JNC CORPORATION

Operating Instructions

Mini-column Cellufine Sulfate

Cellufine™

1. Description

Mini-column Cellufine Sulfate is a prepacked, easy to use column for Cellufine Sulfate affinity chromatography. Cellufine Sulfate is an affinity medium designed for the concentration, purification and depyrogenation of virus, viral coat proteins and microbial antigens, and specific proteins such as blood coagulation factors. The Cellufine Sulfate mini-columns are packed with Cellufine Sulfate gels. These gels are based on a spherical, rigid cellulose beads functionalized with a low concentration of sulfate esters. The low density of sulfate groups gives the gel unique chromatographic selectivity that, in some cases, is similar to immobilized heparin.

Due to Cellufine Sulfate's low exclusion limit of 3 kD, large molecules adsorb primarily on the packing's exterior, resulting in rapid adsorption and desorption times. Its superior rigidity allows high flow rates, and thus, rapid processing times. Because pyrogens have no affinity for Cellufine Sulfate, the gel can typically be depyrogenated with several column volumes of purified and depyrogenated, water.

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 Sulfate characteristics

Column volumes	1 ml and 5 ml
Column dimensions (i.d. x h)	6.7 mm x 30 mm (1 ml) 14.6 mm x 30 mm (5 ml)
Ligand	Sulfate ester
Degree of substitution	700 µg/dry gel
Binding capacity	3 mg/ml
Particle diameter	40 to 130 µm
Bead 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

2. Operating Guidelines

General Operation

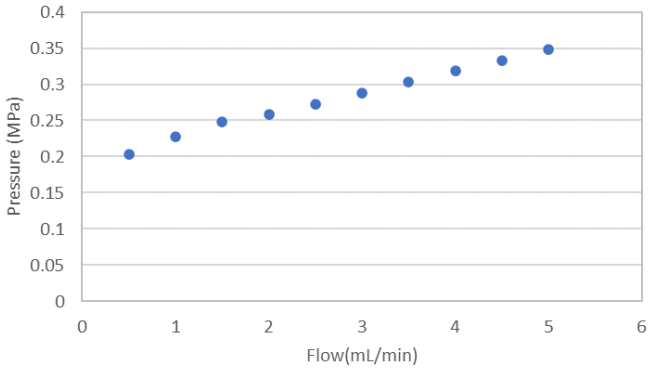
(1) Equilibrate column with adsorption buffer

(2) Load sample (The sample should be adjusted to the composition of the 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 Sulfate measured by AKTA system (Cytiva).



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: 0.01 M sodium phosphate, 0.1 M NaCl, pH 7.5. Depending on the application, other buffer ions may be used. In general, adsorption strength varies inversely with pH and ionic strength. Increasing ionic strength slightly can aid in removing loosely bound contaminants. Non-ionic detergents (Tween®20, Triton® X, etc.) may also be added to improve solubility.

Elution buffer: In general use mobile phase consisting of adsorption buffer containing 1 to 2 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 a 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

(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 Sulfate 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 0.05 to 0.15 N NaOH at 2 to 10 °C, then wash with 2.0 to 3.0 M NaCl until pH drops below 9. Wash the column again with starting buffer until equilibrated.

5. **Scaling up**

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

6. **Storage**

Wash the column with 5 to 10 column volumes 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**

Preparation of endotoxin-free bacteriophages.
Cell Mol Biol Lett. 9(2) 253-9. (2004)
Boratynski J, Syper D, Weber-Dabrowska B, Lusiak-Szelachowska M, Pozniak G, Gorski A.

Development of Vero cell-derived inactivated Japanese encephalitis vaccine.
Biologicals. 30(4) 303-14. (2002)

Sugawara K, Nishiyama K, Ishikawa Y, Abe M, Sonoda K, Komatsu K, Horikawa Y, Takeda K, Honda T, Kuzuhara S, Kino Y, Mizokami H, Mizuno K, Oka T, Honda K.

Purification of a functional gene therapy vector derived from Moloney murine leukaemia virus using membrane filtration and ceramic hydroxyapatite chromatography.
Biotechnol Bioeng. 80 (4) 445-53. (2002)
Kuiper M, Sanches RM, Walford JA, Slater NK.

Scaleable chromatographic purification process for recombinant adeno-associated virus (rAAV). J Gene Med. 2(6)444-54. (2000)
O'Riordan CR, Lachapelle AL, Vincent KA, Wadsworth SC.

8. **Further information**

For further information,

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

9. **Ordering information**

Product	Quantity	Product number
Mini-column Cellufine Sulfate	5 x 1 ml	19845-51
Mini-column Cellufine Sulfate	1 x 5 ml	19845-15
Cellufine Sulfate	10 ml	676943324
Cellufine Sulfate	50 ml	19845
Cellufine GH-25	100 ml	670000327
Mini-column Cellufine GH-25	5 x 5 ml	19711-55

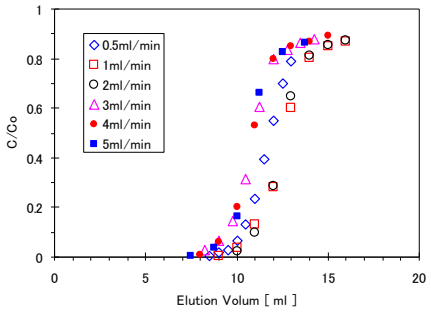


Fig 1. Binding capacity of Lysozyme on CapCell Sulfate 1mL with various flow rate.

Column : Cellufine Sulfate Mini-Column,1 ml

Sample: Lysozyme 1 g/ml in 0.01 M Na-phosphate, pH7

Detection: UV 280 nm

10. **Contact us**

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