

Affinity Chromatography Media
(Endotoxin Removal)

Cellufine[®] ET clean

Technical Data Sheet



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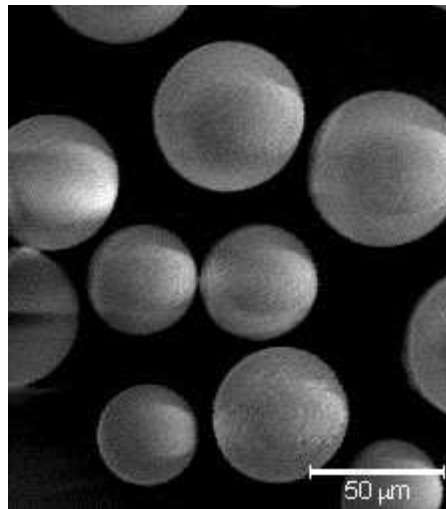
Cellufine web: <http://www.jnc-corp.co.jp/fine/en/cellufine/>

Introduction

The **Cellufine™ ETclean** is poly(ϵ -lysine) immobilized **Cellufine™** (cellulose spherical beads). The beads bind and remove endotoxin from your sample solution. The poly(ϵ -lysine) is a microbial poly(amino acid) that consist of 30-35 lysine residues produced by *Streptomyces albulus*. The poly(ϵ -lysine) as ligand and the cellulose beads act as matrix ands are products of Chisso Corporation.

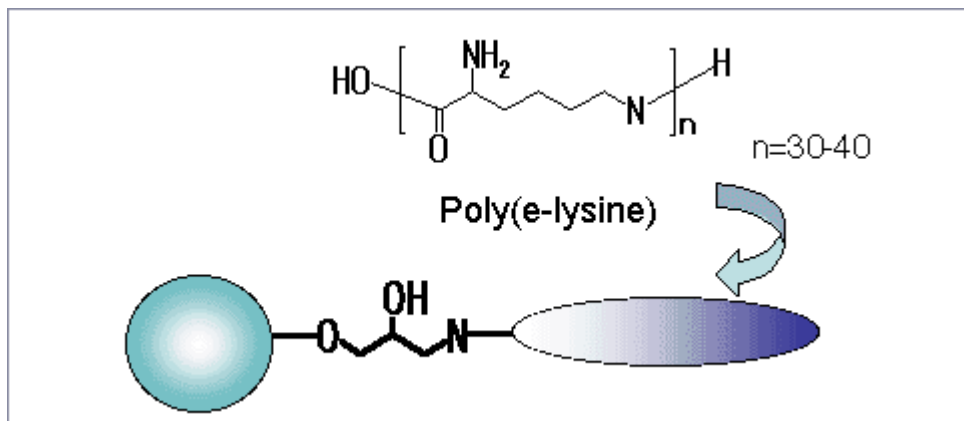
The **Cellufine™ ETclean** endotoxin removing beads were developed jointly by Kumamoto University and Chisso. The poly(ϵ -lysine) was immobilized onto chloromethylxirane-activated cellulose beads. The beads are a stable affinity beads that are resistant against the cleanup solutions, which include 0.2 M sodium hydroxide and 2 M sodium chloride.

The **Cellufine™ ETclean** can remove endotoxin from a cellular product solution at physiological pH, ionic strength of $\mu = 0.02-1.0$, and 0 -25C°.



Electron micrograph of
Cellufine™ ETclean-S beads.

