Binding capacity | Up to 60 mg HSA/ml |
| Operational Flow rate | Up to 500 cm/hr |
| Operating pressure | Up to 3 bar (45 psi) |
| Operating pH | pH 2 – pH 14 (Intermittent) |
| pH stability | Long term (3 months) pH 3 – pH 13 |
| Chemical stability | All commonly used aqueous buffers and co-solvents. |
| Sanitization | 0.5 – 1.0 M sodium hydroxide, 25°C |
| Storage | 20% ethanol/80% 0.1 M NaCl (v/v) |

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ProMetic offers a range of larger pack sizes for supply of bulk resins into development and regulated cGMP manufacturing processes. For more information on Mimetic Blue® adsorbents and supply related matters please do not hesitate to contact us.

CONTACT US

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For sales questions, contact: sales@prometicbiosciences.com.

For tech support questions, contact: techsupport@prometicbiosciences.com.

ORDER

MIMETIC BLUE® P6XL SLURRY FOR COLUMN PACKING

| | |
|------------------------------------|------------|
| Mimetic Blue® 1 P6XL (25 ml) | 3090-00025 |
| Mimetic Blue® 1 P6XL (100 ml) | 3090-00100 |
| Mimetic Blue® 1 P6XL (500 ml) | 3090-00500 |
| Mimetic Blue® 1 P6XL (1000 ml) | 3090-01000 |
| Mimetic Blue® SA P6XL (25 ml) | 3120-00025 |
| Mimetic Blue® SA P6XL (100 ml) | 3120-00100 |
| Mimetic Blue® SA P6XL (500 ml) | 3120-00500 |
| Mimetic Blue® SA P6XL (1000 ml) | 3120-01000 |
| Mimetic Blue® SA HL P6XL (25 ml) | 3125-00025 |
| Mimetic Blue® SA HL P6XL (100 ml) | 3125-00100 |
| Mimetic Blue® SA HL P6XL (500 ml) | 3125-00500 |
| Mimetic Blue® SA HL P6XL (1000 ml) | 3125-01000 |

MIMETIC BLUE® P6XL PRE-PACKED COLUMNS

(for attachment to chromatography workstations)

| | |
|-------------------------------------|------------|
| Mimetic Blue® 1 P6XL (5 x 1 ml) | 4090-00001 |
| Mimetic Blue® SA P6XL (5 x 1 ml) | 4120-00001 |
| Mimetic Blue® SA HL P6XL (5 x 1 ml) | 4125-00001 |

MIMETIC BLUE® P6XL PURAPLATE™

(96 column plate for rapid screening and purification method development)

| | |
|-------------------------------------|------|
| Mimetic Blue® 1 P6XL PuraPlate™ | 2234 |
| Mimetic Blue® SA P6XL PuraPlate™ | 2220 |
| Mimetic Blue® SA HL P6XL PuraPlate™ | 2231 |

MIMETIC BLUE® ELISA KIT

| | |
|-------------------------------------|------|
| ELISA Kit (Mimetic Blue® 1/Blue SA) | 2090 |
|-------------------------------------|------|