Operating Instructions:

Mini-column Cellufine ET clean (Ver.5.1)

1. Description

Cellufine® ETclean can remove endotoxin from a cellular product solutions at physiological pH, ionic concentration of 0.02-1.0 mol/L, and 0 - 25°C. The Mini-column Cellufine® ETclean L&S is a pre-packed, easy to use chromatography column for endotoxin removal. They are packed with Cellufine® ETclean L&S media which consist spherical, rigid cellulose beads with immobilized poly(ε-lysine). The poly(ε-lysine) gives the media unique chromatographic selectivity based on mixed mode interaction with cationic ligand groups and hydrophobic sites on the cellulose beads. Cellufine® ETclean is stable in cleaning solutions, which include 0.2 M sodium hydroxide and 2 M sodium chloride.

The poly(ε-lysine) is a microbial poly(amino) acid with 30-35 lysine residues produced by Streptomyces albulus. Both the poly(ε-lysine) as ligand and the cellulose beads act as matrix are products of JNC Corporation.

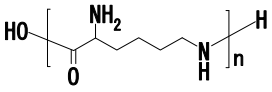

Fig.1 Structure of poly(ε-lysine)

2. Characteristics

ETclean has two grades. ETclean L has large pore size and it can remove endotoxine under 10pg/mL level. ETclean L tends to adsorb acidic protein in the solution of low salt concentration. ETclean S has small pore size and it provide 99% of recovery of proteins. ETclean S can remove endotoxine 10-80 pg/ml, the performance is depend on the samples

Name	pore size (exclusion limit)
Cellufine® ETclean S	2,000
Cellufine® ETclean L	≧2×10^6

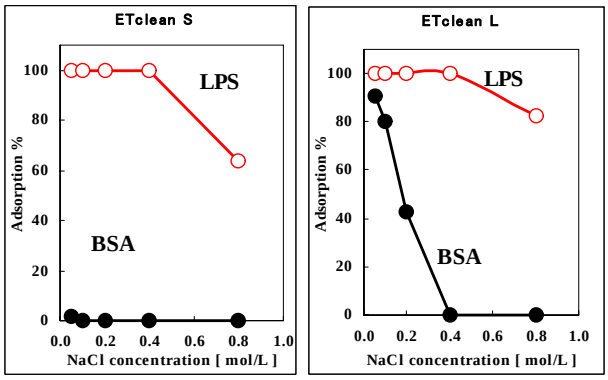

Fig.2 Selective adsorption of endotoxin (LPS) from a bovine serum albumin (BSA) solution by Cellufine® ETclean.

Selective adsorption of endotoxin was determined using a batchwise method with 0.2 g of the wet beads and 2 ml of a sample solution (BSA: 500 µg/ml, E. coli O111: B4 LPS: 100 ng/ml, pH 7.0, ionic conc. 0.05-0.8mol/L).

Table1. Selective removal of endotoxin from a protein solution by Cellufine® ETclean.

proteins	pI	before treatment LPS pg/mL	ETclean S after treatment LPS pg/mL	ETclean S after treatment Protein Recv.%	ETclean L after treatment LPS pg/mL	ETclean L after treatment Protein Recv.%
Ovalbumin	4.6	28,000	81	99	<10	95
BSA	4.9	32,000	45	99	<10	97
Myoglobin	6.8	4,500	18	99	<10	98
γ-globulin	7.4	5,600	20	99	<10	97
Cytochrome C	10.6	1,500	15	99	<10	98

The removal of endotoxin(LPS) was determined by a batchwise method with 0.3 ml of wet adsorbent and 2 ml of a protein solution (1 mg/ml) containing natural endotoxin.

Cellufine® Mini-columns are made of polypropylene tubes and polyethylene frits. The columns are fitted with luer adaptors to facilitate connection to a syringe, a peristaltic pump, or to a chromatography system.

