Affinity Chromatography Media
(Endotoxin Removal)

Cellufine® ET clean

Technical Data Sheet

JNC CORPORATION

Life Chemicals Division

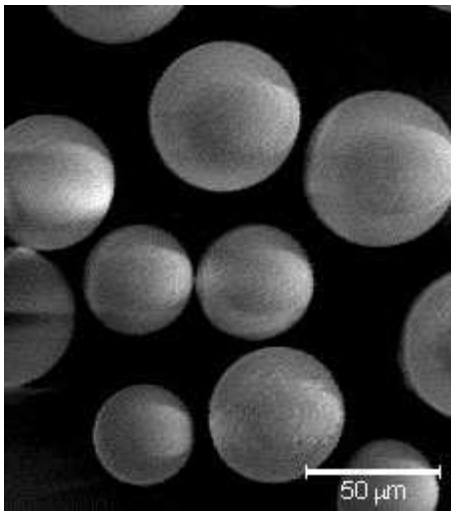
2-1, Otemachi 2-Chome
Chiyoda-ku, Tokyo 100-8105 JAPAN
Phone +81-3-3243-6150
Fax +81-3-3243-6219
e-mail: **cellufine@jnc-corp.co.jp**
https://www.jnc-corp.co.jp/fine/en/cellufine/

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.

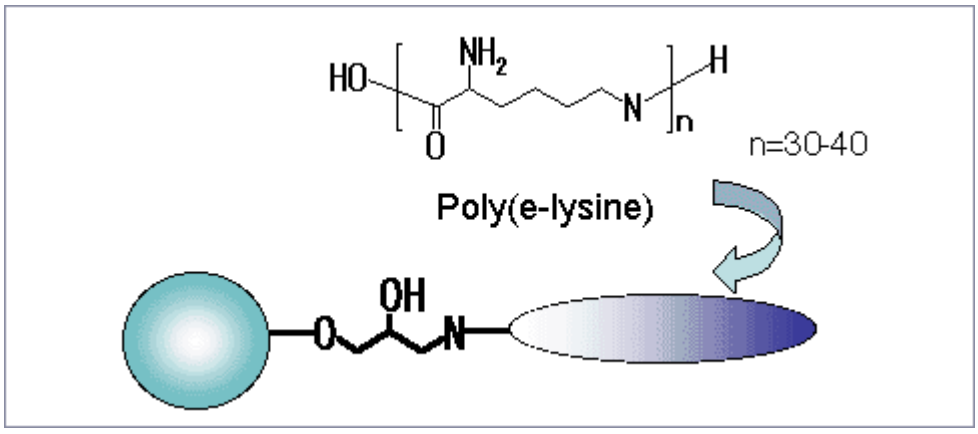
The **Cellufine™ ETclean** endotoxin removing beads were developed jointly by Kumamoto University and Chisso. The poly(ε-lysine) was immobilized onto chloromethyloxirane-activated cellulose beads. The beads are a stable affinity beads that are resistant against the cleanup solutions, which include 0.2 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 μ = 0.02-1.0, and 0 -25C°.



Electron micrograph of
Cellufine™ ETclean-S beads.

