Hydrophobic Interaction Chromatography Media

Cellufine Phenyl EX

Technical Data Sheet

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Technical DATA Sheet

Cellufine Phenyl EX

For removing aggregates of antibody

Cellufine Phenyl EX is a chromatography resin that can efficiently remove aggregates of antibody drugs using our unique ligand modification technology. When the antibody sample after protein A chromatography in the first step is passed through Cellufine phenyl EX, the antibody aggregates are captured to the column, but most of the antibody monomers are recovered as they are in the flow-through fraction. Cellufine Phenyl EX can be used for flow-through purification that selectively adsorbs such as aggregates.

Cellufine Phenyl EX base resin

Cellulose, a natural polysaccharide, possesses unique crystalline molecular structure differing from non-crystalline polysaccharides such as agarose. Thus Cellufine has highly porous structure as shown in the pictograph (Fig. 1). Due to the characteristics of the cellulose, Cellufine has high mechanical strength and excellent mass transfer.

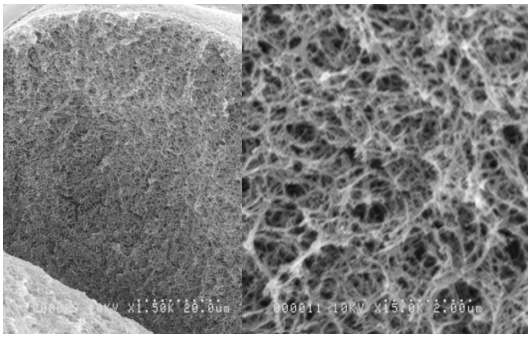

Fig 1. SEM analysis of Cellufine base resin

Partial structure of Cellufine Phenyl EX

Ligand structure for Cellufine MAX HIC media are described in Fig.2.

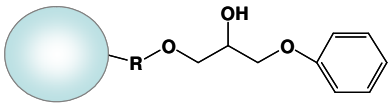

Fig.2. Ligand Structure of Cellufine MAX HIC

Characteristics of Cellufine Phenyl EX

The basic characteristics of Cellufine Phenyl EX and other HIC resins are shown in Table 1. All Cellufine HIC resins are based on 90 μm (average) cross-linked cellulose beads. Cellufine HIC resins are designed for use in bio-pharmaceuticals manufacturing processes. JNC manufactures multiple types of hydrophobic interaction chromatography resin.

	Phenyl EX	MAX Phenyl	MAX Phenyl LS
Matrix	Cross-linked Cellulose	Highly Cross-linked Cellulose	
Particle size	40～130 μm		
Ligand type	Phenyl		
BSA adsorption capacity (mg/ml)	13	11	4
BSA elution efficiency (%)	30	40	90
Operating pressure	< 0.2 MPa	< 0.3 MPa	
pH stability	pH 2 - 13		
Storage	20 % ethanol		

Table 1. Characteristics of Cellufine HIC resins

Pressure-flow Properties of Cellufine Phenyl EX

Cellufine Phenyl EX enable high-flow operation, which is essential to efficient purification of biopharmaceuticals. The figures below show pressure-flow velocity curves of Cellufine Phenyl EX (Fig. 3). Cellufine Phenyl EX is operable at practical flow velocities and pressures.

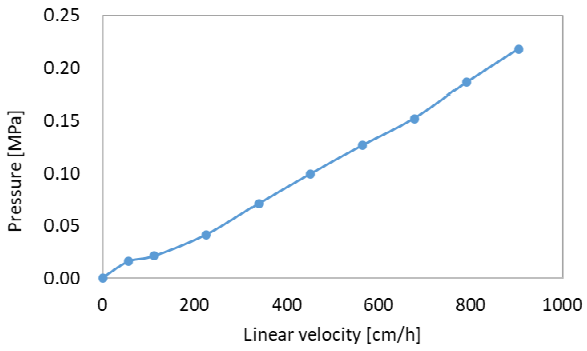

Fig.3 Pressure-flow property of Cellufine Phenyl EX

Column: I.D.2.6 cm x H 19.3cm

Mobile phase: Pure water, 23 - 25 ℃

Resin was packed with a compression factor 1.35. System pressure was excluded from the data.

Model Protein Separation Performance

The optimum amount of ligand for HIC resins vary depending on the application. Fig. 4 shows Model protein separation with Cellufine Phenyl EX and Cellufine MAX Phenyl. Protein separation studies show that relative binding strength are Phenyl EX > MAX Phenyl.

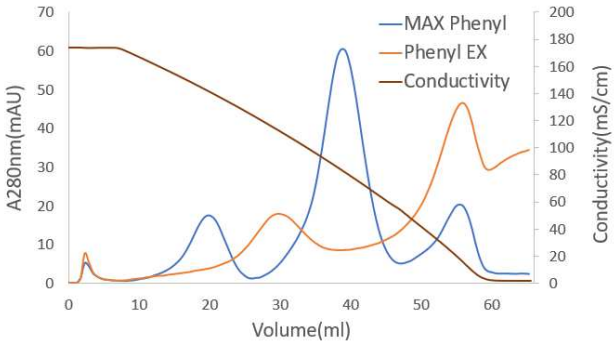

Fig.4 Model Proteins Separation

Column: I.D. 6.6 mm × H 50 mm

Buffer A: 10 mM Phosphate buffer, pH 7, 1.5 M Ammonium sulphate

Buffer B: 10 mM Phosphate buffer, pH 7

Proteins: Ribonuclease A, Cytochrome C, Lysozyme

Purification of antibody aggregates

Cellufine Phenyl EX is the best chromatography resin for removing aggregates from monoclonal antibodies in flow-through mode. Antibody aggregates were removed using the monoclonal antibody purified with a protein A column (Fig.5).

