JNC CORPORATION

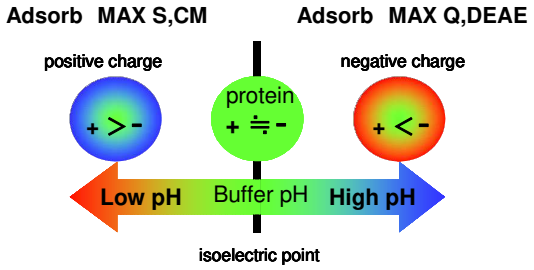
Operating Instructions

Mini-Column Cellufine MAX S-r, S-h, Q-r, Q-h, Q-hv, CM, DEAE, GS

Cellufine™ MAX

1. Description

Mini-columns Cellufine MAX S-r, S-h, Q-r, Q-h, Q-hv, CM, DEAE,GS are prepacked, easy to use columns for Cellufine MAX Ion Exchange chromatography (IEX) .Cellufine MAX IEX are designed for concentration and purification of large molecules such as proteins, enzymes and polysaccharides. The Cellufine MAX IEX mini-columns are packed with Cellufine MAX IEX media. Cellufine MAX 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 MAX 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	S-r, S-h, GS: $-SO_3^-$ Q-r, Q-h: $-N^+(CH_3)_3$ DEAE: $-C_2H_4N^+(C_2H_5)_2$ CM: $-CH_2COO^-$
Ion exchange capacity	S-r: 0.09 – 0.21 meq/ml S-h: 0.10 – 0.22 meq/ml Q-r: 0.10 – 0.20 meq/ml Q-h: 0.13 – 0.22 meq/ml Q-hv: 0.04 – 0.07 meq/ml CM: 0.09 – 0.22 meq/ml DEAE: 0.12 – 0.22 meq/ml GS: 0.09 – 0.15 meq/ml
Dynamic Binding capacity (300 cm/h, 10% break through)	S-r:>110 mg/ml (IgG) S-h:>180 mg/ml (IgG) Q-r:>110 mg/ml (BSA) Q-h:>180 mg/ml (BSA) Q-hv:>120 mg/ml (BSA) CM: >80 mg/ml (IgG) DEAE: >100 mg/ml (BSA) GS: >50 mg/ml(IgG)
Particle diameter	ca. 40 to 130 µm
Matrix structure	highly cross-linked cellulose with dextran scaffold
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 (Long term)	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: Low ion strength (10 mM to 50 mM) buffer containing 10 mM to 50 mM NaCl 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 adsorption buffer containing around 0.5 M to 1M 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 < 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 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.

4. Regeneration and Depyrogenation

Cellufine MAX IEX 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 0.1 M to 0.5 M NaOH (for anion

exchanger) 0.1 N HCl (for cation exchanger) at 2 – 10 °C, or 0.2 M NaOH + 95% EtOH, then wash with 2.0 – 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 spleencells Otani, HajimeNakaya, *et al*

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 MAX S-r, 1 ml	5 x 1 ml	20300-51
Mini-column Cellufine MAX S-r, 5 ml	5 x 5 ml	20300-55
Mini-column Cellufine MAX S-h, 1 ml	5 x 1 ml	20400-51
Mini-column Cellufine MAX S-h, 5 ml	5 x 5 ml	20400-55
Mini-column Cellufine MAX Q-r, 1 ml	5 x 1 ml	20500-51
Mini-column Cellufine MAX Q-r, 5 ml	5 x 5 ml	20500-55
Mini-column Cellufine MAX Q-h, 1 ml	5 x 1 ml	20600-51
Mini-column Cellufine MAX Q-h, 5 ml	5 x 5 ml	20600-55
Mini-column Cellufine MAX Q-hv, 1 ml	5 x 1 ml	22100-51
Mini-column Cellufine MAX Q-hv, 5 ml	5 x 5 ml	22100-55
Mini-column Cellufine MAX CM, 1 ml	5 x 1 ml	20900-51
Mini-column Cellufine MAX CM, 5 ml	5 x 5 ml	20900-55
Mini-column Cellufine MAX DEAE, 1 ml	5 x 1 ml	21000-51
Mini-column Cellufine MAX DEAE, 5 ml	5 x 5 ml	21000-55
Mini-column Cellufine MAX GS, 1 ml	5 x 1 ml	21300-51
Mini-column Cellufine MAX GS, 5 ml	5 x 5 ml	21300-55
Cellufine MAX S-r	100 ml	20300
Cellufine MAX S-h	100 ml	20400
Cellufine MAX Q-r	100 ml	20500
Cellufine MAX Q-h	100 ml	20600
Cellufine MAX Q-hv	100 ml	22100
Cellufine MAX CM	100 ml	20900
Cellufine MAX DEAE	100 ml	21000
Cellufine MAX GS	100 ml	21300
Cellufine GH-25	100 ml	670 000 327
Mini-column Cellufine GH-25, 5 ml	5 x 5 ml	19711-55

10. **Contact information**

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