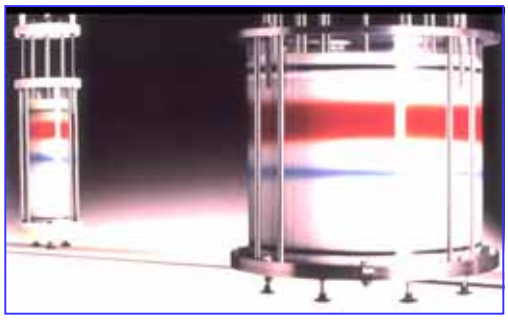
Affinity Chromatography Media

Cellufine® Sulfate

Technical Data Sheet



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Cellufine® Sulfate

For concentration, purification and depyrogenation of virus, viral/microbial antigens and heparin-binding proteins

Advances in vaccines and clinical diagnostics have created an increasing demand for large volumes of highly purified and concentrated virus and viral or microbial antigens. Cellufine Sulfate affinity media is a simple, rapid and effective means for concentration, purification and depyrogenation of these important products.

Cellufine Sulfate eliminates cumbersome, time-consuming and potentially unsafe classical ultra-centrifugation and density gradient methods. It can also provide a significant improvement in concentration and purity. Cellufine Sulfate can reduce or eliminate the expense, ligand leakage and reproducibility problems associated with immobilized dextran sulfate, chondroitin sulfate or heparin.
Elution of the bound product is affected through simple stepwise or gradient increases in ionic strength.

Features

- Affinity for a wide range of live, killed or disrupted viruses, viral or microbial antigens and heparin-binding proteins.
- Closed column operation assures safety and product sterility Endotoxins do not bind, allowing a rapid and contaminant free depyrogenation.
- Rigid, high-strength beads.
- Autoclavable

Benefits

- More effective than ultracentrifugation at removing contaminants from culture media and host cells
- Avoids excessive product handling and safety concerns, particularly with viral preparations
- Simultaneous concentration and purification improve yield, reduce processing steps, time and costs
- Gentle binding and elution conditions provide high capacity and product yield
- Resists compression, providing rapid flow for high-speed processing, even in large columns, making it easily scalable
- Resistant to chemical depyrogenation with base and chemically sterilizable with formalin

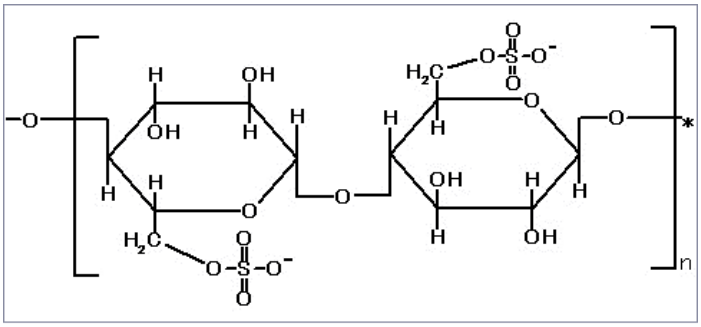

Figure 1
Partial Structure of Cellufine Sulfate

Characteristics	
Support Matrix	Cellulose
Particle Size	ca. 40 – 120 µm
Particle Shape	Spherical
Gel Exclusion Limit	ca. 3kD
Activated Group	Sulfate Ester
Total Sulfur	>700 µg/g dry
Protein Binding Capacity:	
Lysozyme	>3 mg/ml
Hepatitis B Surface Antigen	7 mg/ml
Environmental Resistance	Resistant to 0.1M NaOH, 0.1 % of 37 % Formalin
Operating Pressure	<2 bar (30 psi) In suspension at neutral pH;
Autoclavable	30 min at 121 °C
Supplied	Suspension in 20 % Ethanol

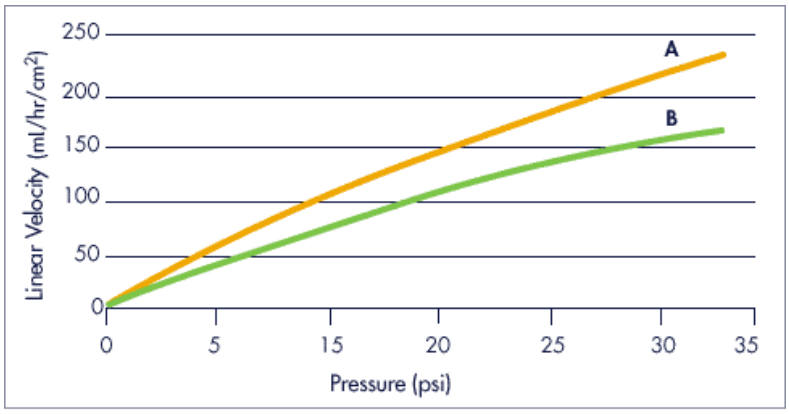

Figure 2
Pressure/Flow Curves

The nearly rigid properties of the spherical cellulose support matrix allow outstanding flow properties, particularly in large production columns.