Cellufine Phenyl EX can be worked with low electrical conductivity and has high agglomerate removal properties. In this study, the conductivity of the sample was adjusted to 6 mS / cm. For this reason, it is possible to suppress corrosion of pipes and buffer precipitation due to high salt concentration, which is used with ordinary HIC resins.

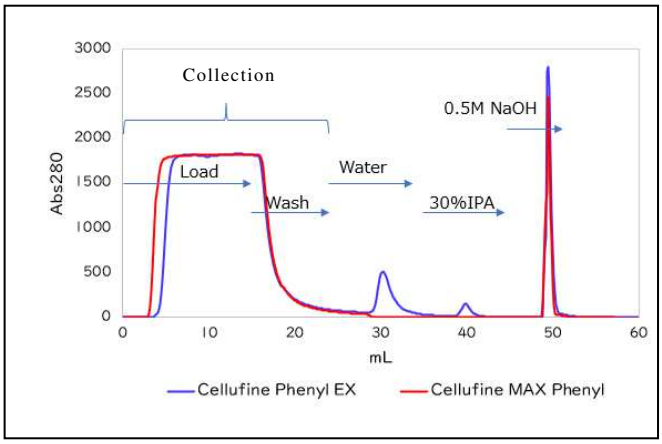

Fig.5 Removal of aggregates from monoclonal antibodies

Column: 1 mL Mini-Column

Flow rate: Residence time 4 min（75 cm/h）

Sample: Purified mAb with Protein A resin 6.6 mg/mL, pH6, 6 mS/cm

Antibody load: 93 mg mAb/ mL_cv

Equilibration/ wash : 20 mM AcOH-Tris + NaCl, pH6,

Resin	Aggregate (Before load) [%]	Aggregate (After load) [%]	Recovery [%]
Phenyl EX	3.6	0.4	87
MAX Phenyl		1.3	99

Table 2 The amount of aggregates after Phenyl EX

As shown in Table 2, the monoclonal antibody aggregate was present at 3.6% before column loading, but it was reduced to 0.4% by passing Cellufine Phenyl EX.

Repeat use

Cellufine Phenyl EX can be used repeatedly. For cleaning-in-place, use a cleaning solution containing 0.5 M sodium hydroxide and 30% isopropanol. By performing cleaning-in-place with an appropriate cleaning solution, the adsorption performance did not change even after repeated use 60 times (Fig. 6).

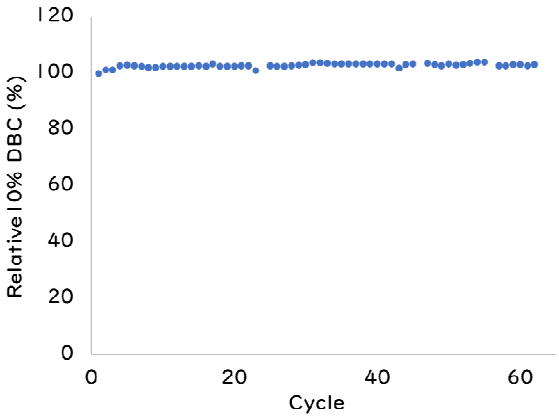

Fig. 6 Performance after repeated use

Column: 1 mL Mini-Column
Sample: human gamma globulin 2 mg/mL, in equilibration buffer
Equilibration: 20 mM Phosphate buffer, 0.5 M Ammonium Sulphate, pH7.0
Elution: 20 mM Phosphate buffer, pH7.0
CIP : 0.5M NaOH+30% IPA（10 CV、Exposure time: 20 min）

Chemical Stability

Cellulose is well-known as a natural product having chemical and physical stability. Thus, since Cellufine is derived from cellulose, it also is stable to chemicals and caustic and acidic solutions.

Usable chemicals
✓Ethanol (70%)
✓ Isopropyl alcohol (30%)
✓ Guanidine hydrochloride (6M)
✓ Urea (6M)
✓ NaOH (0.5M)
✓ Detergents
✓ Autoclave (121 ºC, 20 min)

Storage

After cleaning-in-place, replace the resin with 20% ethanol and store at 2-25 ° C. For long-term storage, it is recommended refrigerating at about 4 ° C.

Cellufine

Ordering Information

Product Name	Pack size	Catalogue No.
Cellufine Phenyl EX	1ml x 5 (Mini-Column)	22000-51
	5ml x 5 (Mini-Column)	22000-55
	100 ml	22000
	500 ml	22001
	5 lt	22002
	10 lt	22003
Cellufine MAX Phenyl	1ml x 5 (Mini-Column)	20700-51
	5ml x 5 (Mini-Column)	20700-55
	100 ml	20700
	500 ml	20701
	5 lt	20702
	10 lt	20703
Cellufine MAX Phenyl LS	1ml x 5 (Mini-Column)	20800-51
	5ml x 5 (Mini-Column)	20800-55
	100 ml	20800
	500 ml	20801
	5 lt	20802
	10 lt	20803

Purchase order・Technical support contacts

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