Hydrophobic Interaction Chromatography Media

Cellufine MAX Butyl HS

Technical Data Sheet

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Cellufine MAX Butyl HS is a hydrophobic interaction chromatography resin with butyl groups immobilized on the surface. The butyl group is immobilized at a higher concentration than conventional MAX butyl. Specifically designed to be optimized for the purification of polysaccharide vaccines.

High Flow Rate typed Media

Cellufine MAX is a Cellufine chromatography resin that can be used at high flow rates. Using JNC's unique cross-linking technology, we have designed a highly robust resin that can be used at high flow rates.

Cellufine MAX base resin

Cellulose, which is the raw material of Cellufine MAX Butyl HS, is a natural polysaccharide, but unlike agarose, which has an amorphous structure, it has a unique crystal structure. Thus, Cellufine has distinctive pore structure as shown in the pictograph (Fig. 1). Cellufine MAX series has the largest pore size among all Cellufine series. Due to the characteristics of these base materials, it has high mechanical strength and excellent mass transfer in pores.

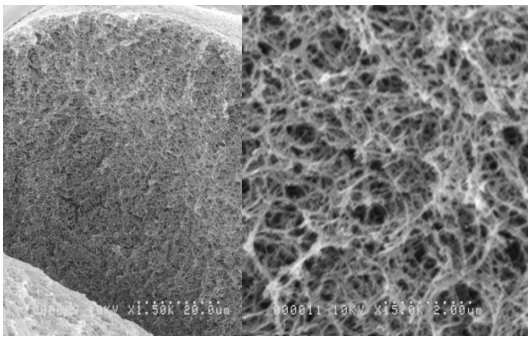


Fig 1. SEM analysis of Cellufine MAX base resin

Ligand structure of Cellufine MAX Butyl HS

Ligand structure for Cellufine MAX Butyl HS is described in Fig.2.

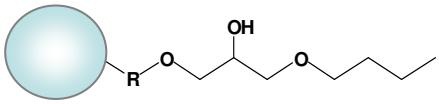


Fig.2 Ligand Structure of Cellufine MAX Butyl HS

Characteristics of Cellufine MAX Butyl HS

Table 1 shows the basic characteristics of Cellufine MAX Butyl HS. The resin is highly cross-linked cellulose particles with an average of 90um. Cellufine MAX Butyl HS is designed for use in biopharmaceutical manufacturing processes. There are several lineups of chromatography resin with butyl groups in Cellufine brand. Table 1 shows a comparison of each feature.

	MAX Butyl	**MAX Butyl HS**
Base resin	Highly cross-linked cellulose	
Particle diameter	40 – 130 µm (ca. 90 µm	
Ligand	Low conc. butyl group	High conc. butyl group
BSA capacity (mg/ml)	9	13
BSA elution efficiency (%)	70	36
Operating pressure	< 0.3 MPa	
pH stability (30 ℃, 1 week)	pH 2～13	pH 2～13
Chemical stability	Stable in commonly used buffers	
CIP	1M NaOH	
Storage	20 % Ethanol	

Table 1 Features of Cellufine MAX Butyl HS

Flow pressure property

Cellufine MAX Butyl HS enable high-flow operation, which is essential to efficient purification of biopharmaceuticals. The figures below show pressure-flow velocity curves of Cellufine MAX Butyl HS (Fig. 3).

All Cellufine MAX HIC media are operable at practical flow velocities and pressures.

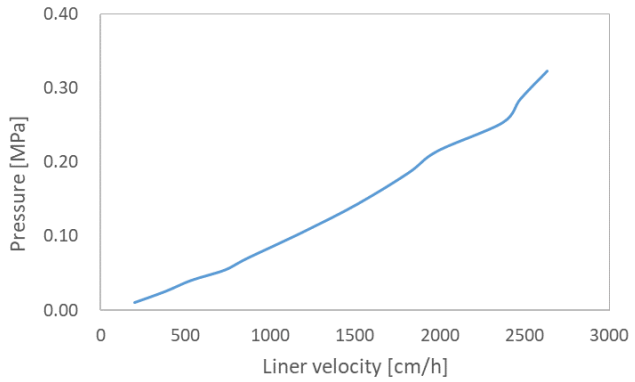


Fig. 3 Pressure-flow curve of Cellufine MAX Butyl HS
Column: 2.2 cm ID x 20 cm H
Mobile phase: pure water, 24°C

Model Protein Separation Performance for Cellufine MAX Butyl HS

Cellufine MAX Butyl HS is designed for high separation of target proteins. Figure 4 shows the protein separation behavior of Cellufine MAX Butyl and Cellufine MAX Butyl HS. From this result, it can be seen that the strength of protein adsorption is MAX butyl HS > MAX butyl.

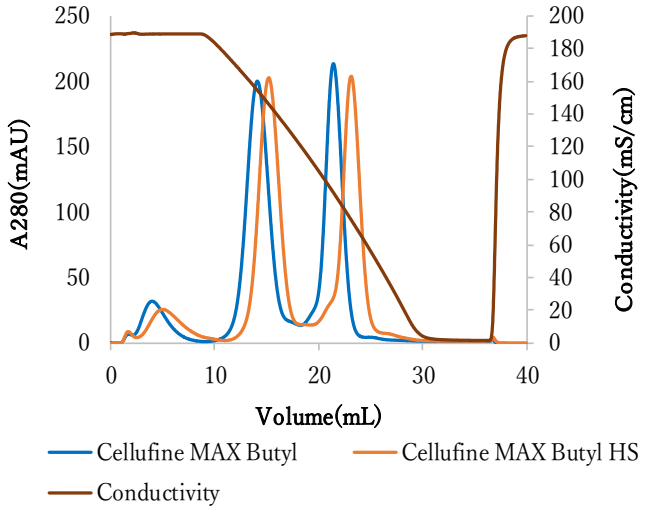


Figure 4 Separation characteristics of model proteins

Column: 6.6 mm ID x 30 mm L (1.0 ml)
Buffer A: 10 mM Na phosphate, 1.5 M ammonium sulfate, pH 7.0
Buffer B: 10 mM Na phosphate, pH 7.0
Protein: Ribonuclease A, Cytochrome C, Lysozyme

Application: Purification of polysaccharide vaccine

Cellufine MAX Butyl HS is optimally designed for the purification of polysaccharide vaccines. Here is an example of purification of capsular polysaccharide from *Streptococcus pneumonia* serotype 19F (ATCC49619).

