amsbio

Cellufine®

LIQUID CHROMATOGRAPHY MEDIA

- ✓ION EXCHANGE
- ✓AFFINITY
- ✓HYDROPHOBIC INTERACTION
- ✓GEL FILTRATION

CELLUFINE®
MINI-COLUMN

An effective tool for protein purification

Cellufine® Mini-Columns offer convenience and expedience for isolating, purifying and concentrating biomolecules from aqueous samples.

Products available as a mini-column:

Cellufine® Sulfate
Cellufine® ETclean L
Cellufine® ETclean S
Cellufine® PB
Cellufine® Chelate
Cellufine® Phosphate
Cellufine® A-500
Cellufine® Q-500
Cellufine® C-500
Cellufine® Butyl
Cellufine® Phenyl

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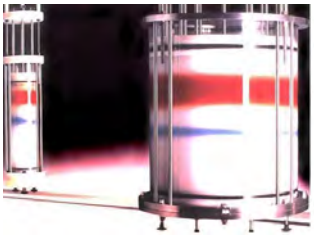

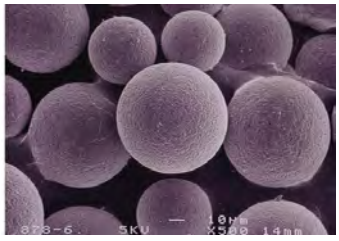

WHAT IS CELLUFINE®?

Cellufine® is the liquid chromatography media for the purification of proteins, enzymes and other bio-active substances. 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. Leaking from this matrix is much less than that from the synthetic polymer media.

Cellufine® product range covers all liquid chromatography modes:

- ✓ Ion Exchange
- ✓ Affinity
- ✓ Hydrophobic Interaction chromatography
- ✓ Gel Filtration

Figure 1: A range of Cellufine® Products

In addition, AMSBIO can provide custom made grades corresponding to user requests, such as the ion exchanger made from big beads and the affinity with a designated ligands.

The production of ® is guaranteed by ISO 9001 and 14000.

ION EXCHANGE CHROMATOGRAPHY

For capturing and intermediate purification of proteins, peptides and enzymes.

Cellufine® Ion Exchangers are based on spherical particles manufactured from cross linked cellulose. Each offers excellent flow properties, mechanical stability and chemical resistance. These ion exchangers are ideally suited for both laboratory and process scale chromatography of proteins, peptides and other biomolecules. Applications include the purification of antibodies, growth factors, albumin, enzymes, nucleic acids, etc.

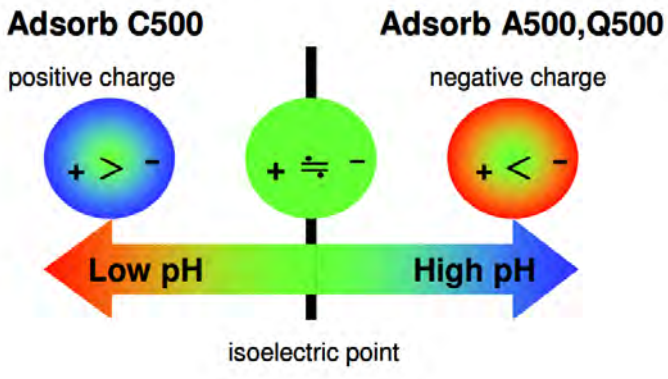

Figure 2: Separation of proteins is based on the isoelectric point.

Type	Particle size (μm)	Ion Exchange Capacity meq/g)	Capacity	
			BSA（mg/ml）	Lysozyme （mg/ml）
A-200	40-130	0.8-1.1	≧ 80	
A-500	40-130	1.1-1.4	≧ 60	
A-800	40-130	0.6-1.0	≧ 45	
C-500	40-130	0.9-1.2		≧ 70
Q-500	40-130	1.2-1.9	≧ 10	

Cellufine® MAX S, Q, CM, DEAE

High Flow Rate, High Binding Capacity:

Cellufine® MAX is the new, high-flow, Cellufine® media. Advanced cross-linking technologies have created more robust base beads operable at high flow and pressure. Further, Cellufine® MAX ion exchange (IEX) media are made using surface modification techniques that dramatically increase ligand availability, which translates to higher dynamic binding capacities. Cellufine MAX IEX media are offered in six products, including both anion and cation chemistries.

