Affinity Chromatography Media

Matrex® Cellufine™ Sulfate

For Concentration, Purification and Depyrogenation of Virus, Viral/Microbial Antigens, 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. Matrex Cellufine Sulfate affinity media is a simple, rapid and effective means for concentration, purification and depyrogenation of these important products.

Matrex 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. Matrex Cellufine Sulfate offers flow properties superior to hydroxyapatite and can reduce or eliminate the expense, ligand leakage and reproducibility problems associated with immobilized dextran sulfate, chondroitin sulfate or heparin.

Matrex Cellufine Sulfate consists of a rigid spherical cellulose matrix of 3,000 Dalton exclusion limit, with a low concentration of sulfate ester functionality on the 6-position of cellobiose (see Figure 1). This low density of cation exchange groups provides affinity binding properties for a wide range of viruses and antigens. In normal physiological buffers, virus particles or viral/microbial antigens bind to the surface of the beads due to an affinity interaction. Pyrogens and most contaminating proteins, as well as nucleic acids and endotoxins, pass through the column unbound.

Elution of the bound product is effected through simple stepwise or gradient increases in ionic strength. Additionally, Matrex Cellufine Sulfate is similar enough to heparin and dextran sulfate to simulate these materials in many protein affinity purification applications.

A wide variety of lipid-enveloped viruses have been found to bind to the gel, including Influenza, Hepatitis B, Enchephalitis (Japanese), Herpes, Rabies.

FEATURES

- Affinity for a wide range of live, killed or disrupted viruses, viral or microbial antigens and heparin-binding proteins.
- More effective than ultracentrifugation at removing contaminants from culture media and host cells.
- Closed column operation assures safety and product sterility.
- Avoids excessive product handling and safety concerns, particularly with viral preparations.
- Endotoxins do not bind, allowing a rapid and contaminant free depyrogenation.
- Simultaneous concentration and purification improve yield, reduce processing steps, time and costs.
- Gentle binding and elution conditions provide high capacity and product yield.
- Rigid, high-strength beads resist compression, providing rapid flow for high-speed processing, even in large columns, making it easily scaleable.
- Autoclavable, resistant to chemical depyrogenation with base and chemically sterilizable with formalin.

MILLIPORE

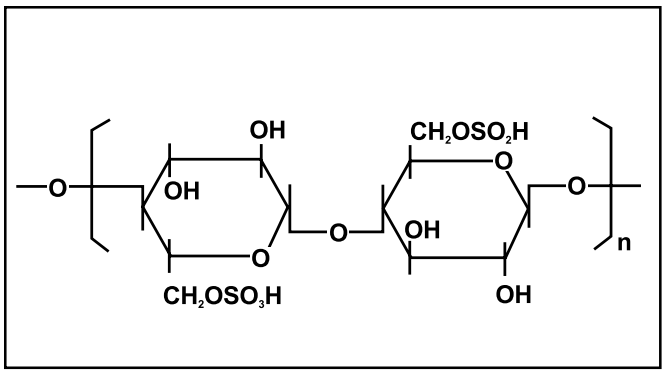

Figure 1
Partial Structure of Cellufine Sulfate

CHARACTERISTICS

Support Matrix:	Cellulose
Particle Size:	44 - 105µm
Particle Shape:	Spherical
Gel Exclusion Limit:	3kD
Activated Group:	Sulfate Ester
Total Sulfur:	>700µg/g dry
Protein Binding Capacity	
Lysozyme:	>3mg/ml
Hepatitis B Surface Antigen:	7mg/ml
Environmental Resistance:	Resistant to 0.1M NaOH, 0.1% of 37% Formalin
Operating Pressure:	<2 bar (30 psi)
Autoclavable:	In suspension at neutral pH: 30 min at 121˚C
Supplied:	Suspension in 20% Ethanol

FLOW PROPERTIES

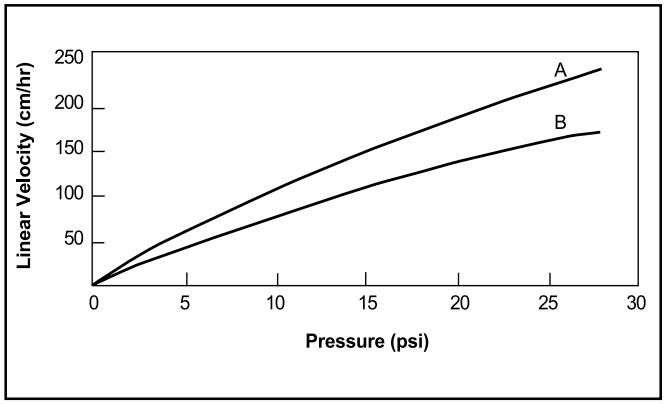

Figure 2
Pressure/Flow Curves

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

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

APPLICATIONS

Virus, Viral/Microbial Antigens

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

Viruses Rabies* Influenza* Japanese Enchephalitis* Feline Leukemia Feline Herpes Feline Calicivirus Respiratory Syncytial Virus Human Herpes Simplex Human Measles Human Parainfluenza
Viral/Microbial Antigens Herpes Simplex gA and gB Glycoprotein Subunits* Hepatitis B Surface Antigen Filamentous Hemagglutinin from *B. pertussis** Leucocytosis Promoting Factor Hemagglutinin*
* These applications are covered by US and foreign process patents. Please inquire regarding details and licensing arrangements.

