Operating Instructions

Mini-column Cellufine® Sulfate (Ver. 2.1)

Mini-column Cellufine Sulfate , 1 mL & 5 mL ver.2

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

Mini-column Cellufine are made of polypropylene tube and polyethylene frits. The columns can be connected a syringe, a peristaltic pump, or in a chromatography system with luer adaptors.

Table 1. Mini-column Cellufine Sulfate characteristics

Column volumes	1ml and 5ml
Column dimensions (i.d. x h)	9mm x 18 mm (1ml) 13mm x 44mm (5ml)
Ligand	Sulfate ester
Degree of substitution	700µg/dry gel
Binding capacity	3mg/ml
Particle diameter	44 to 105 µm
Bead structure	Spherical Cellulose
Maximum back pressure	0.2MPa(1 ml)
Maximum flow rate	10ml/min(1 ml)
Recommend flow rate	5ml/min(1 ml)
pH stability	3 to 12
Storage	+4ºC to +10ºC in 20% ethanol

Note: Need max flow and pressure values for 5 ml column

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

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 – 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 – 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 GH25.

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 – 10 column volumes of adsorption buffer.

(6) Elute with 5 – 10 column volumes of elution buffer.

4. Regeneration and Depyrogenation

Cellufine Sulfate 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 0.05 – 0.15 N NaOH at 2 – 10 ºC, then wash with 2.0 – 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 – 10 column volumes 20% ethanol .Store the column in 20% ethanol at +2 ºC to +8 ºC.

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.

9. Ordering information

Product	No. Supplied	Code. No.
Mini-column Cellufine Sulfate	5 x 1 ml	19845-51
Mini-column Cellufine Sulfate	1 x 5 ml	19845-15
Cellufine Sulfate	10 ml (bulk)	676943324
Cellufine Sulfate	50 ml (bulk)	19845
Cellufine GH-25	100ml	670000327
Mini-column Cellufine GH-25	5 x 5ml	19711-55

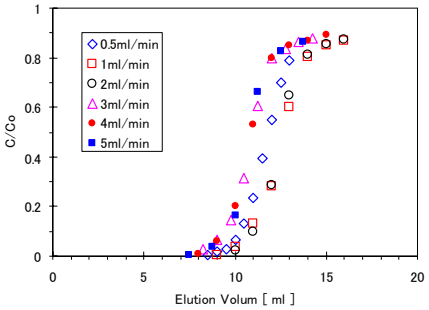


Fig 1. Binding capacity of Lysozyme on CapCell Sulfate 1mL with various flow rate.

Column : Cellufine Sulfate Mini-Column,1mL
Sample: Lysozyme 1mg/ml in 0.01M Na-phosphate, pH7
Detection: UV 280 nm

Appendix : Column connection

Cellufine Mini-column has luer adaptors.
You can connect up soft tube and rigid 1/16”(inch) tube with luer fittings.
The 1/16” tube is used by many chromatography systems.
It is possible to connect Cellufine Mini-column to a chromatography system using the Luer Tight™ Fittings.

1. For soft tube “Soft tube Fittings”

   (a) Connect tube with male luer


Fig.1 Male luer

   (b) Feed buffer and purge air in the tube.
   (c) Connect male luer with top of the column
   (d) Take off plug of the bottom of column
   (e) Connect female luer with bottom of the column.

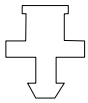


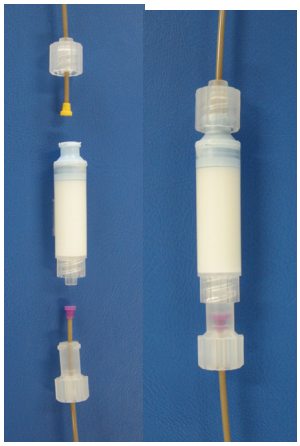
Fig.2 Female luer

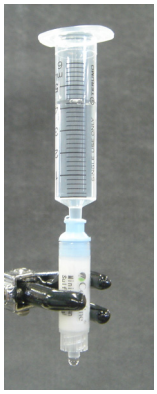
   (f) Connect tube with female luer.

2. For 1/16” tube “Luer Tight™ Fittings”

We have employed the Luer Tight™ Fittings of UPCHURCH SCIENTIFIC.
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.


Picture 1. The example of connection of a flexible tube


Picture 2. The example of connection of a rigid tube(PEEK).


Picture 3. Syringe is directly connectable with Cellufine Mini-column.

Luer Tight™ Fittings is UPCHURCH SCIENTIFIC product.

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