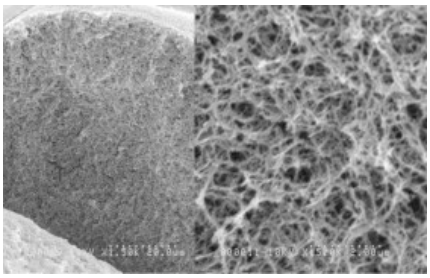

Figure 3; Cellufine pore structure

Cellufine MAX Base Resin:

Cellulose, natural polysaccharide, possesses unique crystalline molecular structure differing from non-crystalline polysaccharides such as agarose. Cellufine has unique pore structure as shown in the pictograph (figure 2 above).

Cellufine® A-200/A-500/A-800 — **DEAE**

Cellufine® C-500 — **CM**

Cellufine® Q-500 — **QA**

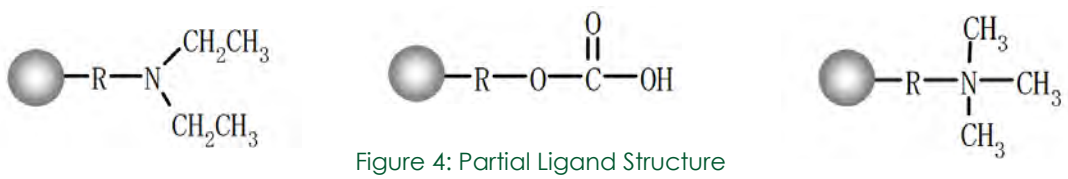

Figure 4: Partial Ligand Structure

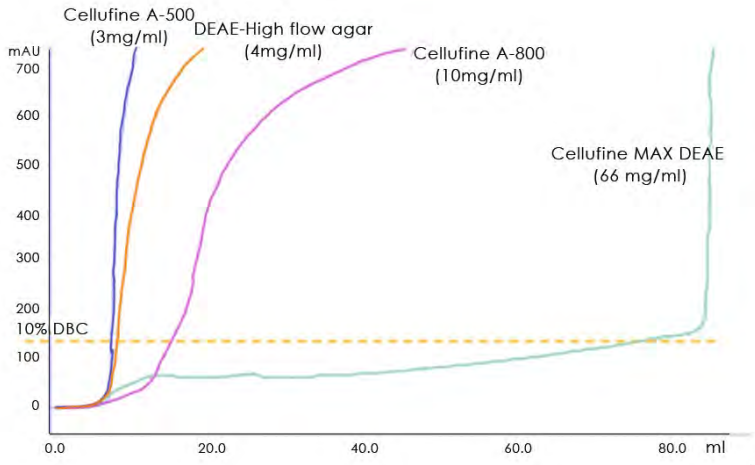

Figure 5: The new Cellufine® MAX series offers the largest pore size of all Cellufine® chromatography media. The benefit of such pore size in Cellufine® MAX IEX media provides superior strength and excellent mass transfer. This is seen in the break-through curves for thyroglobulin, a very large protein .

Partial Structure of Cellufine® Max IEX Media:

Ligand structure for Cellufine® MAX IEX media are described in Fig. 4. S, Q, CM and DEAE are correspondingly strong cation, strong anion, weak cation and weak anion exchangers. Two sub-types, h and r, are available for Cellufine® MAX S and Q.

