JNC CORPORATION

Operating Instructions

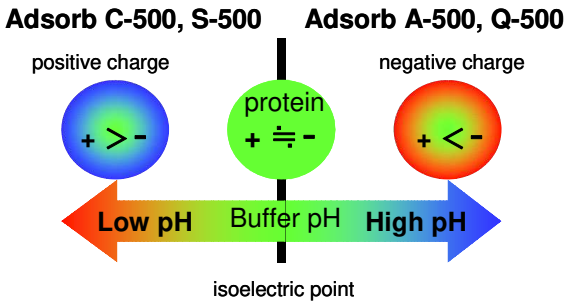
Mini-Column Cellufine

A-200, A-500, A-800, Q-500, C-500 and S-500

Cellufine™

1. Description

Mini-columns Cellufine A-200, A-500, A-800, Q-500, C-500 and S-500 are prepacked, easy to use columns for Cellufine Ion Exchange chromatography (IEX) .Cellufine IEX are designed for concentration and purification of large molecules such as proteins, enzymes and polysaccharides. The Cellufine IEX mini-columns are packed with Cellufine IEX media. Cellufine media are based on spherical and rigid cellulose beads functionalized with charge 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 IEX 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	A-200: Diethyaminoethyl A-500: Diethyaminoethyl A-800: Diethyaminoethyl Q-500: Quaternary ammonium C-500: Carboxymethyl S-500: Sulfobutyl
Ion exchange capacity	A-200: 0.8 to 1.1 meq/g A-500: 1.1 to 1.4 meq/g A-800: 0.6 to 1.0 meq/g Q-500: 1.2 to 1.9 meq/g C-500: 0.9 to 1.2 meq/g S-500: 1.1 to 1.5 meq/g
Binding capacity	A-200: ≧80 mg/ml (BSA) A-500: ≧60 mg/ml (BSA) A-800: ≧45 mg/ml (BSA) Q-500: ≧*10mg/ml (BSA) C-500: ≧70 mg/ml (Lysozyme) S-500: ≧110 mg/ml (Lysozyme)
Particle diameter	ca 90 µm
Matrix structure	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

*Assayed with high salt concentration buffer

2. Operating Guidelines

General Operation

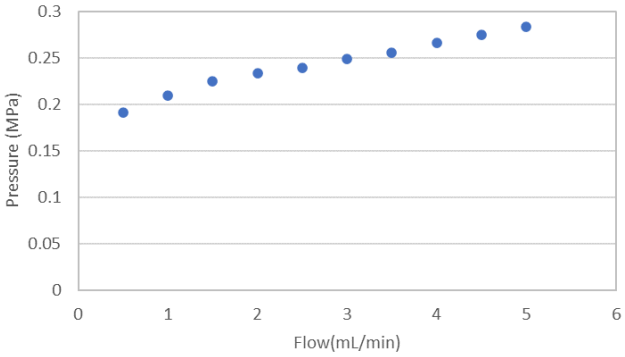
(1) Equilibrate column with adsorption buffer

(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

Typical flow property of Cellufine IEX (Cellufine A-500 as example) measured by AKTA system (GE Healthcare).



System: Akta avant 25

Flow Ristrictor FR-902:in line

Mobile phase: Water

Temperature:20-25 ℃

connection piping: ID 5mm x 20 cm

Recommended Buffers

Adsorption buffer: Low ion strength (10 mM to 50 mM) buffer is recommended. Phosphate, acetate or Tris, etc. can be used. Depending on the application, different buffer ions may be used. In general, adsorption strength varies inversely with pH and ionic strength. A slight Increase of ionic strength can aid in removing closely bound contaminants. Non-ionic detergents (Tween®20, Triton® X, etc.) may be also added to improve solubility.

Elution buffer: In general elution buffer containing more than 0.5 M NaCl or KCl. The exact concentration can be determined by gradient elution. Step gradients are typically employed for preparative applications.

Sample Preparation

Prepare samples at concentration of 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 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 air introduction of air into the column.

(2) Remove the outlet plug (end of the column).

(3) Wash out the preservative (20% ethanol) 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.

(6) Elute with 5 to 10 column volumes of elution buffer (gradient elution or step gradients).

4. **Regeneration and Depyrogenation**

Cellufine IEX 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 0.1 N NaOH (for anion exchanger) 0.1 N HCl (for cation exchanger) at 2 – 10 °C, then wash with 2.0 to 3.0 M NaCl until pH drops to 7. Wash the column again with starting buffer until equilibrated.

5. **Scaling up**

Two or three of Cellufine IEX 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 cool and dark place.

Note: To prevent leakage it is essential to ensure that the end plugs are tight.

7. **Reference**

(AEX)

Biosci Biotechnol Biochem. 2004, 68 (6) pp1299-305

A chitinase indispensable for formation of protoplast of Schizophyllum commune in basidiomycete-lytic enzyme preparation produced by Bacillus circulans KA-304.Yano S, *et al*.

Toxicon. 2000, 38 (3) pp463-8.

Purification and some properties of a tetrodotoxin binding protein from the blood plasma of kusafugu, Takifugu niphobles. Matsui T, *et al*

Infect Immun. 1999, 67 (8) pp 4014-8.

New exfoliative toxin produced by a plasmid-carrying strain of Staphylococcus hyicus. Sato H, *et al*

Insect Biochem Mol Biol. 1997, 27 (8-9) pp 757-67.

Purification and characterization of Bombyx mori chitinases. Koga D, *et al*

(CEX)

Arch Biochem Biophys. 1996, 328(1) pp 165-72.

Purification and molecular characterization of a novel b5-type cytochrome of the parasitic nematode, Ascaris suum. Yu Y, *et al*

Anim. Sci. Technol.1995, 66(6) pp 513-22

Purification and characterization of Japanese quail (Coturnix japonica) egg white proteins with inhibitory effects on Tlymphocyte mitogen-induced proliferative responses of mouse spleen cells Otani, HajimeNakaya, *et al*

8. **Further information**

For further information, visit https://www.jnc-corp.co.jp/fine/en/cellufine/

9. **Ordering information**

Product	Quantity	Product number
Mini-column Cellufine A-200, 1 ml	5 x 1 ml	19611-51
Mini-column Cellufine A-200, 5 ml	5 x 5 ml	19611-55
Mini-column Cellufine A-500, 1 ml	5 x 1 ml	19805-51
Mini-column Cellufine A-500, 5 ml	5 x 5 ml	19805-55
Mini-column Cellufine A-800, 1 ml	5 x 1 ml	19800-51
Mini-column Cellufine A-800, 1 ml	5 x 5 ml	19800-55
Mini-column Cellufine Q-500, 1 ml	5 x 1 ml	19907-51
Mini-column Cellufine Q-500, 5 ml	5 x 5 ml	19907-55
Mini-column Cellufine C-500, 1 ml	5 x 1 ml	19800-51
Mini-column Cellufine C-500, 5 ml	5 x 5 ml	19800-55
Mini-column Cellufine S-500, 1 ml	5 x 1 ml	21200-51
Mini-column Cellufine S-500, 5 ml	5 x 5 ml	21200-55
Cellufine A-200	100 ml	676 980 327
Cellufine A-500	100 ml	675 980 327
Cellufine A-800	100 ml	673 980 327
Cellufine Q-500	100 ml	675 982 327
Cellufine C-500	100 ml	675 983 327
Cellufine S-500	100 ml	21200
Cellufine GH-25	100 ml	670 000 327
Mini-column Cellufine GH-25, 5 ml	5 x 5 ml	19711-55

10. **Contact us**

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