Partial Structure

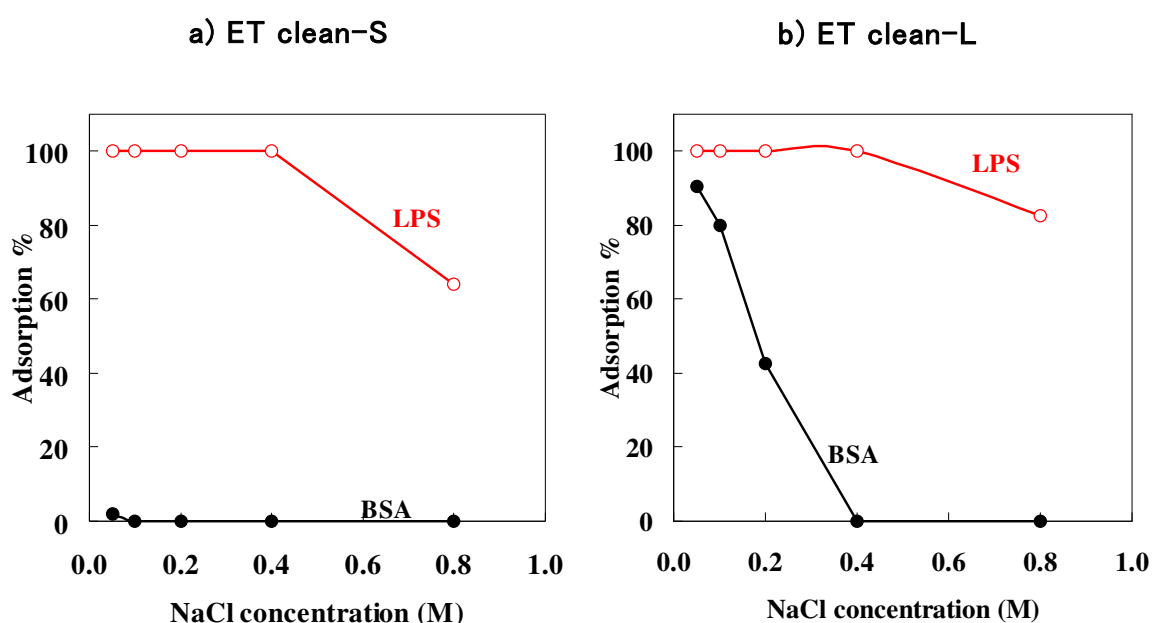


Characteristics

Name	Supplied	Wet Bead Diameter	Pore Size*
Cellufine ET clean S	a slurry in 20 % ethanol	ca. 40-130 μm	M_{lim} 2000
Cellufine ET clean L			$>M_{\text{lim}}$ 2×10^6

*The pore size (molecular weight exclusion; M_{lim}) of the beads was estimated from calibration curves obtained by size exclusion chromatography. Pullulan and maltose were used for the M_{lim} determination.

Selective adsorption of endotoxin (LPS) from a bovine serum albumin (BSA) solution by Cellufine ET clean media.



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 $\mu\text{g/ml}$, *E. coli* O111: B4 **LPS**: 100 ng/ml, pH 7.0, NaCl concentration of mol/L = 0.05-0.8).

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

Sample Solution		Cellufine ET clean S (NaCl = 0.05M, pH 7.0)		Cellufine ET clean L (NaCl = 0.4M, pH 7.0)		
Compound	pI	Concentration of endotoxin before treatment (pg/ml)	Concentration of endotoxin after treatment (pg/ml)	Recovery of protein after treatment (%)	Concentration of endotoxin after treatment (pg/ml)	Recovery of protein after treatment (%)
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

Application Data

Endotoxin Removal Example

BSA / ET clean L

Column chromatography

Column size : 1 X 1.1 cm (I.D.) (1.1ml)

Flow rate 0.17 ml / min (10cm / h)

Buffer 50 mM PB, pH 7 + 0.15 mol NaCl aq

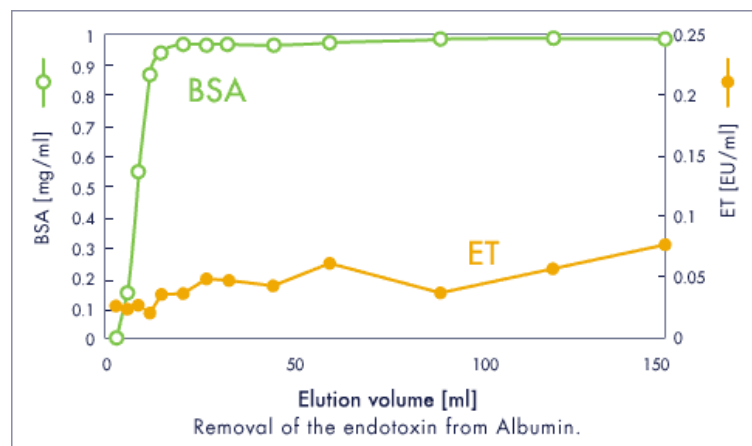
Assay

Protein Abs. at 280 nm

ET LAL rate assay

Injection sample (150 ml)

BSA 1 mg/ml ET 100 EU/ml



Lysozyme / ET clean L

Column chromatography

Column size 10 x 0.9 cm (I.D.) (9.6 ml)

Flow rate 0.5 ml / min (47 cm / h)

Buffer 1 mM Tris-HCl, pH 7.3

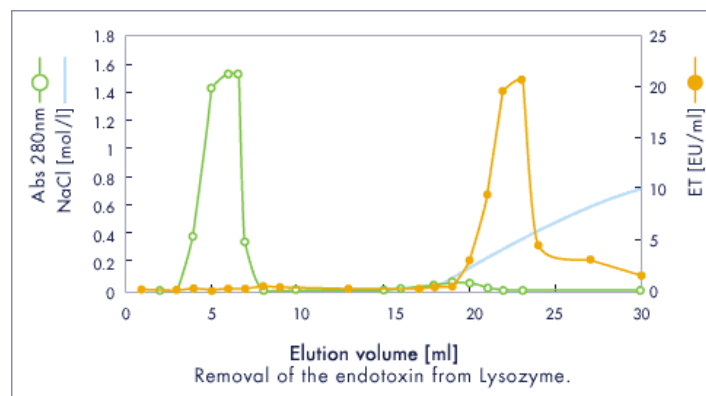
Gradient 0 → 1.0 mol / l NaCl aq.