The differences between X-h and X-r type Cellufine® MAX strong ion exchange media (X) are due to the design of the media. The X-h type is designed for higher binding capacity than the X-r type by optimizing the ligand content and dextran scaffold

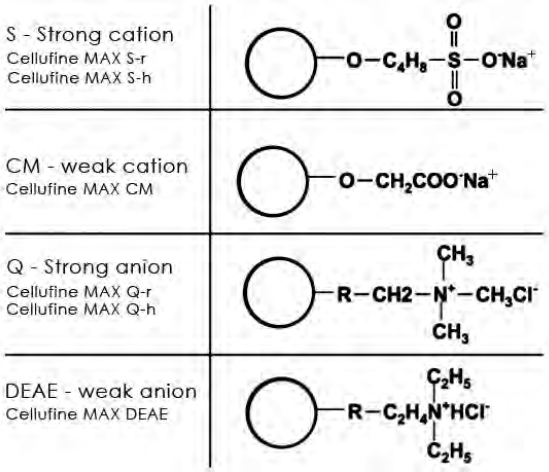

Figure 6: Ligand Structure of Cellufine® MAX IEX

DEAE Weak Anion		QA Strong Anion	
Cellufine® A-200 (*)	40-130µm	Cellufine® Q-500 (*)	40-130µm
Cellufine® A-500(*)	40-130µm	Cellufine® MAX Q-r (*)	40-130µm
Cellufine® A-800(*)	40-130µm	Cellufine® MAX Q-h(*)	40-130µm
Cellufine® MAX DEAE (*)	40-130µm		
CM Weak Cation		**S Strong Cation**	
Cellufine® C-500 (*)	40-130µm	Cellufine® MAX S-r (*)	40-130µm
Cellufine® MAX CM (*)	40-130µm	Cellufine® MAX S-h(*)	40-130µm

AFFINITY

For high specificity separations of a wide range of molecules.

Cellufine® Sulfate

For concentration, purification and depyrogenation of virus, viral/microbial antigens and heparin-binding proteins. Especially, for purification of Influenza virus, manufacture of influenza vaccine.

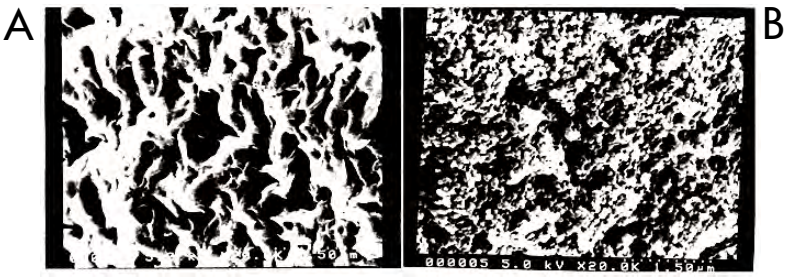

Figure 7: (A) The surface of Cellufine® Sulfate (before influenza adsorption) . (B)The surface of Cellufine® Sulfate after influenza adsorption. The data representing from professor Kida.(Hokkaido Univ. Graduate school of

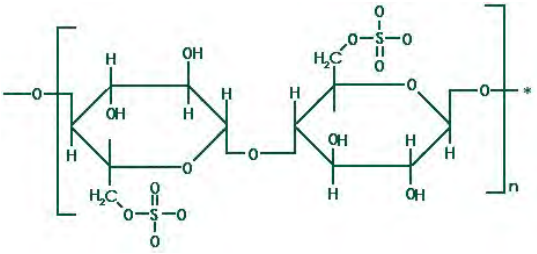

Figure 8: Partial structure of Cellufine® Sulfate

Characteristics	
particle size	40-130μm
ligand	sulfate ester
ligand conc.	approx. 8μM/ml
capacity	lysozyme >3mg/ml HBsAg 6-8mg/ml

Cellufine® PB: (Phenyl Borate)

Cellufine® PB is an affinity medium designed for concentration, purification of glycoprotein, glycated protein, saccharide. This media are based on a spherical, rigid cellulose beads functionalized with phenyl borate. The phenyl borate groups give unique chromatographic selectivity for cis-diol groups of target molecule.

