

# aminophenylboronate affinity adsorbents

Aminophenylboronate A6XL  
Aminophenylboronate A6XL LL  
Aminophenylboronate P6XL



Aminophenylboronate affinity adsorbents are used for purification of a diverse range of macromolecules which possess 1,2-diols, 1,3-diols, 1,2-hydroxy acids and 1,2-hydroxylamine groups. These functionalities are present in glycoproteins, carbohydrates, nucleic acids (nucleosides, nucleotides and RNA's), and polyphenols (catechols, flavonoids). Aminophenylboronate Agarose affinity adsorbents are particularly suited to the purification of glycoproteins, or the removal of glycoprotein and carbohydrate impurities from non-glycosylated molecules.

These adsorbents can also be used for the purification and removal of certain enzymes such as proteases and hydrolases where the boronic acid group has affinity for the active site.

The chemically stable affinity ligand is bonded to 6% cross-linked agarose beads to produce a highly robust adsorbent which is resistant to concentrated sodium hydroxide and suitable for use in downstream purification process applications and incorporation into diagnostic assays.

## KEY BENEFITS

- Affinity purification and removal of glycoproteins and glycans
- High purity m-aminophenylboronic acid ligand
- Ligand binds selectively to carbohydrate groups with cis-diols
- High dynamic binding capacity
- Robust, long life adsorbent
- Sanitisable with NaOH allowing multiple cycles
- Highly reproducible batch-to-batch manufacture to ISO 9001 standard
- Supported with comprehensive Regulatory Support Files
- Multiple end users in regulated biopharmaceutical manufacturing and medical diagnostics

## ADVANTAGES

### OPTIMISED PRODUCTS

All of ProMetic BioSciences Ltd's (PBL) Aminophenylboronate affinity products have been optimised for use in various bioseparation applications. All products share the same robust ligand and coupling chemistry.

### DIFFERENT SUPPORT MATRIX OPTIONS

Aminophenylboronate products are available based on two different beaded agarose materials. Aminophenylboronate P6XL utilises PBL's proprietary PuraBead® P6XL near-monodisperse cross-linked agarose beads. PuraBead® products have very uniform particle size which enables reproducible column packing and high flow rates at low back-pressure. Consequently Aminophenylboronate P6XL is the adsorbent of choice for bioprocess applications. Aminophenylboronate A6XL is PBL's original product with a wider particle size distribution. This product is applicable to uses where large packed beds are not required such as small-scale process applications and diagnostics.

### DIFFERENT LIGAND DENSITIES

Aminophenylboronate A6XL is available in two different ligand densities (normal and Low Ligand). For purification applications the normal ligand density product (P6XL/A6XL) provides optimal purification and column capacity. However, for diagnostic/analytical applications the Low Ligand (LL) version provides enhanced selectivity for glycoproteins.

### ADSORBENT STABILITY

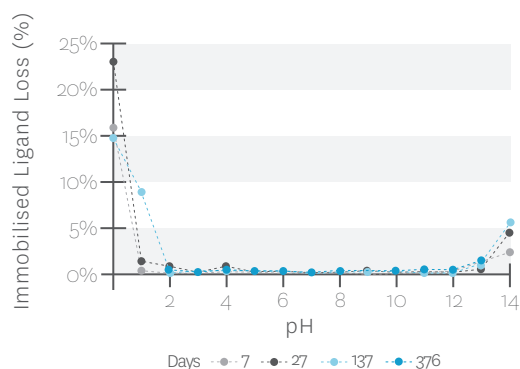
PBL's Aminophenylboronate adsorbents can be subjected to pH's in the range of pH 2.0 - pH 14.0 without significant ligand loss. This enables routine use of 0.5 M NaOH for column cleaning and sanitisation.

## pH STABILITY

Our Aminophenylboronate adsorbents are stable across the pH range of pH 3.0 - pH 13.0 (continuous exposure) and compatible with intermittent exposure to both pH 2.0 and pH 14.0.

FIGURE 1

pH stability profile for Aminophenylboronate A6XL incubated in a range of test solutions from pH 0.0 (1 M HCl) to pH 14.0 (1 M NaOH) at ambient room temperature. Samples of supernatant were collected and assayed for the presence of ligand at time intervals ranging from 1 week to 1 year continuous exposure.