Sample preparation

Streptococcus pneumonia serotype 19F (ATCC49619) was inoculated into a sheep blood agar medium and cultured under anaerobic conditions for 16 hours, then inoculated into 2,000 mL of Brain Heart Infusion medium and cultured at 37° C. for 20 hours.

10% sodium deoxycholate was added to the culture medium and incubated at 37° C for 16 hours to lyse the bacteria. After centrifugation (12,000 rpm, 15 minutes, 4°C), the supernatant was collected. Further, this supernatant was filtered through a 0.45 µm cellulose acetate membrane filter. The filtrate was concentrated by ultrafiltration (Vivaflow 200, Milli Q, MWCO 100k)

Ammonium sulfate corresponding to 50% of the saturation solubility was added to the samples and incubated at 4° C for 16 hours. The pellet was removed by centrifugation (12,000 rpm, 15 min, 4 ℃), and the supernatant was subjected to ultrafiltration (Vivaflow 200, MilliQ, MWCO 100k) to change the buffer. The solution was filtered through a 0.2 µm membrane filter to obtain a load sample.

Chromatography

Chromatography was performed according to the following method with Cellufine MAX Butyl HS.

Column: 6.7 mmID x 30 mm (1.06 mL)
Flow rate: 0.5 mL/min (RT 2 min, 36 cm/hr),
1 mL/min for equilibration steps
Buffer A: 10 mM Sodium phosphate, pH7.0
Buffer B: 10 mM Sodium phosphate, pH7.0,
2.0 M Ammonium sulfate

Process	Solution	Volume
Equilibration	Buffer B	5 CV
Sample loading	Sample solution	40 CV
Elution 1	Buffer A	10 CV
Elution 2	Pure water	20 CV
Wash	Buffer A	5 CV
CIP	0.5M NaOH	5 CV
Equilibration	Pure water	20 CV

Purification result

Polysaccharides (Ps) were quantified using the Anthrone Sulfate Method. Protein (Pr) was quantified by the Bradford method using a protein assay kit (Bio-Rad). For nucleic acids (NA), the absorbance at 260 nm was measured using BioSpec nano (Shimazu) and calculated as 1 AU=50 μg/mL.

	Ps μg/mL	Ps Recovery%	Ps Purity%	Pr μg/mL	NA μg/mL
Load	726	-	46	73	772
After	518	89	54	0	436

Chemical stability and cleaning in place

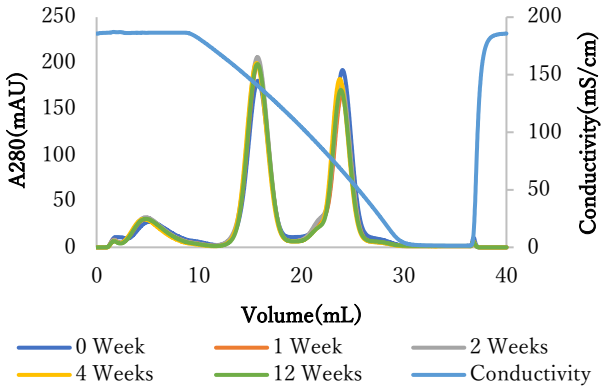
Cellulose is known as a chemically and physically stable natural compound. Since Cellufine is derived from cellulose, it exhibits stability against chemicals, acidity, and alkalinity. A 0.5 M to 1.0 M NaOH aqueous solution can be used for cleaning in place (CIP) of Cellufine MAX Butyl HS. After use, the resin should be washed and stored at 2-25°C in 20% ethanol.

Usable chemicals, etc.

- ✓ Ethanol (70%)
- ✓ Isopropanol (30%)
- ✓ Guanidine hydrochloride (6M)
- ✓ Urea (6M)
- ✓ NaOH (0.5M)
- ✓ Surfactant
- ✓ Autoclave (121 ℃, 20 min)

Repeatability

After immersing Cellufine MAX Butyl HS in 1 M NaOH, the separation properties were evaluated using model proteins. Separation characteristics do not change even after 12 weeks, and it can be seen that separation performance does not change even after repeated cleaning in place.



Column: 6.6 mm ID x 30 mmL (1.0 ml)
Buffer A: 10 mM Na phosphate, 1.5 M ammonium sulfate, pH 7.0
Buffer B: 10 mM Na phosphate, pH 7.0
Protein: Ribonuclease A, Cytochrome C, Lysozyme

Fig.5 Separation characteristics of model proteins after immersion in 1 M NaOH

Ordering Information

Products	Pack size	Catalogue No.
Cellufine MAX Butyl HS	1ml x 5 (Mini-Column)	22200-51
	5ml x 5 (Mini-Column)	22200-55
	100 ml	22200
	500 ml	22201
	5 lt	22202
	10 lt	22203
Cellufine MAX Butyl	1ml x 5 (Mini-Column)	21100-51
	5ml x 5 (Mini-Column)	21100-55
	100 ml	21100
	500 ml	21101
	5 lt	21102
	10 lt	21103

Purchase / Technical Support Request

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