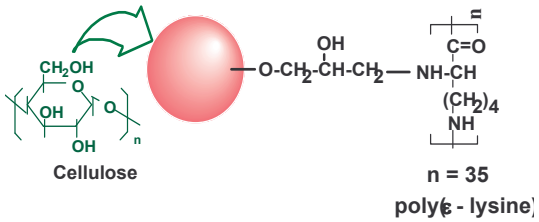
Endotoxine Removal

Cellufine ETClean

Cellufine is the liquid chromatography media for the purification of proteins, enzymes and other bio-active substance. Since it is made from spherical cellulose particles having high chemical stability, high mechanical strength and bio-compatibility, it is suitable for the production in pharmaceutical and food industry. And the leaking from this matrix is much less than that from the synthetic polymer media.



Beads Structure

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 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°.

Two types of resins are available ; **ETclean-S** and **ETclean-L.**

ETclean-S has a small pore structure to exclude proteins and other macromolecules.

ETclean-L has a large pore structure that permits proteins and large endotoxin aggregates access to the matrix surface as well as the internal pores.

The type of resin used depends on the amount of endotoxin contained within the samples.

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

Sample solution		Cellufine ET clean S (μ = 0.05, pH 7.0)		Cellufine ET clean L (μ = 0.40, pH 7.0)	
Compound	Concentration of endotoxin before treatment (pg/ml)	Concentration of endotoxin after treatment (pg/ml)	Recovery of protein after treatment (pg/ml)	Concentration of endotoxin before treatment (pg/ml)	Recovery of protein after treatment (pg/ml)
Ovalbumin	28000	81	99	<10	95
BSA	32000	45	99	<10	97
Myoglobin	4500	18	99	<10	98
□-Globulin	5600	20	99	<10	97
Cytochrome C	1500	15	99	<10	98

Adsorbing capacity of Endotoxin

Cellufine ET clean S 185 μg Endotoxin/ml of gel
Cellufine ET clean L 480 μg Endotoxin/ml of gel

Instructions for Use

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 Etclean, 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 buffers using dialysis, diafiltration or desalting chromatography such as Cellufine GH-25. Prepare samples at concentration of 1-20 mg/ml in the adsorption buffer.

[Batchwise Method]

The Cellufine ET clean beads must be washed before use with about 20 volumes of adsorbent of 0.2 M NaOH e.q., followed by 2 M NaCl e.q., endotoxin-free water, and then PBS, respectively, by a batchwise method.

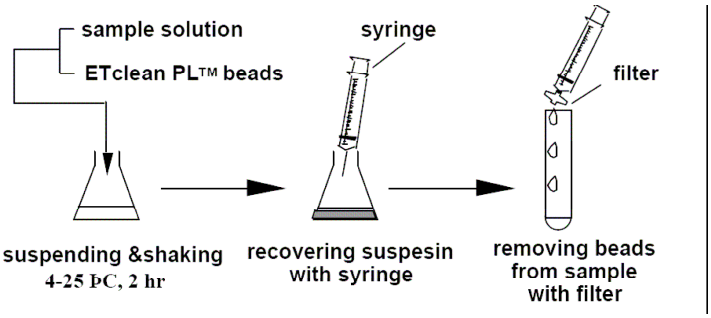
Standard Protocole
(1) Place 5 ml of Cellufine ET clean beads into a glass-buchner funnels with fritted disc (pore size : 30 µm). Add 25 ml of 0.2 M NaOH solution in it, and suspend the mixture with a spatula. Stand the suspension for a few minutes and then remove the solution by vacuum. Repeat this washing assay 4-5 times.

(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 Millipore filter (0.8 µm) 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).



Column Method

(1) Wash the Cellufine ET clean column with 5 column volumes of 0.2 M NaOH e.q., followed by 2 M NaCl e.q., and then endotoxin-free water, respectively.
(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 0.1-0.2 ml/min at 4-25 °C.
(4) Collect the effluent 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.

Sample Conditions

The endotoxin-adsorbing capacity of the Cellufine ET clean beads was strongly depend on Mlim: the adsorbing capacity increased from 500 to 1000 µg (LPS from E. coli O111:B4) per ml of wet beads, while the Mlim increased from $2.0x10^3$ to $>2x10^6$ at pH 7.0 and ionic strength of µ=0.17. Although Cellufine ET clean L, having the large Mlim of $>2x10^6$, 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 follow :

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

(2) To reduce endotoxin from a sample solution containing neutral or basic protein with pI 7.0-10.5, you can use Cellufine ET clean-L beads with a large pore size at pH 7-9 and ionic strength of 0.1-0.4.

Tolerance : To stock the beads, keep each beads (in 25% ethanol) in the fridge at 4°C. The beads must be regenerated before each use, including first time use.

Produit	Quantité	Référence
Cellufine ETclean L 53-125 µm	**10 ml**	**681984324**
Cellufine ETclean L 53-125 µm	**50 ml**	**681984326**
Cellufine ETclean S 44-105 µm	**10 ml**	**682985324**
Cellufine ETclean S 44-105 µm	**50 ml**	**682985326**

for research use only, not intended for diagnostic use.

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