Characteristics	
Ligand	Phenyl borate
boron contents	700μg/dry gel
Binding capacity (Conalbumin)	10mg/ml
Bead Matrix	Spherical Cellulose
pH Stability	3 to 12
Storage	+2°C to +8°C in 20% ethanol

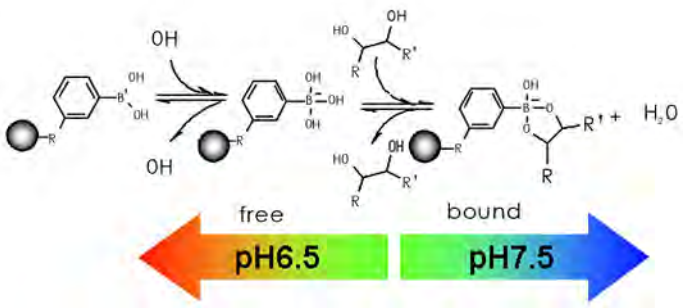

Figure 9: Structure of the ligand and Mechanism of binding cis-diol groups.

Cellufine® Phosphate

Cellufine® Phosphate is an affinity media for nucleic acid relate protein, dehydrogenase, phosphate relate protein, cation exchange chromatography.

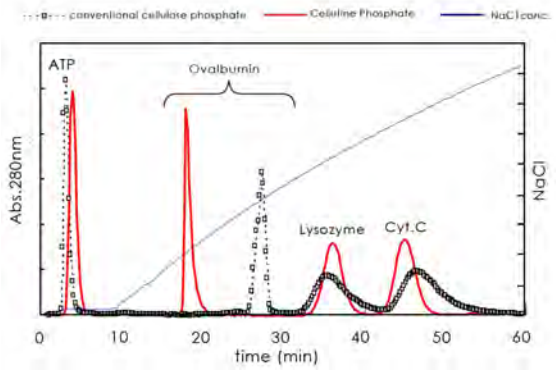

Figure 10: The example of separation of the mixed protein by Cellufine® Phosphate

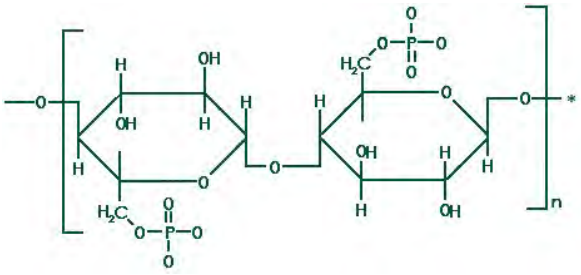

Figure 11: Partial structure of Cellufine® Phosphate

Characteristics	
Support Matrix	Cellulose
Ligand	Phosphate ester
Ligand conc.	2 - 4 meq/g-dry
Adsorption Capacity	≧2 0mg/ml-gel (lysozyme)

Cellufine® Chelate

Cellufine® Chelate is designed for immobilized metal chelate affinity chromatography of proteins and peptides. This media comprised of spherical cellulose beads to which iminodiacetic acid (IDA) has been immobilized. Its superior rigidity allows high flow rates and thus, rapid processing times. When exposed to metal salts, IDA readily complexes with the cation.

Characteristics	
particle size	120-210μm
ligand	iminodiacetic acid
capacity	Zn^{2+} 22-30μmol/ml Cu^{2+} 35-45μmol/ml
Operating Pressure	<2 bar (29 psi)
Supplied	Suspension in 20% EtOH

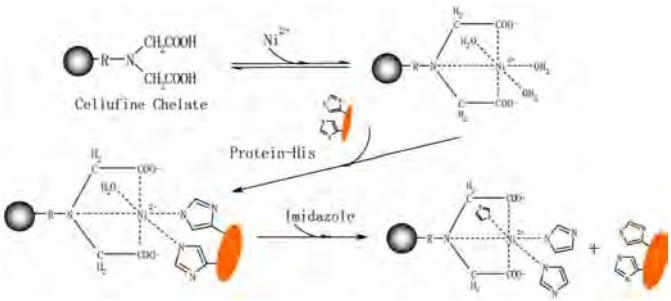

Figure 12: Adsorption and desorption mechanism of Cellufine® Chelate IMAC.

