

# JNC CORPORATION

Operating Instructions:

Mini-Column Cellufine PB



## 1. Description

Mini-column Cellufine PB is a prepacked, easy to use column for Cellufine PB affinity chromatography. Cellufine PB is an affinity medium designed for concentration, purification of glycoprotein, glycosylated protein, saccharide. The Cellufine PB mini-columns are packed with Cellufine PB media. This media are based on a spherical, rigid cellulose beads functionalized with phenyl borate. The phenyl borate groups give a unique chromatographic selectivity for cis-diol groups of target molecule.

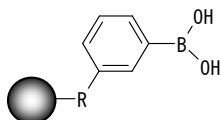


Fig.1 Structure of the ligand.

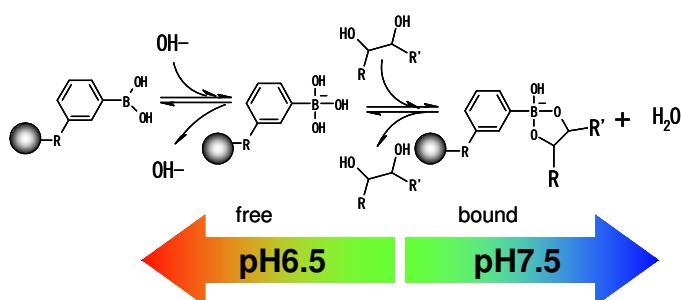


Fig.2 Mechanism of binding cis-diol groups.

## Column

Cellufine Mini-columns are made of polypropylene tube and UHMW-PE frits. The columns can be connected to chromatography system with 10-32UNF thread for connection of 1/16 inch OD tubing.

Table 1. Mini-column Cellufine PB characteristics

Column volumes	1 ml and 5 ml
Column dimensions (i.d. x L)	6.7 mm x 30 mm (1 ml) 14.6 mm x 30 mm (5 ml)
Ligand	Phenyl borate
Boron Contents	700 µg/dry gel
Binding capacity(Conalbumin)	10 mg/ml
Particle diameter	ca. 40 to 130 µm
Bead matrix	Spherical Cellulose
Pressure limit	0.4 MPa (4 bar)
Recommend flow rate	0.1 – 1.0 ml/min (1 ml) 0.1 – 5.0 ml/min (5 ml)
pH stability	3 – 12
Storage	Cool and dark place in 20% ethanol

## 2. Operating Guidelines

### General Operation

- (1) Equilibrate column with adsorption buffer
- (2) Sample load (preferably in adsorption buffer.)
- (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 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<sup>®</sup>20, Triton<sup>®</sup> 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

Prepare samples at concentration 1 to 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.

## 3. Purification

- (1) Fill the pump tubing or syringe outlet with adsorption buffer. Remove the inlet plug (top of the column) and connect the column to the pump tubing, or syringe, “dripping the buffer” to avoid introducing air into the column.
- (2) Remove the outlet plug (end of the column).
- (3) Wash out the preservative and equilibrate the column with 10 column volumes of adsorption buffer.
- (4) Apply the sample, using a syringe or by pumping it on the column.
- (5) Wash with 5 to 10 column volumes of adsorption buffer. Elute with 5 to 10 column volumes of elution buffer.

#### 4. Regeneration and Depyrogenation

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

#### 5. Scaling up

Two or three of Cellufine PB Mini-columns can be connected in series.

#### 6. Storage

Wash the column with 5 to 10 column volumes of 20% ethanol. Store the column in 20% ethanol at cool and dark place.  
Note: To prevent leakage it is essential to ensure that the end plugs are tight.

#### 7. Reference

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

#### 8. Further information

For further information, visit  
<http://www.jnc-corp.co.jp/fine/en/cellufine/index.html>

#### 9. Ordering information

Product	Quantity	Product number
Mini-column Cellufine PB, 1 ml	5 x 1 ml	20251
Mini-column Cellufine PB, 5 ml	1 x 5 ml	20215
Cellufine PB	50 ml	683986326
Cellufine GH-25	100 ml	670000327
Mini-column Cellufine GH-25	5 x 5 ml	19711-55

#### 10. Contact us

Asia & Others: JNC Corporation Chemical Division Life  
Chemicals Department  
2-2-1 Otemachi, Chiyoda-ku, Tokyo 100-815, Japan  
Tel: +81-3-3243-6150, Fax: +81-3-3243-6219  
E-mail : [cellufine@jnc-corp.co.jp](mailto:cellufine@jnc-corp.co.jp)

America & Europe: JNC America Incorporated  
555 Theodore Fremd Ave., Suite C-206, Rye, New York 10580,  
U.S.A.  
Tel: 914-921-5400 Fax: 914-921-8822  
E-mail : [cellufine@jncamericany.com](mailto:cellufine@jncamericany.com)