JNC CORPORATION

Operating Instructions

Mini-Column Cellufine MAX DexS-HbP and MAX DexS-VirS

Cellufine™ MAX

1. Description

Cellufine MAX media are based on highly cross-linked spherical and rigid cellulose beads optimized for high flow applications. Cellufine MAX DexS resins have pseudo Heparin affinity characteristics for concentration and purification of heparin binding proteins and virus particles. Two Cellufine MAX DexS resins having different lengths of Dextran Sulfate polymer are avirable; a) DexS-HbP developed for purification of **h**eparin **b**inding **p**roteins. and b) DexS-VirS, for purifying **vir**us and virus like particles Mini-column characteristics of both resins are summarized in Table 1.

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 MIX 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	MAX DexS-HbP; Dextran sulfate Sulfur contents)≥ 36 μmol/ml MAX DexS-VirS; Dextran sulfate Sulfur contents)≥ 74 μmol/ml
Particle diameter	ca. 90 μm
Bead structure	Highly cross-linked 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	4 – 12
Storage	Cool and dark place in 0.1M Phosphate buffer, 20% ethanol

2. Operating Guidelines

General Operation

(1) Equilibrate column with adsorption buffer

(2) Load sample (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 10mM to 50mM 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 1 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 introducing 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-wise).

(Cleaning and sanitization)

If necessary, all resins are able to be used such as standard cleaning or sanitizing solution, NaOH (0.1 to 0.5 M), or 70% ethanol or non-ionic detergents or combinations, etc. After cleaning, the media should be re-equilibrated.

4. Regeneration and Depyrogenation

Cellufine MAX DexS resins are typically regenerated and depyrogenated with water. If this is not sufficient, regenerate more aggressively with 3 to 10 column volumes of 0.1 N NaOH at 2 to 10 °C, then wash with water until pH drops to near neutral. Washing with 2 to 4 column volume of ethanol, acetone etc., can be also helpful. After wash the mini column with water and finally with starting buffer until equilibrated.

5. **Storage**

Wash the column with 5 to 10 column volumes 20% ethanol. Store the column in phosphate buffer- 20% ethanol at cool and dark place.

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

6. Further information

For further information, visit

https://www.jnc-corp.co.jp/fine/en/cellufine/

7. Ordering information

Product name	Quantity	Product number
Mini-column Cellufine MAX DexS-HbP, 1 ml	5 x 1 ml	21700-51
Mini-column Cellufine MAX DexS-HbP , 5 ml	1 x 5 ml	21700-15
Mini-column Cellufine MAX DexS-VirS, 1 ml	5 x 1 ml	21800-51
Mini-column Cellufine MAX DexS-VirS, 5 ml	1 x 5 ml	21800-15
Cellufine MAX DexS-HbP	50 ml	21701
Cellufine MAX DexS-VirS	50 ml	21801
Cellufine GH-25	100 ml	670 000 327
Mini-column Cellufine GH-25	5 x 5 ml	19711-55

8. Contact us

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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.