Cellufine® ET clean LS

The Cellufine® ETclean is poly(ε-lysine) immobilized Cellufine® (cellulose spherical beads). The beads bind and remove endotoxin from your sample solution.

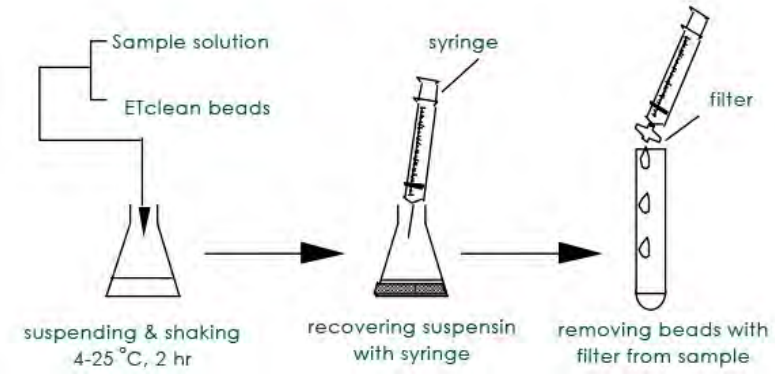

Figure 13: Batchwise Method

Products	Particle size μm	Capacity μg/mL	Exclusion limit
Cellufine® ET clean-S	40 - 130	280	$<10^3$
Cellufine® ET clean-L	40 - 130	480	2×10^6

Removal of LPS from a protein solution by ET-Clean

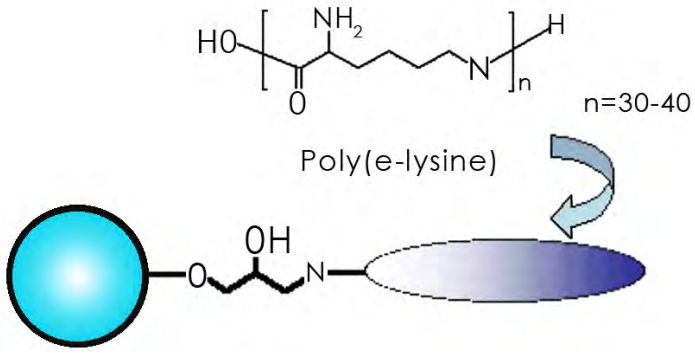

Figure 14: The removal of LPS was determined by a batch wise methods with 0.3 mL of wet adsorbent and 2mL of a protein solution containing natural LPS(protein:1mg mL^{-1},pH=7.0,m=0.05 or 0.40).

Sample solution			ET-Clean S (μ=0.05 , pH7.0)		ET-Clean L (μ=0.40 , pH7.0)	
protein	pI	LPS con. in protein solution pg mL^{-1}	Remain LPS pg mL^{-1}	Recovery of Protein %	Remain LPS pg mL^{-1}	Recovery of Protein %
BSA	4.9	32000	45	99	<10	97
γ-Globuline	8.4	5600	20	99	<10	97
CytochromeC	10.6	1500	15	99	<10	98

Cellufine® Amino

Cellufine® Amino is a primary amine activated support for the covalent immobilization of carboxyl containing proteins and ligands. The exclusion properties of Cellufine® Amino are similar to those of 4 % agarose gels. With the use of a condensation agent, ligands (protein, etc.) can be easily coupled via the reactive amine moiety.