Column A: 90 x 200 mm
Column B: 350 x 200 mm

Virus, Viral/Microbial Antigens

Viruses	Viral/Microbial Agents
• Rabies • Influenza • Japanese Enchephalitis • Feline Leukemia • Feline Herpes • Feline Calicivirus • Respiratory Syncytial Virus • Human Herpes Simplex • Human Measles • Human Parainfluenza	• Herpes Simplex gA and gB Glycoprotein Subunits • Hepatitis B Surface Antigen • Filamentous Hemagglutinin from *B. pertussis* • Leucocytosis Promoting Factor Hemagglutinin

Table 1
There are many applications of Cellufine Sulfate in the concentration or purification of viral and microbial antigens, proteins and viruses.

Purification of Rabies Virus

The example in Figure 3 illustrates the high degree of concentration, purification and yields obtained with Cellufine Sulfate on typical viral preparations.

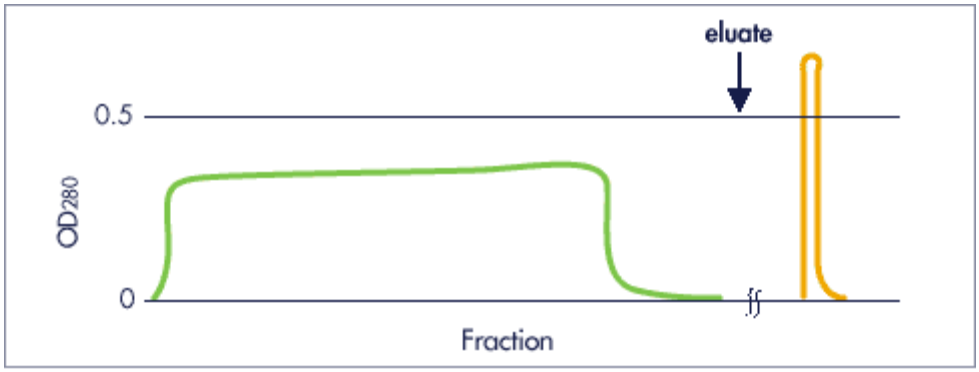

Figure 3
Purification of Rabies virus from chick embryo tissue culture fluid

Column : 50 x 70 mm (140 ml)
Buffer : 0.01M Phosphate (pH 7.2)
Eluant : 1M NaCl/0.01M Phosphate (pH 7.2)

	Load	Eluate
Volume (ml)	4,200	50
Virus titer	32	4,096
Protein (μg/ml)	8.5	14
Yield (%)	100	152
Purification factor		79x
Concentration factor		126x

Table 2
Concentration and Purification of virus with Cellufine Sulfate

Purification of Influenza Virus

Hen's egg allantoic fluid was loaded directly onto a 33.3 mL gel bed and 94.5% virus was recovered in the eluate fraction.

	Volume (ml)	Virus Titer	TCA-N μg/ml	Recovery (%)	Fold Purification
Allantoic Fluid	4200	77	337.1	100	1
Wash	6700	1	209.2	2.1	-
Eluate	170	1797	448.0	94.5	20.1

Table 3
Purification of Influenza virus from hen's egg allantoic fluid

Column : 50 x 170 mm
Buffer : 0.01M Phosphate pH 7.4
Wash : 0.01M Phosphate pH 7.2 + 0.2M NaCl
Elution : 0.01M Phosphate pH 7.0 + 1.5M NaCl

Antigenic Protein Purification and Depyrogenation

Cellufine Sulfate is ideal for depyrogenating virus and other microbial extracts because it does not bind endotoxins. Figure 4 shows the purification of filamentous hemagglutinin (FHA) from the whooping cough bacterium Bordetella pertussis.