Table 1

Purification of Rabies Virus

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

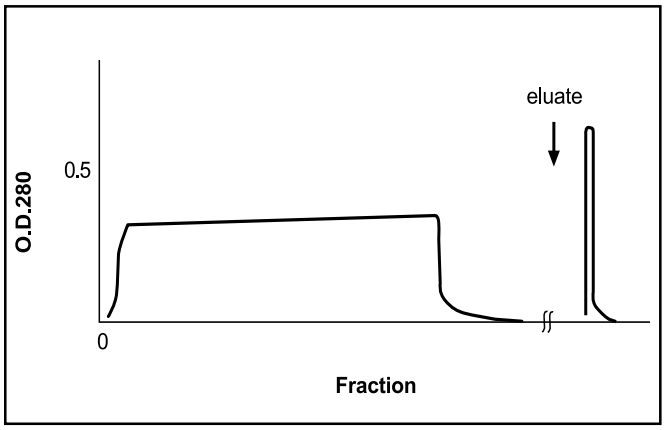

Figure 3
Purification of Rabies virus from chick embryo tissue culture fluid

Column: 50 x 70mm (140ml)
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 (ug/ml)	8.5	14
Yield (%)	100	152
Purification Factor	-	79x
Concentration Factor	-	126x
Concentration and Purification of Virus with Cellufine Sulfate		

Table 2

Purification of Influenza Virus

Hen's egg allantoic fluid was loaded directly onto a 333ml gel bed and 94.5% virus was recovered in the eluate fraction.

	Volume ml	Virus titer	TCA-N ug/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

Column: 50 x 170mm
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

Matrex 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*.

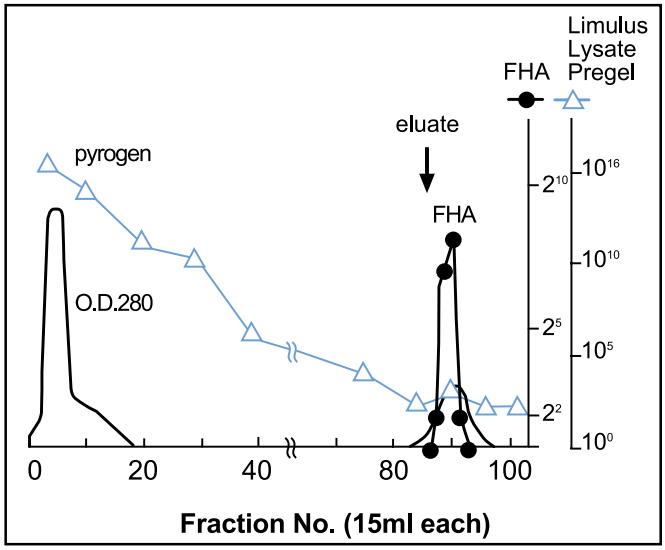

Figure 4
Purification of filamentous hemagglutinin from B. pertussis

Column: 16 x 70mm (20ml)
Sample: 800ml *B.pertussis* culture fluid (endotoxin liter > 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: 20 x
Concentration Factor: 28x (30ml product)
Endotoxin: Below standard level by *Limulus* lysate, rabbit pyrogen and mouse toxicity tests

Protein Purification

Matrex 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

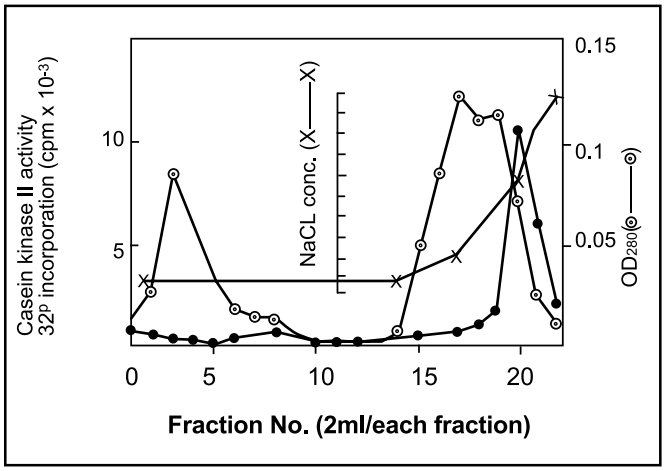

Figure 5

Column: 10 x 20mm
Sample: 7ml
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

Type	Quantity	Catalogue No.
Matrex Cellufine Sulfate	50ml	19845
Matrex Cellufine Sulfate	500ml	19846
Matrex Cellufine Sulfate	5 liters	19847
Matrex Cellufine Sulfate	10 liters	19849
Matrex Cellufine Sulfate	20 liters	19848

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