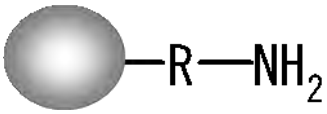

Figure 15: The scheme of the structural formula of Cellufine® Amino. Coupling with carboxyl group and aldehyde group.

Characteristics	
Active Group	(-NH2)
Active Group conc.	15-20μmol/ml
particle size	120-210μm
Amino Density (μM/ml)	15 - 20

Cellufine® Formyl

Cellufine® Formyl is an aldehyde activated support for the covalent immobilization of amine containing proteins and ligands. As with all Cellufine® products, the base support consists of spherical cellulose beads which exhibit superior rigidity and chemical stability relative to classical agarose gels.

Characteristics	
Activated Group	formyl (-CHO)
Formyl group conc.	10-15μmol/ml-gel
particle size	120-210μm
Delivery Condition	0.2 M Acetate buffer, pH3.0

The coupling of protein ligands having primary amino groups to Cellufine® Formyl proceeds via a Schiff's-base intermediate followed by reduction with reducing agent $NaCNBH_3$ (SCBH), $(CH_3)_3NBH_3$ (TMAB) and $NaBH_4$ (SBH) as illustrated below in Figure16:

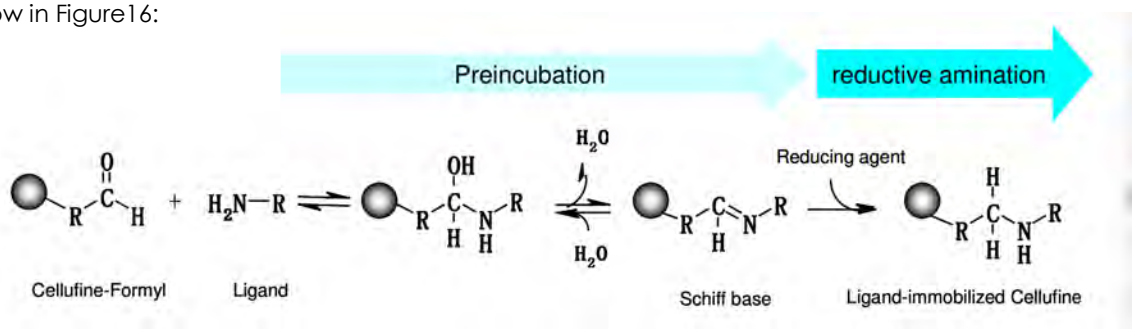

Figure 16: Cellufine formyl reaction mechanism

Endotoxin Removal		IMAC Metal Binding Molecules	
Cellufine® ETclean L (*)	40-130µm	Cellufine® Chelate (*)	125-210µm
Cellufine® ETclean S (*)	40-130µm		
Nucleic Acid Related Molecules		**Activated Supports**	
Cellufine® Phosphate (*)	40-130µm	Cellufine® Amino	125-210µm
		Cellufine® Formyl	125-210µm
Virus & Heparin Binding Proteins		**Molecules with cis-diols & EPO**	
Cellufine® Sulfate (*)	40-130µm	Cellufine® PB (*)	125-210µm

Mini-Column Cellufine®	Pack Size
Sulfate	1ml
Phosphate	1ml
PB	1ml
Chelate	1ml
ET-clean LS	1ml

HYDROPHOBIC INTERACTION CHROMATOGRAPHY

Hydrophobic Interaction Chromatography (HIC) is a method which separates proteins on the basis of their differential interactions with a mildly hydrophobic surface.

HIC media are porous chromatography particles, manufactured from cross linked cellulose to which either a butyl, phenyl functionality has been covalently bonded via a short spacer.

