Operating Instructions: Mini-Column Cellufine PB (ver.2.1)

Cellufine

Mini-column Cellufine PB, 1 mL and 5 ml.

1. Description

Mini-column Cellufine PB is a prepacked, easy to use column for Cellfine PB affinity chromatography. Cellufine PB is an affinity medium designed for concentration, purification of glycoprotein, glycated protein, scaccharide. 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 unique chromatographic selectivity for cis-diol groups of target molecule.

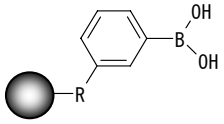


Fig.1 Structure of the ligand.

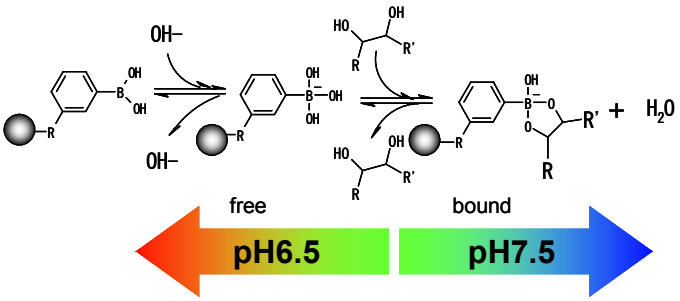


Fig.2 Mechanism of binding cis-diol groups.

Column

Cellufine Mini-columns are made of polypropylene tube and polyethylene frits. The columns can be connected to syringe, peristaltic pump, or in chromatography system with luer adaptors.

Table 1. Mini-column Cellufine PB characteristics

Column volumes	1ml and 5ml
Column dimensions (i.d. x L)	9mm x 18 mm (1ml) 13mm x 44mm (5ml)
Ligand	Phenyl borate
Boron Contents	700µg/dry gel
Binding capacity(Conalbumin)	10mg/ml
Particle diameter	ca. 40 to 130 µm
Bead matrix	Spherical Cellulose
Maximum back pressure	0.2 MPa
Maximum flow rate	10ml/min
Recommend flow rate	5 ml/min
pH stability	3 to 12
Storage	+2° C to +8° C 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®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

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.

3. Purification procedure

(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 (20 % EtOH) 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 – 10 column volumes of adsorption buffer.

(6) Elute with 5 – 10 column volumes of elution buffer.

4. 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.

5. **Scaling up**

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

6. **Storage**

Wash the column with 5 – 10 column volumes of 20% ethanol. Store the column in 20% ethanol at +2ºC to +8 ºC.

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)

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

Appendix : Column connection

Cellufine Mini-column has luer adaptors.
You can connect up soft tube and rigid 1/16”(inch) tube with luer fittings.
The 1/16” tube is used by many chromatography systems.
It is possible to connect Cellufine Mini-column to a chromatography system using the Luer Tight™ Fittings.

1. For soft tube “Soft tube Fittings”

   (a) Connect tube with male luer


Fig.1 Male luer

   (b) Feed buffer and purge air in the tube.
   (c) Connect male luer with top of the column
   (d) Take off plug of the bottom of column
   (e) Connect female luer with bottom of the column.

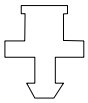


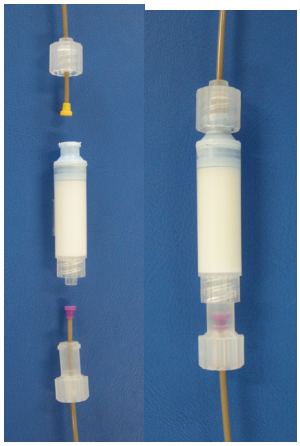
Fig.2 Female luer

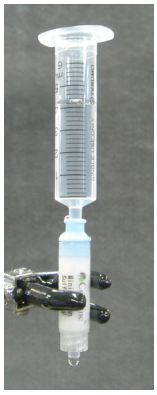
   (f) Connect tube with female luer.

2. For 1/16” tube “Luer Tight™ Fittings”

We have employed the Luer Tight™ Fittings of UPCHURCH SCIENTIFIC.
This product can connect the tube and Cellufine Mini-column, which are generally used to chromatography systems, such as PEEK, Teflon, PP, etc.
Please read the instruction manual attached to this product before using it.


Picture 1. The example of connection of a flexible tube


Picture 2. The example of connection of a rigid tube(PEEK).


Picture 3. Syringe is directly connectable with Cellufine Mini-column.

Luer Tight™ Fittings is UPCHURCH SCIENTIFIC product.

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