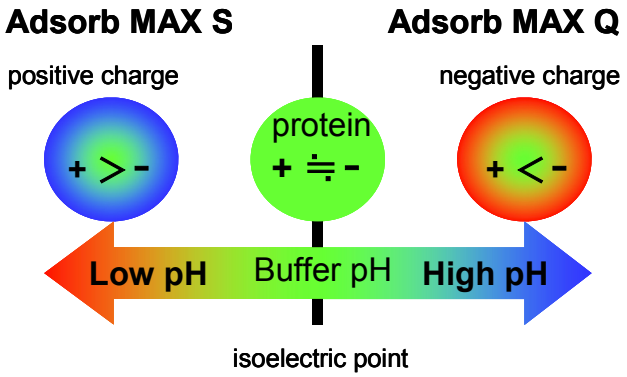
Operating Instructions

Mini-Column Cellufine MAX S-r, S-h, Q-r, Q-h (ver.1)

Cellufine MAX

1. Description

Mini-columns Cellufine MAX S-r, S-h, Q-r, Q-h 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 polyethylene frits. The columns can be connected to syringe, peristaltic pump, or chromatography system with luer adaptors.

Table 1. Mini-column Cellufine MAX IEX characteristics

Column volumes	1 ml and 5 ml
Column dimensions (i.d. x L)	9mm x 18 mm (1ml) 13mm x 44mm (5ml)
Ligand	S-r, S-h: $-SO_3^-$ Q-r, Q-h: $-N^+(CH_3)_3$
ion exchange capacity	S-r: 0.12 - 0.24 m eq/ml S-h: 0.13 - 0.25 m eq/ml Q-r: 0.17 - 0.24 m eq/ml Q-h: 0.13 - 0.20 m eq/ml
Dynamic Binding capacity (300cm/h, 10% brake through)	S-r:>130 mg/ml (IgG) S-h:>180 mg/ml (IgG) Q-r:>110 mg/ml (BSA) Q-h:>180 mg/ml (BSA)
Particle diameter	40 to 130 μm
Matrix structure	highly cross-linked cellulose with dextran scaffold
Maximum back pressure	0.2 Mpa
Maximum flow rate	10 ml/min
Recommend flow rate	5 ml/min
pH stability	3 to 12
Storage (Long term)	+2 °C to +25 °C 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 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 0.5M 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 air introduction of air into the column.

(2) Remove the outlet plug (end of the column).

(3) Wash out the preservative (20 % EtOH) 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 (gradient elution or step gradients).

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 0.5M NaOH (for anion exchanger) 0.1 N HCl (for cation exchanger) at 2 – 10°C, or 0.2M 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 +2 ºC to +8 ºC. .

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*

9. **Ordering information**

Product	Quantity	Product number
Mini-column Cellufine MAX S-r, 1ml	5 x 1 ml	20300-51
Mini-column Cellufine MAX S-r, 5ml	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, 1 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
Cellufine MAX S-r	100ml	20300
Cellufine MAX S-h	100ml	20400
Cellufine MAX Q-r	100ml	20500
Cellufine MAX Q-h	100 ml	20600
Cellufine GH-25	100 ml	670 000 327
Mini-column Cellufine GH-25, 5 ml	5 x 5 ml	19711-55

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 Min-column to a chromatography system using the Lure 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.

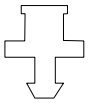


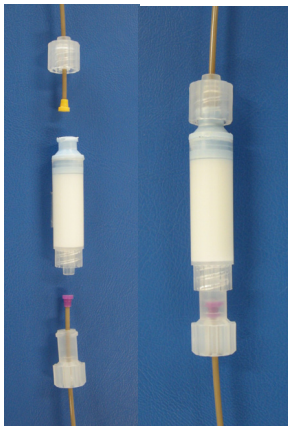
Fig.2 Female luer

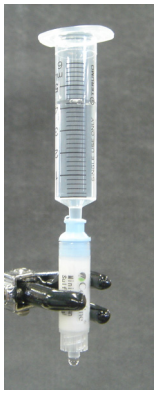
   (f) Connect tube with female luer.

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.