Operating Instructions

Affinity Chromatography Media for Endotoxin removal

Cellufine® ET clean

Description

The Cellufine ET clean 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 JNC Corporation Corporation.

Physical-Chemical Characteristics

Product Name	Supplied	Particle size	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.

Column Packing

1. Calculate volume required for the desired bed dimension.
2. Prepare a 40 – 60 % (v/v) slurry with water, 0.1 M NaCl solution or the appropriate buffer. Allow the gel to equilibrate at ambient temperature for one hour.
3. Gently stir or place under vacuum to degas.
4. With column outlet closed, carefully pour the slurry into column. Depending on the volume, a filler tube may be necessary.
5. With the inlet open to release air, insert and affix the top adjuster assembly at the slurry interface.
6. Open the column outlet and begin pumping elution buffer at rate 10 % – 20 % greater than the operational flow rate.
7. After the bed stabilizes, close the column outlet. Then with the inlet open, reposition the end cell on top of the bed. Equilibrate with 10 column volumes of adsorption buffer before sample loading.

Bed Compression

Compression factor (Cf) = Settled bed height* / Packing bed height

* Gravity settled volume (after 24 hours)

As Cellufine media are strong, it obtains good packing results to compress little.

Target compression

Cf of ET CleanS ≒1.1

Cf of ET CleanL ≒ 1.2

Supplementation (HETP & Asymmetry)

■HETP

•Well packed column HETPs will be in the range of 2 - 4 X average particle diameter

•For 75 µ beads, the HETP target is 0.015 to 0.030 cm

■Asymmetry

•A good working range is 0.8 - 1.5

•As < 1.0 typically indicates column packed to “hard”

•As > 1.0 typically indicates column flow restriction of some sort

Operating Guidelines

General Operation

1. CIP for endotoxin free. Wash the Cellufine ET clean column with 5 column volumes of alkali solution. It leaves as it is required time until endotoxin becomes free. Followed by wash with endotoxin-free water.

   ➢ Note

   Alkali solution and require time for endotoxin free.

Alkali solution	Require time for endotoxin free
0.2mol/l NaOH	16hr or over night
0.2mol/l NaOH in 20% EtOH	3 to 5 hr
0.2mol/l NaOH in 95%EtOH	1hr

2. Equilibrate the column with 5 column volumes of a suitable endotoxin-free buffer.
3. Apply sample through the column at a flow rate of 10 to 50cm/h at 4-25 ˚C.
4. Collect the effuluent and determine the endotoxin content of the effluent, as a sample solution after endotoxin-removing treatment.
5. The column can be reused after washing with a cleanup method of (1) to (2). We had already checked that the beads can be regenerated 5 times.

Recommended Buffers

Generally, 10mM to 50mM sodium phosphate buffer or Tris-HCl buffer, neutral pH can use it well. As long as a sample is stable, simply endotoxin-water is sufficient to use.

Since acid protein may be adsorbed on ET-clean, salt concentration is raised when protein adsorbs. By buffer pH lower than protein *pI*, the protein is hard to adsorb on ET-clean.

Sample Preparation and Load

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

The protein may not be recovered if protein is added to superfluous ET-clean by very low concentration and very small amount.

Selection guide

The endotoxin-adsorbing capacity of the Cellufine ET clean beads was strongly depend on “exclusion limit” (abbreviates to Mlim.) the adsorbing capacity increased from 500 to 1000 x 10^{-6}g (LPS from *E. coli* O111:B4) per ml of wet beads, while the M_{lim} increased from 2.0 x 10^3 to >2 x 10^6 at pH 7.0 and NaCl concentration of 0.17M. Althogh Cellufine ET clean-L, having the large M_{lim} of >2 x 10^6, show the greatest endotoxin-removing activity, ionic binding of components other than endotoxin may occur by entry of the components into the pore of the beads.

The beads must be selected as follows:

(1) To reduce endotoxin from a sample solution containing acidic protein with *p*I 4.0 - 6.5, you can use Cellufine ET clean-S beads with a small pore size at pH 5 - 7 and NaCl concentration of 0.1 - 0.4 M.

