Affinity Chromatography Media
(Endotoxin Removal)

**Cellufine® ET clean**

Technical Data Sheet

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**JNC CORPORATION**

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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 chloromethylloxirane-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 -25°C.

![Electron micrograph of Cellufine™ ETclean-S beads.](image)

Partial Structure

![Partial Structure of Cellufine™ ETclean-S beads.](image)
Characteristics

<table>
<thead>
<tr>
<th>Name</th>
<th>Supplied</th>
<th>Wet Bead Diameter</th>
<th>Pore Size*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellufine ET clean S</td>
<td>a slurry in 20% ethanol</td>
<td>ca. 40-130 μm</td>
<td>M&lt;sub&gt;lim&lt;/sub&gt; 2000</td>
</tr>
<tr>
<td>Cellufine ET clean L</td>
<td></td>
<td></td>
<td>M&lt;sub&gt;lim&lt;/sub&gt; &gt;2×10&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*The pore size (molecular weight exclusion; M<sub>lim</sub>) of the beads was estimated from calibration curves obtained by size exclusion chromatography. Pullulan and maltose were used for the M<sub>lim</sub> determination.

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

a) ET clean-S

![Graph a) ET clean-S](image)

b) ET clean-L

![Graph b) ET clean-L](image)

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 ET clean beads.

<table>
<thead>
<tr>
<th>Compound</th>
<th>pI</th>
<th>Concentration of endotoxin before treatment (pg/ml)</th>
<th>Concentration of endotoxin after treatment (pg/ml)</th>
<th>Recovery of protein after treatment (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovalbumin</td>
<td>4.6</td>
<td>28,000</td>
<td>81</td>
<td>99</td>
</tr>
<tr>
<td>BSA</td>
<td>4.9</td>
<td>32,000</td>
<td>45</td>
<td>99</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>6.8</td>
<td>4,500</td>
<td>18</td>
<td>99</td>
</tr>
<tr>
<td>γ-globulin</td>
<td>7.4</td>
<td>5,600</td>
<td>20</td>
<td>99</td>
</tr>
<tr>
<td>Cytochrome C</td>
<td>10.6</td>
<td>1,500</td>
<td>15</td>
<td>99</td>
</tr>
</tbody>
</table>

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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

![BSA and ET elution profile](image)

**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

![BSA and ET elution profile](image)
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


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

<table>
<thead>
<tr>
<th>Cellufine ET clean L</th>
<th>Cellufine ET clean S</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pack Size</strong></td>
<td><strong>Catalogue No.</strong></td>
</tr>
<tr>
<td>Mini-Column 1ml x 5</td>
<td>20051</td>
</tr>
<tr>
<td>Mini-Column 5ml x 1</td>
<td>20015</td>
</tr>
<tr>
<td>10 ml</td>
<td>681 984 324</td>
</tr>
<tr>
<td>50 ml</td>
<td>681 984 326</td>
</tr>
<tr>
<td>500 ml</td>
<td>681 984 328</td>
</tr>
<tr>
<td>5 Liters</td>
<td>681 984 330</td>
</tr>
<tr>
<td>10 Liters</td>
<td>681 984 335</td>
</tr>
</tbody>
</table>

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