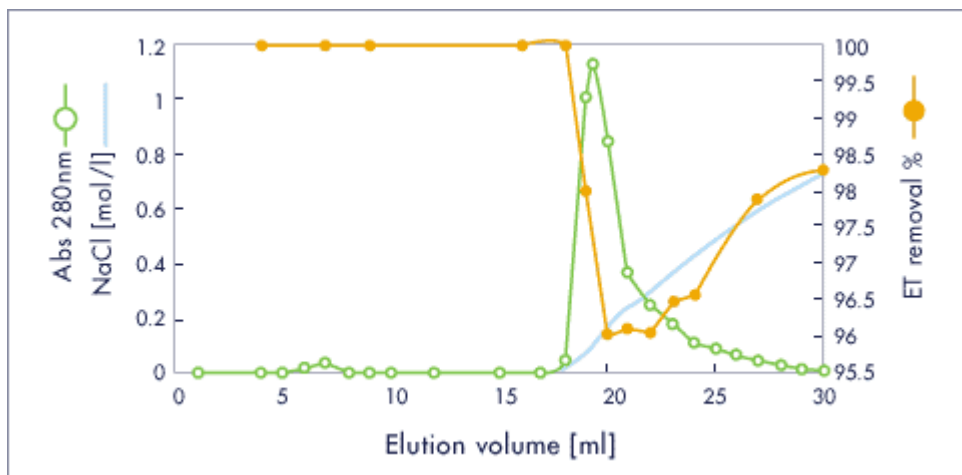
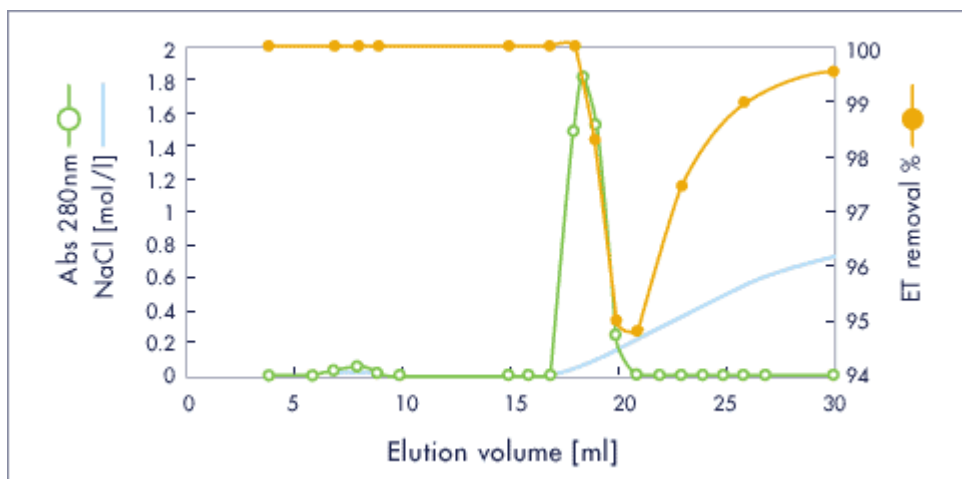
Assay

Protein Abs. at 280 nm

ET LAL rate assay

Injection sample (1ml) : 14 mg / ml



Insulin chain A / ET clean L
Injection sample (1ml) : 13 mg / ml, 309 EU / ml

Tranferrin / ET clean L
Injection sample (1ml) : 13 mg / ml, 2982 EU / ml

References

- 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.

Cellufine ET clean was developed by Kumamoto Univ & JNC Corp Joint Project.

Ordering Information

Cellufine ET clean L		Cellufine ET clean S	
Pack Size	Catalogue No.	Pack Size	Catalogue No.
Mini-Column 1ml x 5	20051	Mini-Column 1ml x 5	20151
Mini-Column 5ml x 1	20015	Mini-Column 5ml x 1	20115
10 ml	681 984 324	10ml	682 985 324
50 ml	681 984 326	100ml	682 985 326
500 ml	681 984 328	500 ml	682 985 328
5 Liters	681 984 330	5 Liters	682 985 330
10 Liters	681 984 335	10 Liters	682 985 335

Contact us

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