(2) To reduce endotoxin from a sample solution containing neutral or basic protein with *p*I 7.0 - 10.5, you can use Cellufine ET clean-L beads with a large pore size at pH 7 - 9 and NaCl concentration of 0.1 - 0.4 M

Storage

Short term (2 weeks or less), bulk and column can be stored in 1 M NaCl in neutral buffer at 2 – 4 ˚C. Longer term storage can be conducted under identical conditions; however, a preservative (e.g. 0.1 % formalin, 0.05 % chloreton or 0.02 % sodium azide) should be added to the buffer. Store at 2 – 8 ˚C. Do not freeze.

Shelf Lifetime:

10 years from date of manufacture

Batchwise Method

The Cellufine ET clean beads must be endotoxin-free. See “General Operation”.

(Example)

(1) Place 5 ml of Cellufine ET clean beads into a glass-buchner funnels with fritted disc (pore size: 30 micron-m). Add 25 ml of alkali solution in it, and suspend the mixture with a spatula. Stand the suspension for a required time until endotoxin becomes free and then remove the solution by vacuum.

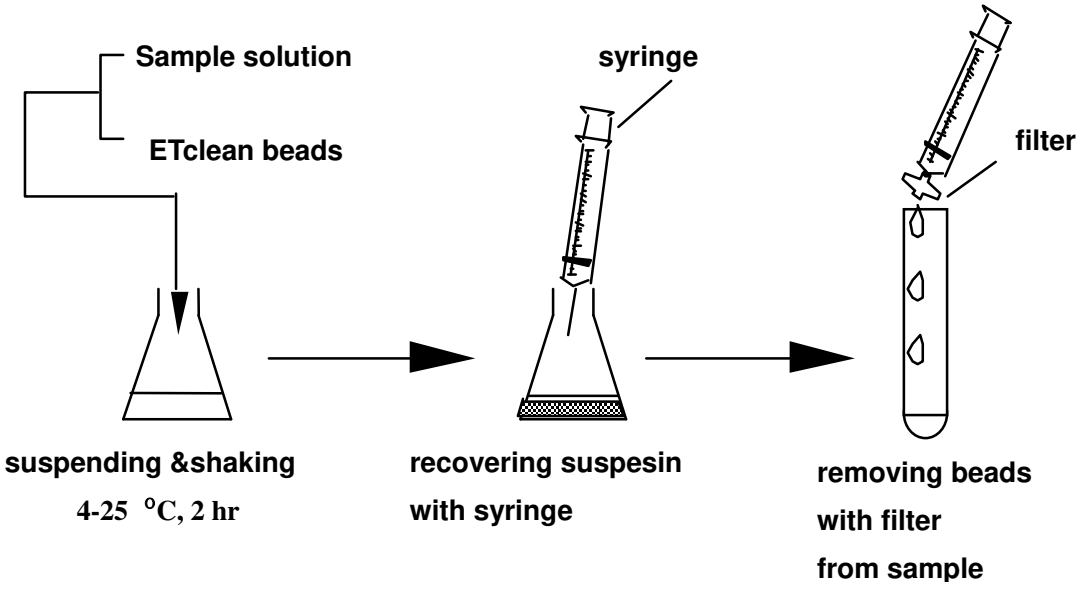
Alkali solution	Require time for endotoxin free
0.2mol/l NaOH	16hr or over night
0.2mol/l NaOH in 20% EtOH	3 to 5 hr
0.2mol/l NaOH in 95%EtOH	1hr

(2) Wash the beads, then, with other cleanup solutions (2 M NaCl e.q., endotoxin-free water, and then equilibrate buffer, respectively) by a similar method of (1).

(3) Suspend 0.2- to 0.4-g portion of wet adsorbent (after removing equilibrate buffer by vacuum) into a flask with 2 ml of sample solution. Shake the suspension for 2 h at 4-25 ˚C and filter it through a membrane filter (0.8 micron-m) to remove the beads.

(4) determine the endotoxin content of the filtrate obtained, as a sample solution after endotoxin-removing treatment.

(5) The beads can be regenerated before each use, with the washing method of (1) to (2).



supplement

ET clean L can easy to remove LPS from buffers.

ET clean L easily reduces LPS to low concentrations compared with polymyxin-immobilized agarose gel or DEAE Cellufine A-500(anion-exchanger).

Materials & Method

Column: 9mm I.D. x 100mm

Pump: Peristaltic with silicon tube

Buffer: 1M sodium phosphate, pH 7.0 (spiked 2.6 EU/ml LPS)

LPS Removal (pre-washing)

Column & media: wash with 5CV 0.2 M NaOH; let stand for 16 hours, then wash with endotoxin-free water.

