

JNC CORPORATION

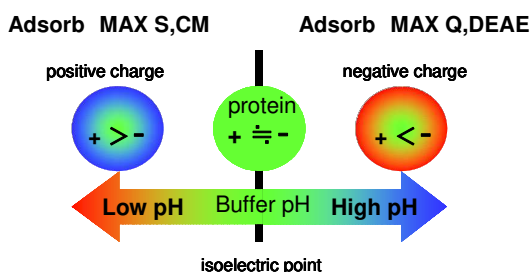
Operating Instructions

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



1. Description

Mini-columns Cellufine MAX S-r, S-h, Q-r, Q-h, 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: $-\text{SO}_3^-$ Q-r, Q-h: $-\text{N}^+(\text{CH}_3)_3$ DEAE: $-\text{C}_2\text{H}_4\text{N}^+(\text{C}_2\text{H}_5)_2$ CM: $-\text{CH}_2\text{COO}^-$ |
| 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 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) 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 kusahugu, 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 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 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 | 5 x 5 ml | 19711-55 |
| Cellufine GH-25, 5 ml | | |

10. Contact information

Asia & Others: JNC Corporation

Life Chemicals Division

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

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