Cellufine® Butyl

Cellufine® Phenyl

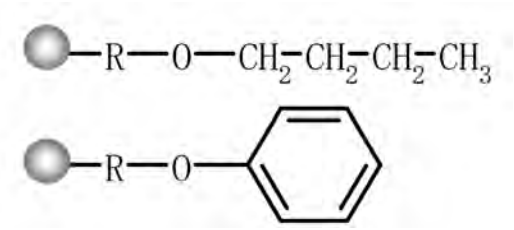

Figure 17

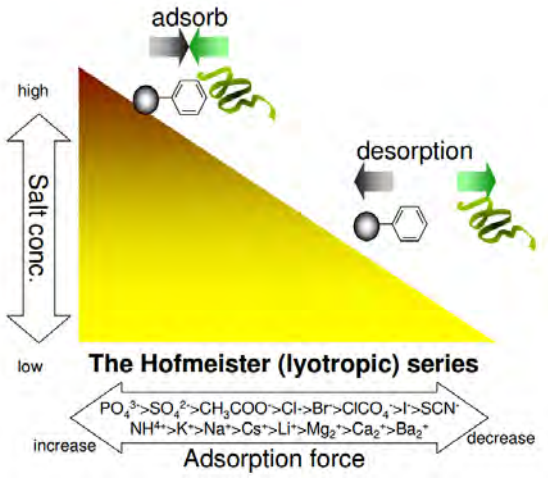

Figure 18: Effect of salt concentration and various ions on adsorption.

Cellufine® MAX is a 2nd generation Cellufine® media with high flow characteristics. A new, highly cross-linked base resin for Cellufine® MAX series. Cellufine® MAX hydrophobic (Phenyl) chromatography is now available in 2 types, which differ in functional ligand density; a standard type and low ligand density type LS.

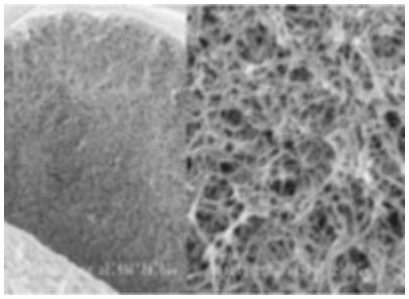

Figure 16: SEM analysis of Cellufine® MAX base resin

Cellulose, natural polysaccharides, possesses unique crystalline molecular structure differing from non-crystalline polysaccharides such as agarose. Cellufine® has unique pore structure with the new MAX series offering the largest pore size of all Cellufine® chromatography media. The benefit of such pore size is that it provides superior strength and excellent mass transfer.

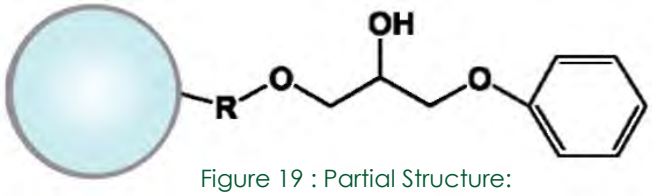

Figure 19 : Partial Structure:
Hydrophobic (Phenyl) | Cellufine® MAX Phenyl | Cellufine® MAX Phenyl LS

Type	Cellufine® MAX Phenyl	Cellufine® MAX Phenyl LS
Matrix	Highly cross-linked cellulose	
Particle size	ca. 40~130 µm	
Ligand type	Phenyl	
BSA adsorption capacity (mg/ml)	14	6
Polyclonal IgG 10% DBC (mg/ml)	31	19
Operating pressure	< 0.3 MPa	
pH stability	pH 2~13	
Storage	20 % ethanol	

Product	Pack Size
Cellufine® Butyl (*)	40-130µm
Cellufine® Phenyl(*)	40-130µm
Cellufine® MAX Phenyl(*)	40-130µm
Cellufine® MAX Phenyl L S (*)	40-130µm
Cellufine® MAX Butyl (*)	40-130µm

Gel Filtration:

Gel Filtration (size exclusion chromatography), has proven to be an effective and simple purification method for proteins and other biomolecules. However, the traditionally slow flow rates and limiting resolving power of most gel filtration media have restricted its usefulness. Cellufine® GCL media overcome these limitations. The mechanical strength of the spherical cellulose matrix allows high flow rates, even in large industrial columns.