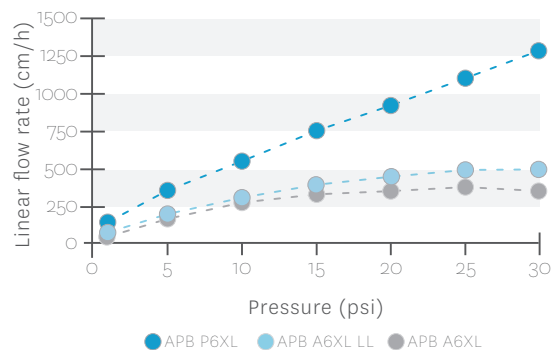


## FLOW RATES

The uniform particle size of Aminophenylboronate P6XL enables operation of this adsorbent at high linear flow rates. Operational flow rates up to 500 cm/h may be used, though flow rates during column loading and elution are typically in the range 50 - 200 cm/h.

FIGURE 2

Pressure-flow curves for Aminophenylboronate A6XL, A6XL LL and P6XL (10 cm diameter column, ~20 cm bed length).



## BINDING CAPACITIES

Binding capacities of Aminophenylboronate P6XL/A6XL are in the range 10 - 20 g/L for glycoproteins and up to 30 g/L for carbohydrates.

## OPERATING CONDITIONS

Binding of glycoproteins to Aminophenylboronate adsorbents is optimal at low to medium ionic strength (10 – 300 mM) and in mildly alkaline pH conditions (pH range of pH 8.0 - pH 9.5). Buffers prepared using phosphate and glycine are recommended for column equilibration. Flow rates of between 50 – 150 cm/h are recommended for binding. Adsorbent binding capacity is dependent on the nature of the sample and typically it is in the range of 10 – 20 g/L for glycoproteins which should be attainable without excessive process development. Elution of the bound protein from Aminophenylboronate adsorbents is achieved either by using a low pH buffer or by the use of a competing diol. Elution by reduction of pH can be achieved using 0.1 M glycine-HCl or 0.1 M acetate buffer, pH 4.0. In the case of acid labile proteins, elution can be performed with a neutral pH buffer using a suitable competing diol such as 20 – 200 mM sorbitol.

STEP	CONDITION
Equilibration/Packing buffer	50 mM sodium phosphate, pH 8.0 buffer
Protein preparation	Prepare protein in equilibration buffer. Pre-filter at 0.45 µm or less.
Operating flow rate	50 - 200 cm/h
Elution	20 - 200 mM sorbitol or 0.1 M glycine-HCl or 0.1 M acetate buffer, pH 4.0
CIP	0.5 - 1.0 M sodium hydroxide
Re-equilibration	Equilibration buffer
Sanitization	0.5 M NaOH
Storage	20% ethanol

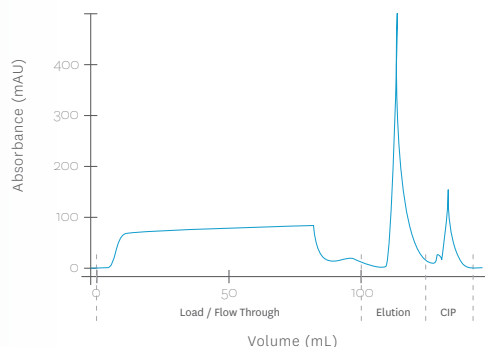
## APPLICATIONS

### PURIFICATION OF FETUIN

Fetuin is a major 59 kDa glycoprotein in the serum of fetal calves containing three N-linked and O-linked carbohydrate side chains. Its purification from crude commercial preparation of fetuin on Aminophenylboronate A6XL column is illustrated.

**FIGURE 3**

Column chromatography of Fetuin. Column Height: 3.9 cm; Column Volume: 3 mL; Linear Flow Rate: 100 cm/h; Equilibration & wash buffer: 50 mM sodium phosphate, pH 8.0; Elution: 50 mM sodium phosphate, 200 mM sorbitol, pH 8.0; CIP: 0.5 M NaOH.

