JNC CORPORATION

Operating Instructions

Mini-column Cellufine ET clean S and L

Cellufine™

1. Description

Mini-column Cellufine ET clean 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 ET clean L&S is a pre-packed, easy to use chromatography column for endotoxin removal. They are packed with Cellufine ET clean 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 ET clean 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.

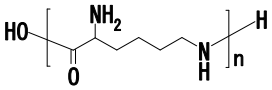

Fig. 1 Structure of poly (ε-lysine)

2. Characteristics

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

Name	Pore size (exclusion limit)
Cellufine ET clean S	2,000
Cellufine ET clean L	≧2×10^6

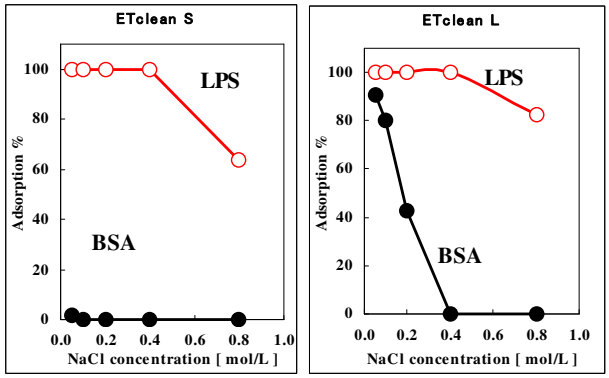

Fig. 2 Selective adsorption of endotoxin (LPS) from a bovine serum albumin (BSA) solution by Cellufine ETclean.

Selective adsorption of endotoxin was determined using a batch wise 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 – 0.8 mol/l).

Table 1. Selective removal of endotoxin from a protein solution by Cellufine ET clean.

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 batch wise method with 0.3 ml of wet adsorbent and 2 ml of a protein solution (1 mg/ml) containing natural endotoxin.

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 2. Mini-column Cellufine ETclean Characteristics

Column volume	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	poly(ε-lysine)
Particle diameter	ca. 40 – 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	2 – 14
Chemical stability	0.2 M NaOH/20 – 95% ethanol
Storage	Cool and dark place in 20% ethanol

3. **Operating Guidelines**

General Operation

(1) Wash out the preservative with 5 – 10 ml of pure water.
(2) Regenerate the ET clean by washing 5 – 10 ml of 0.2 mol NaOH in 95% ethylalcohol, and let stand for 3 hours. (Note: ET clean 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 ET clean.

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 ET clean, 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 0.2 M NaOH, it is need let stand overnight for regeneration.

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.

4. **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 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.

5. **Scaling Up**

Two or three of Cellufine ET clean 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**

The Cellufine ET clean 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)

8. **Further Information**

For further information, visit
http://www.jnc-corp.co.jp/fine/en/cellufine/index.html

9. **Ordering information**

Product	Quantity	Product number
Mini-column Cellufine ET clean L, 1 ml	5 x 1 ml	20051
Mini-column Cellufine ET clean S, 1 ml	5 x 1 ml	20151
Mini-column Cellufine ET clean L, 5 ml	1 x 5 ml	20015
Mini-column Cellufine ET clean S, 5 ml	1 x 5 ml	20115
Cellufine ET clean S	10 ml	681984324
Cellufine GH-25	100 ml	670000327
Mini-column Cellufine GH-25	5 x 5 ml	19711-55

10. **Contact us**

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