For purification of biomolecules and proteins by molecular size:

Cellufine® GCL-2000	40-130µm

For salt and solvent removal, and buffer exchange:

Cellufine® GH-25 (*)	40-130µm

Note:
Cellufine® - spherical highly cross-linked cellulose
Customised grades an bonded chemistries are available upon a justified development agreement
Cellufine® 2nd generation
(*) Available in 1 & 5ml prepacked mini-columns

Cellufine® GCL-200

This media offer an extraordinarily broad selection of fractionation ranges: from the unique Cellufine® GCL-2000 for very high molecular weight protein complexes.

Cellufine® GH-25

Cellufine® GH-25 desalting media is based on porous, spherical, cellulose particles. The sharp 3kD exclusion limit allows proteins to pass through the column in the void volume while retarding smaller molecular weight solutes in the internal pores.

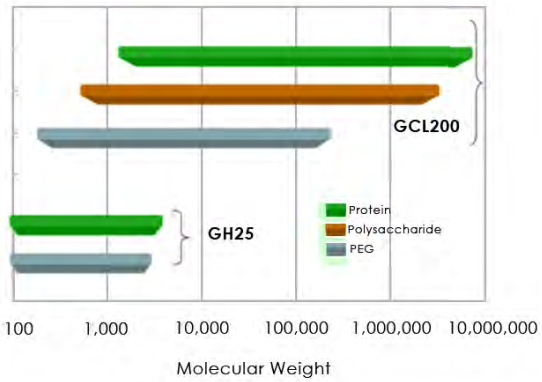

Figure 20: Molecular weight range for gel filtration media.

CELLUFINE® PRODUCT LIST

Affinity Media		
Cellufine® Sulfate	1ml x 5 (MC) 10ml 50ml 500ml	19845-51 676 943 324 19845 19846
Cellufine® ETclean L	1ml x 5 (MC) 10ml 50ml 500ml	20051 681 984 324 681 984 326 681 984 328
Cellufine® ETclean S	1ml x 5 (MC) 10ml 50ml 500ml	20151 682 985 324 682 985 326 682 985 328
Cellufine® PB	1ml x 5 (MC) 10ml 50ml 500ml	20251 683 986 324 683 986 326 683 986 328
Cellufine® Chelate	1ml x 5 (MC) 10ml 50ml 500ml	19875-51 676 951 324 19875 19876
Cellufine® Amino	10ml 50ml 500ml	676 945 324 19856 19857
Cellufine® Formyl	10ml 50ml 500ml	676 944 324 19853 19854
Cellufine® Phosphate	1ml x 5 (MC) 10ml 50ml 500ml	19551 684 987 324 684 987 326 684 987 328
Ion Exchange, Hydrophobic Interaction Media and Gel Filtration Media		
Cellufine® A-200	100ml 500ml	676 980 372 19611
Cellufine® A-500	1ml x 5 (MC) 100ml 500ml	19805-51 675 980 327 19805
Cellufine® A-800	100ml 500ml	673 980 327 19800
Cellufine® Q-500	1ml x 5 (MC) 100ml 500ml	19907-51 675 982 327 19907
Cellufine® C-500	1ml x 5 (MC) 100ml 500ml	19800-51 675 983 327 19865
Cellufine® Butyl	1ml x 5 (MC) 100ml 500ml	19905-51 19905 19906
Cellufine® Phenyl	1ml x 5 (MC) 100ml 500ml	19900-51 19900 19901
Cellufine® GH-25	100ml 500ml	670 000 327 19711
Cellufine® GCL-2000	100ml 500ml	672 000 327 19791

Note: MC = Mini-Column

Cellufine™ is the trademark of JNC Corporation, Tokyo, Japan

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