

## Operating Instructions

## Affinity Chromatography Media Cellufine® PB

### Description

Cellufine PB is an affinity chromatography medium designed for concentration, purification of glycoproteins, glycosylated proteins, and saccharides. This media are based on a spherical, rigid cellulose beads functionalized with phenyl borate. The phenyl borate groups give unique chromatographic selectivity for cis-diol groups of target molecule.

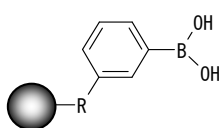


Fig.1 Structure of the ligand.

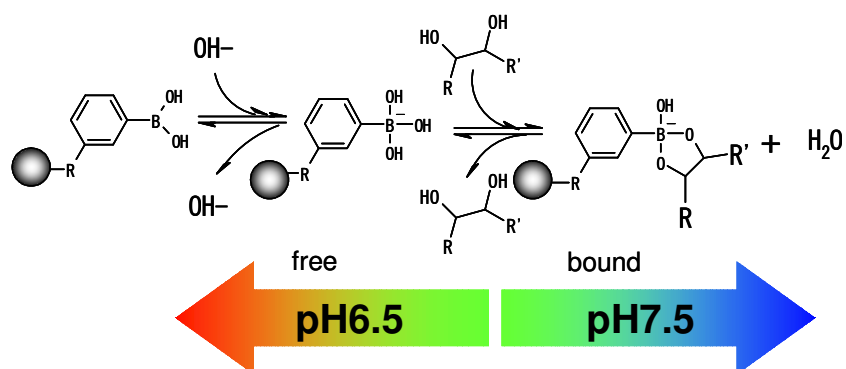


Fig.2 Mechanism of binding cis-diol groups.

### Physical-Chemical Characteristics

Support matrix	cellulose
Particle shape	Spherical
Ligand	Phenyl Borate
Boron Contents ( $\mu\text{g}/\text{dry gel}$ )	$\geq 600$
Binding capacity (Conalbumin)(mg/ml)	$\geq 7$
pH operating range	3-12
Operating pressure	< 2 bar (29 psi)
Supplied	suspension in 20 % EtOH

### Column Packing

1. Calculate volume required for the desired bed dimension.
2. Prepare a 40 – 60 % (v/v) slurry with the appropriate elution buffer (high salt). Allow the gel

to equilibrate at ambient temperature for one hour.

3. Gently stir or place under vacuum to degas.
4. With column outlet closed, carefully pour the slurry into column. Depending on the volume, a filler tube may be necessary.
5. With the inlet open to release air, insert and affix the top adjuster assembly at the slurry interface.
6. Open the column outlet and begin pumping elution buffer at rate 10 % – 20 % greater than the operational flow rate.
7. After the bed stabilizes, close the column outlet. Then with the inlet open, reposition the end cell on top of the bed. Equilibrate with 10 column volumes of adsorption buffer before sample loading.

## Operating Guidelines

### General Operation

1. Equilibrate column with adsorption buffer.
2. Load sample .
3. Wash with several bed volumes of adsorption buffer to remove non-binding contaminants.
4. Elute bound solute(s) with desorption buffer

### Recommended Buffers

Adsorption buffer: 0.01 M sodium phosphate, 0.1 to 0.2 M NaCl, and the pH greater than pH 7.5. Depending on the application, other buffer ions may be used. In general, adsorption strength varies inversely with pH and ionic strength. Slightly Increased ionic strength can help to remove closely bound contaminants. Non-ionic detergents (Tween®20, Triton® X, etc.) may be also added to improve solubility.

Elution buffer: In general the target molecule is eluted at pH less than 6.7. The elution can be also done with boric acid, cis-diol compound such as mannitol or sorbitol.

### Sample Preparation and Load

Prepare samples at concentration 1 – 20 mg/ml, in adsorption buffer. Remove insoluble material by centrifugation or microfiltration. If necessary, exchange sample buffer using dialysis, diafiltration or desalting chromatography such as Cellufine GH-25.

**Flow Rate**

The recommended linear velocity range for Cellufine PB is 50 – 250 cm/h.

**Chemical and Physical Stability**

pH 3 – 12, when operated at temperatures between 2 – 30 °C.

200 time repeat washing using 0.5 M NaOH is possible.

**Regeneration and Depyrogenation**

Cellufine PB is typically regenerated and depyrogenated with high ionic strength (2.0 – 3.0 M) NaCl. If this is not sufficient, regenerate more aggressively with 3 – 10 column volumes of 0.05 – 0.5 N NaOH at 2 – 10°C, then wash with 2.0 – 3.0 M NaCl until pH drops below 9. Wash the column again with adsorption buffer until equilibrated.

**Storage**

Short term (2 weeks or less), bulk and column can be stored in 1 M NaCl in neutral buffer at 2 – 4 °C. Longer term storage can be conducted under identical conditions; however, a preservative (e.g. 20% EtOH or 0.02 % sodium azide) should be added to the buffer. Store at 2 – 8 °C. Do not freeze.

**Shelf Lifetime:**

10 years from date of manufacture

**References**

Weith, H.L., Wiebers, J.L., Gilham P.T. Biochemistry, 9, 4396 (1970)

**Product Ordering Information (Catalogue No.)**

Product Name	Pack Size					
	1 ml x 5 Mini-Column	10 ml	50 ml	500 ml	5L	10L
Cellufine PB	20251	683 986 324	683 986 326	683 986 328	683 986 330	683 986 335

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