Table 2. Mini-column Cellufine® ETclean Characteristics

Column volume	1ml and 5ml
Column dimensions (i.d. x h)	9mm x 18 mm (1ml) 13mm x 44mm (5ml)
Ligand	poly(ε-lysine)
Particle diameter	ca. 40-130 µm
Bead structure	Spherical Cellulose
Maximum back pressure	0.2 Mpa (1 ml)
Maximum flow rate	10 ml/min (1 ml)
Recommend flow rate	0.5-1 ml/min (1 ml)
Chemical stability	0.2 M NaOH/20-95% ethanol
Storage	+2 to +8 ºC in 20% ethanol

3. Operating Guidelines

General Operation

(1) Wash out the preservative with 5-10 mL of pure water.

(2) Regenerate the ETclean by washing 5-10 mL of 0.2 mol NaOH in 95% ethylalcohol, and let stand for 3 hours. (Note: ETclean must be regenerated before every use.)

(3) Wash out the regenerate solution with 5-10 mL of pyrogen-free buffer or water.

(4) Equilibrate column with adsorption buffer

(5) Load sample. (The sample should be adjusted to the composition of the adsorption buffer.)

(6) Collect the passing fraction. If needed, high ionic strength buffers may be used to aid in elution.

(7) Repeat steps 1, 2, and 3 to regenerate the ETclean.

4. Recommended Buffers

Adsorption buffer: 0.01-0.05 M sodium phosphate, Tris-HCl, containing 0.1-0.2 M NaCl, neutral pH. 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 selective elution of protein.

Elution buffer: If the sample is adsorbed on ETclean, the ionic strength of the buffer may be increased to elute the sample.

Regeneration buffer: 95%(v/v) of ethanol, containing 0.2 M NaOH. When using 20%(v/v) of ethanol containing 0.2M NaOH, it is need let stand overnight for regeneration.

5. Sample Preparation

Remove insoluble material by centrifugation or microfiltration. If necessary, exchange sample buffer using dialysis, diafiltration or desalting chromatography such as Cellufine® GH-25. Prepare samples at a concentration of 1 – 20 mg/ml in the adsorption buffer.

6. **Scaling Up**

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

7. **Storage**

Wash the column with 5 – 10 column volumes of 20% ethanol. Store the column in 20% ethanol at +2 to +8 ° C.
Note: To prevent leakage it is essential to ensure that the end plugs are tight.

8. **Reference**

The Cellufine® ETclean was developed jointly by Kumamoto University and JNC Corporation.

1) M. Sakata, M. Todokoro, C. Hirayama, American Biotechnol. Lab., 20 (2002) 36.
2) M. Todokoro, M. Sakata, S. Matama, M. Kunitake, J. Ohkuma, C. Hirayama, J. Liq. Chrom. & Rel. Technol., 25 (2002) 601.
3) Ivars Bemberis, Masayo Sakata, Chuichi Hirayama et al. BioPharm International, January 2005 pp 50-51 (www.biopharminternational.com)

10. ORDERING INFORMATION

Product	No. Supplied	Coed. No.
Mini-column Cellufine® ETclean L, 1 mL	5 x 1 ml	20051
Mini-column Cellufine® ETclean S, 1 mL	5 x 1 ml	20151
Mini-column Cellufine® ETclean L, 5 mL	1 x 5 ml	20015
Mini-column Cellufine® ETclean S, 5 mL	1 x 5 ml	20115
Cellufine® ETclean S	10 ml	681984324
Cellufine® GH-25	100 ml	670000327
Mini-column Cellufine GH-25	5 x 5ml	19711-55

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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 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.

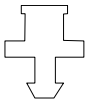


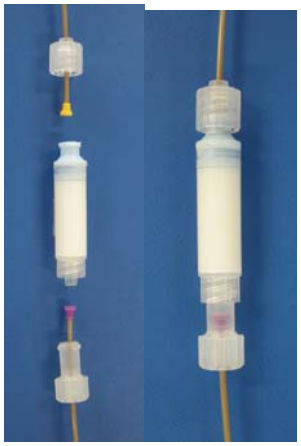
Fig.2 Female luer

   (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.

Cellufine™ is the trademark of JNC Corporation, Tokyo, Japan

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