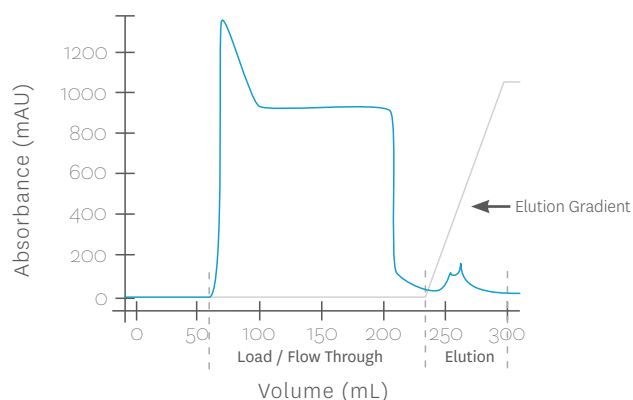


### REMOVAL OF RESIDUAL GLYCOPROTEIN IMPURITIES

Aminophenylboronate adsorbents are particularly useful for depletion of potentially antigenic glycoprotein impurities present in residual host cell proteins (which can be a particular issue for recombinant proteins derived from yeast).

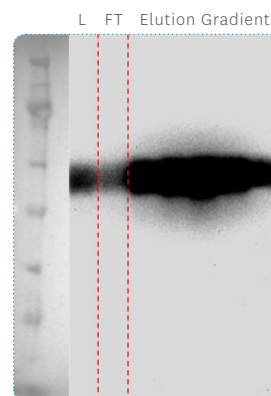
**FIGURE 4A**

Capture of a recombinant glycoprotein expressed in a mammalian cell line using Aminophenylboronate A6XL LL. Column volume: 7.9 mL; Linear Flow Rate: 200 cm/h; Equilibration & wash buffer: 50 mM glycine-NaOH, pH 9.0; Elution: 50 mM sodium phosphate, 25 mM citric acid, 0-200 mM sorbitol gradient, pH 8.0; CIP: 0.5 M NaOH.



**FIGURE 4B**

Western blot of chromatography fractions from the capture and recovery of glycoproteins using Aminophenylboronate A6XL LL (L – Load and FT – Flow through).



## REGULATORY SUPPORT

PBL's Aminophenylboronate affinity adsorbents are supported by regulatory support files.

## BULK MANUFACTURE

PBL can provide Aminophenylboronate Agarose adsorbents in volumes sufficient to meet the requirements of the largest biomanufacturing processes. Single batches of up to 275 litres are produced in PBL's controlled environment manufacturing facility under an ISO 9001 quality system.

## TECHNICAL SUPPORT

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

## PROPERTIES

Ligand	m-Aminophenylboronic acid
Matrix	PuraBead® P6XL (6% near-monodispers cross-linked agarose).
Particle size	100 ± 10 µm
Binding capacity	Up to 30 mg sorbitol/mL
Operational Flow rate	Up to 500 cm/h
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

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

## ORDER

### AMINOPHENYLBORONATE P6XL (SLURRY)

Aminophenylboronate P6XL (25 mL)	3355-00025
Aminophenylboronate P6XL (100 mL)	3355-00100
Aminophenylboronate P6XL (500 mL)	3355-00500
Aminophenylboronate P6XL (1000 mL)	3355-01000

### AMINOPHENYLBORONATE A6XL (SLURRY)

Aminophenylboronate A6XL (25 mL)	0355-00025
Aminophenylboronate A6XL (100 mL)	0355-00100
Aminophenylboronate A6XL (500 mL)	0355-00500
Aminophenylboronate A6XL (1000 mL)	0355-01000

### AMINOPHENYLBORONATE A6XL LL (SLURRY)

Aminophenylboronate A6XL LL (25 mL)	0356-00025
Aminophenylboronate A6XL LL (100 mL)	0356-00100
Aminophenylboronate A6XL LL (500 mL)	0356-00500
Aminophenylboronate A6XL LL (1000 mL)	0356-01000

### AMINOPHENYLBORONATE AGAROSE PRE-PACKED COLUMNS

(for attachment to chromatography workstations)

Aminophenylboronate P6XL (5 x 1 mL)	4355-00001
Aminophenylboronate A6XL (5 x 1 mL)	1355-00001
Aminophenylboronate A6XL LL (5 x 1 mL)	1356-00001

### AMINOPHENYLBORONATE AGAROSE PURAPLATE™

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

Aminophenylboronate P6XL PuraPlate™	2232
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