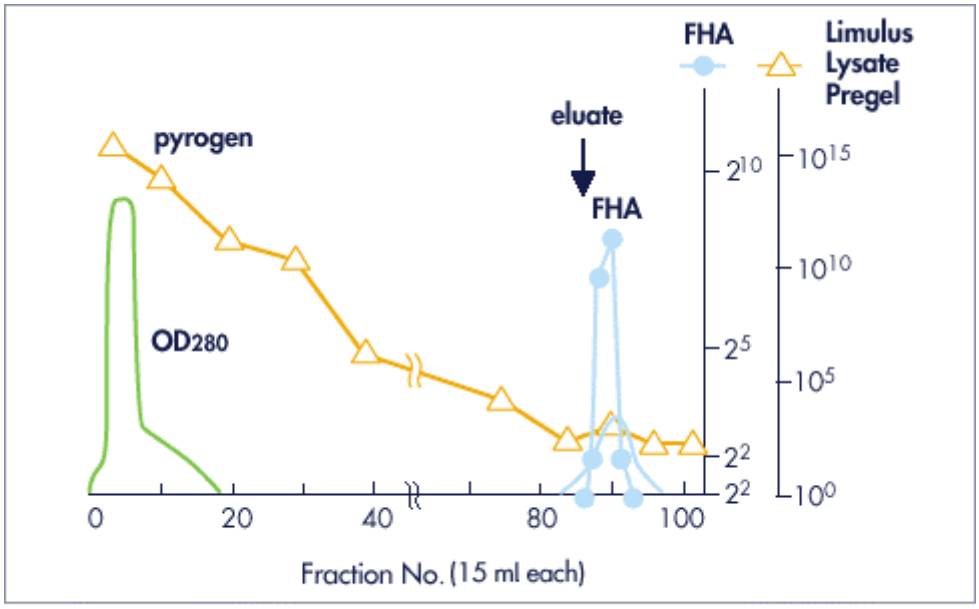

Figure 4
Purification of filamentous hemagglutinin from B. pertussis

Column :	16 x 70 mm (20 ml)
Sample :	800 ml B. pertussis culture fluid (endotoxin titer > 1015 by Limulus lysate test)
Buffer :	0.01M Phosphate (pH 7.6)
Eluant :	1M NaCl/0.01M Phosphate (pH 7.6)
FHA Yield :	94%
Purification Factor :	20x
Concentration Factor :	28x (30 ml product)
Endotoxin :	Below standard level by Limulus lysate, rabbit pyrogen and mouse toxicity tests

Protein Purification

Cellufine Sulfate mimics the affinity of heparin or dextran sulfate for many proteins. It can function as an affinity support for selected plasma proteins, cellular growth factors and lipases. Its capacity is comparable to conventional heparin gels.

Binding Proteins	Non-binding Proteins
• Antithrombin III • β-Lipoprotein • Complement C5, C6, C8 • Complement C3 Activator • Tryspin • Tryspin Inhibitor • Chymotrypsinogen • Lysozyme • Urease • Catalase • Factor IX	• Albumin • α-Lipoprotein • Complement C3, C9 • Complement C1, C3b Inactivators • IgG • Ceruloplasmin • α2-Macroglobulin • RNase • Bacitracin • Glucose Oxidase
Binding and elution are extremely rapid and very fine separations can be generated in gradient mode.	

Table 4

Purification of Partially Purified Casein Kinase II from Calf Thymus

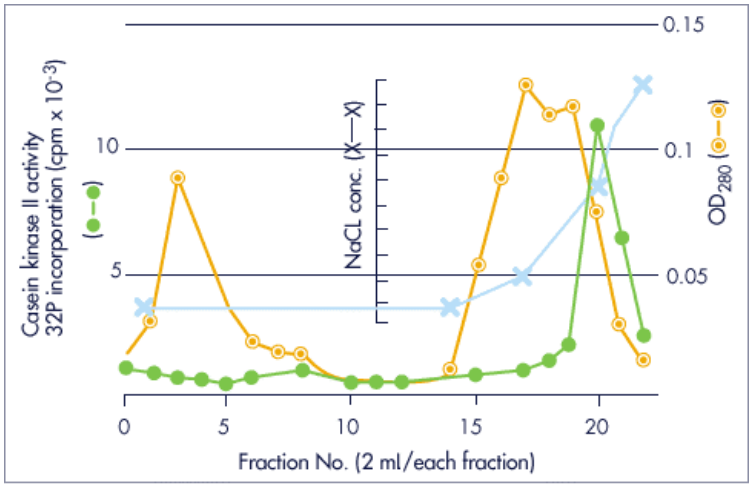

Figure 5

Column : 10 x 20 mm
Sample : 7 ml
Buffer : 50mM Tris-HCl (pH 7.9)
+ 50mM $MgCl_2$
+ 0.1mM EDTA
+ 0.1mM PMS
+ 0.5mM DTT
+ 25 % glycerol
Eluant : 0.05 – 1.0M NaCl in buffer

Ordering Information

Ordering Information		
Description	Quantity	Catalogue No.
Cellufine Sulfate	5 x 1ml (mini-column)	19845-51
	10 ml	676 943 324
	50 ml	19845
	500 ml	19846
	5 Liters	19847
	10 Liters	19849

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