Silicon tubing: wash with 0.5 M NaOH, let stand for 16 hours, then wash with endotoxin-free water.
※0.2 M NaOH-20% EtOH is more effective for endotoxin-free.

ChromatographyFlow rate : 30ml/h [Residence time 12.8min; linear velocity 47cm/h]
Fraction 6.36ml (total 128 tubes)

Assay

LAL (rate assay, EndospecyES-50M Set; SEIKAGAKU CORPORATION)

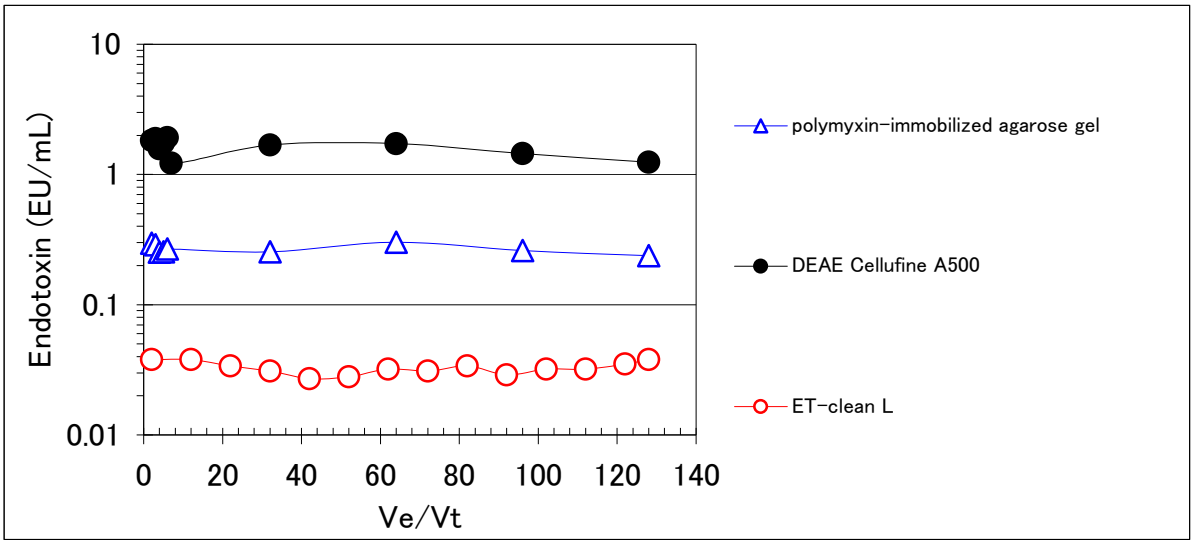


Fig. Comparison of the LPS removal capability from 1M phosphate buffer of ET-clean L, and polymyxin-immobilized agarose gel and DEAE Cellufine A-500.

Product 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
10ml	681 984 324	10ml	682 985 324
50ml	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

Cellufine ET clean was developed by the Joint Project of Kumamoto University & JNC Corporation Corporation.

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9. **Fair Labor Standards** - JNC Corporation represents that the Products or services provided hereunder were produced and/or performed in compliance with the requirements of all sections of the Fair Labor Standard Act of 1938, as amended.

10. **Equal Employment Opportunity** - JNC Corporation is an Equal Opportunity Employer. It does not discriminate in any phase of the employment process against any person because of race, color, creed religion, national origin, sex, age, veteran or handicapped status. The JNC Corporation Equal Opportunity Certificate, which is mailed annually to all vendors and vendees, is incorporated into this contract by reference.

11. **Modifications, Waiver, Termination** - This contract may be modified and any breach hereunder may be waived only by a writing signed by the party against whom enforcement thereof is sought.

12. **Governing Law** - This contract shall be governed by and construed in accordance with the laws (other than those relating to conflict of laws questions) of the Commonwealth of Japan.

13. **Arbitration** - Any and all disputes or controversies arising under, out of or in connection with this contract or the sale or performance of the Products shall be resolved by final and binding arbitration in Tokyo under the rules of the Japan Arbitration Association then obtaining. The arbitrators shall have no power to add to, subtract from or modify any of the terms or conditions of this contract. Any award rendered in such arbitration may be enforced by either party in either the courts of the Commonwealth of Japan District Court for the District of Japan, to whose jurisdiction for such purposes JNC Corporation and Buyer each hereby irrevocably consents and submits.