Partial Structure

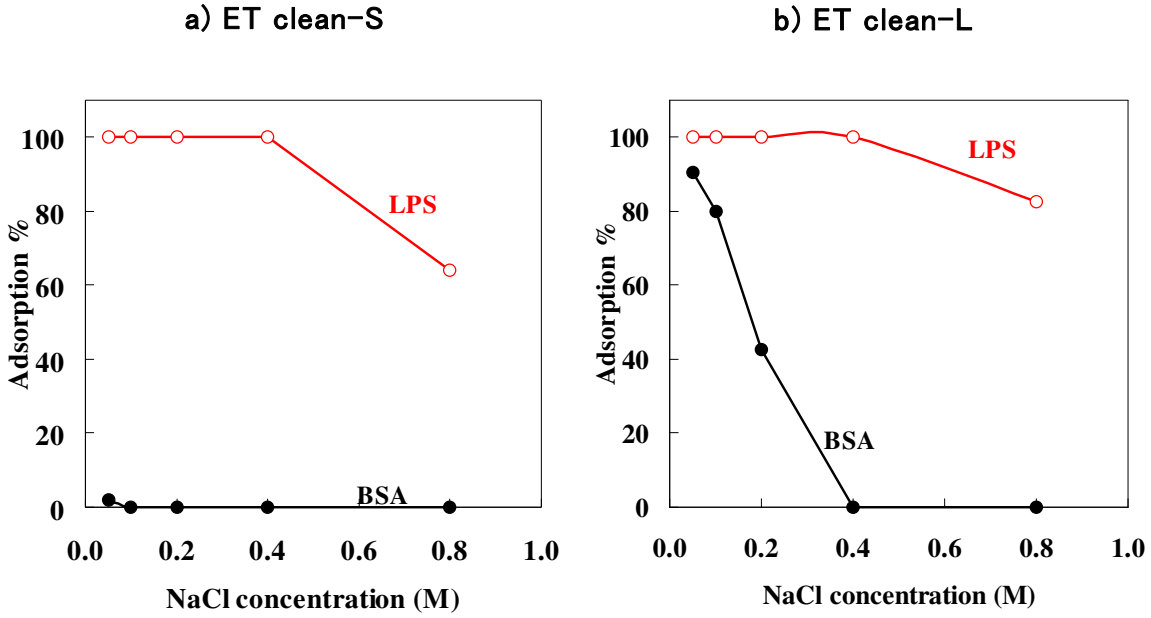


Characteristics

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_{lim} 2x10^6

*The pore size (molecular weight exclution; 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 μg/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**		**Cellufine ET clean L**	
Compound	pI	Concentration of endotoxin before treatment (pg/ml)	(NaCl = 0.05M, pH 7.0) Concentration of endotoxin after treatment (pg/ml)	(NaCl = 0.05M, pH 7.0) Recovery of protein after treatment (%)	(NaCl = 0.4M, pH 7.0) Concentration of endotoxin after treatment (pg/ml)	(NaCl = 0.4M, pH 7.0) 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

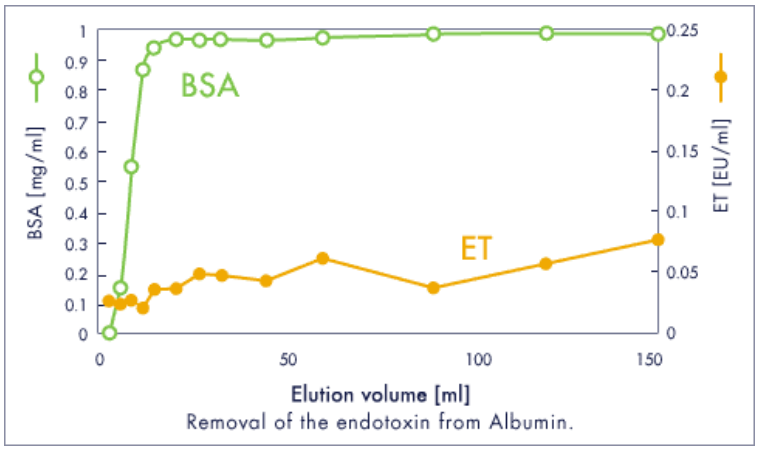
Endotoxin Removal Example

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



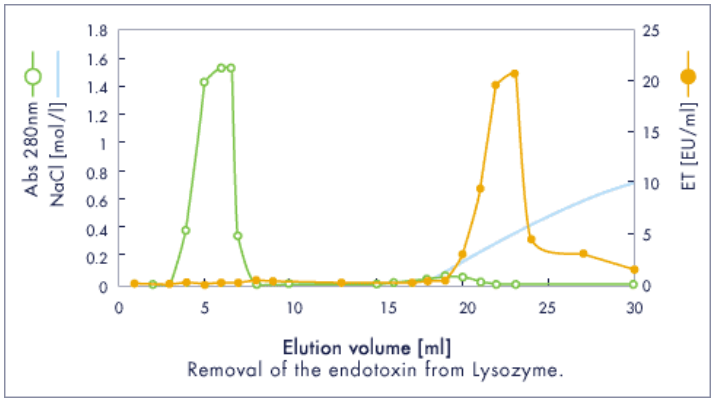
Removal of the endotoxin from Albumin.

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

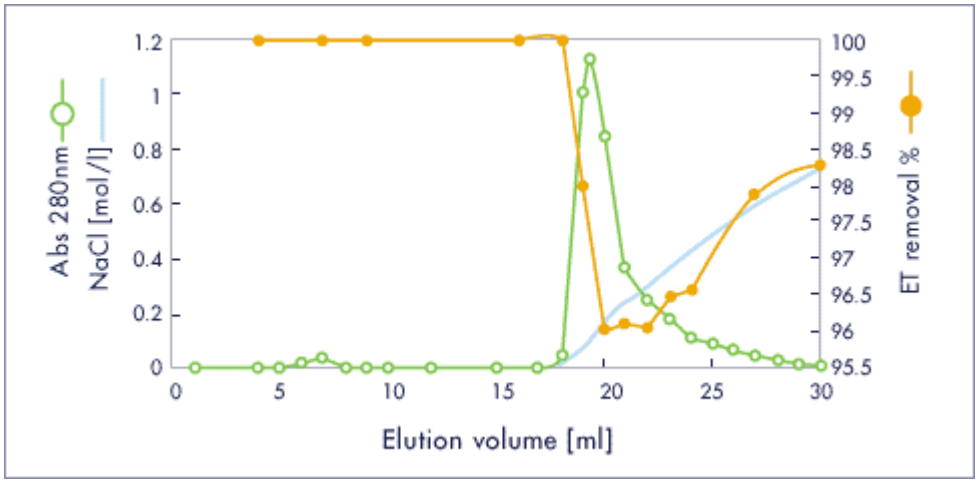
Injection sample (1ml) : 14 mg / ml



Removal of the endotoxin from Lysozyme.

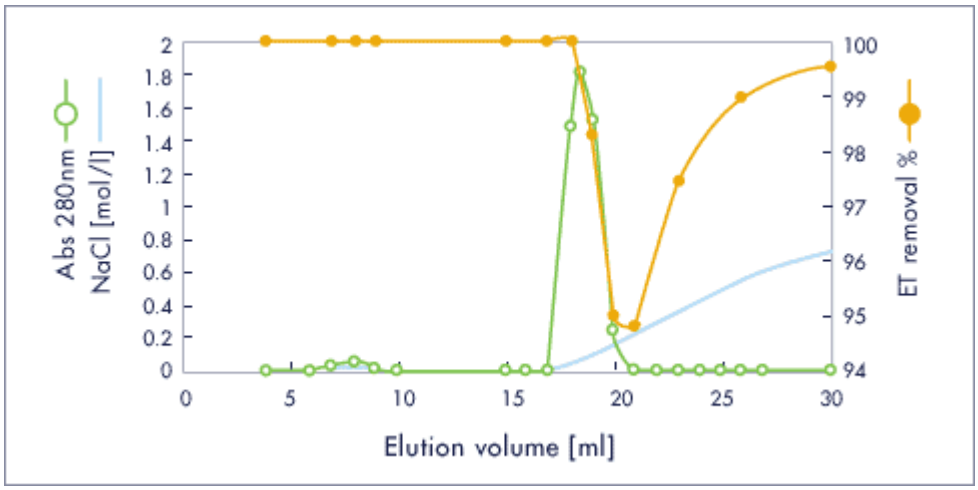
Insulin chain A / ET clean L

Injection sample (1ml) : 13 mg / ml, 309 EU / ml



Tranceferrin / 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

(Technical Support)

(North America & Europe)
JNC America Incorporated
411 Theodore Fremd Avenue, Suite 206
South Rye, NY 10580 USA
TEL: 914-921-5400
FAX: 914-921-8822
E-mail: cellufine@jncamericany.com

(Asia & Others)
JNC Corporation
Chemical Division Life Chemicals Department
2-1, Otemachi 2-Chome, Chiyoda-ku
Tokyo 100-8105 Japan
Tel: +81-3-3243-6150
Fax: +81-3-3243-6219
E-mail: cellufine@jnc-corp.co.jp

(Purchase Orders)

(North America & Europe)
JNC America Incorporated
411 Theodore Fremd Avenue, Suite 206
South Rye, NY 10580 USA
TEL: 914-921-5400
FAX: 914-921-8822
E-mail: cellufine@jncamericany.com

(Asia & Others)
JNC Corporation
Life Chemicals Division
2-1, Otemachi 2-Chome, Chiyoda-ku
Tokyo 100-8105 Japan
Tel: +81-3-3243-6150
Fax: +81-3-3243-6219
E-mail: cellufine@jnc-corp.co.jp