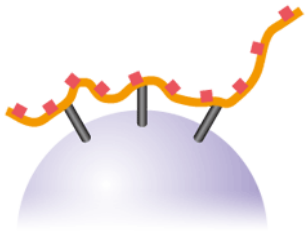
Ion Exchange Chromatography Media

Cellufine MAX Q-hv

Technical Data Sheet



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Cellufine MAX Q-hv is a strong anion exchange chromatography resin. It uses a base resin with highly cross-linked particles and surface modification. Therefore, it has high pressure resistance. Specifically, the ligand concentration is designed to be effective for purification of polysaccharide vaccines.

The Cellufine MAX Q series includes Cellufine MAX Q-r, which has excellent resolution properties, and Cellufine MAX Q-h, which has high adsorption capacity. Cellufine MAX Q-hv has a ligand design aimed at improving the elution of target substances such as large nucleic acids, polysaccharides, and virus particles while maintaining adsorption capacity. Therefore, separation behavior different from conventional MAX Q-r and MAX Q-h can be expected.

The resin characterized by high flow velocity and high adsorption

Cellufine MAX Q-hv is a high flow velocity type resin. Using JNC's unique cross-linking technology, we have designed a highly robust resin that can be used at high flow rates. In addition, we dramatically increased the efficiency of ligand utilization by surface modification technology. This technology has achieved high dynamic binding capacity.

Cellufine MAX base resin

Cellulose, which is the raw material of Cellufine MAX Q-hv, 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). For this reason, the substance to be purified quickly diffuses into the pores, exhibiting excellent adsorption capacity.

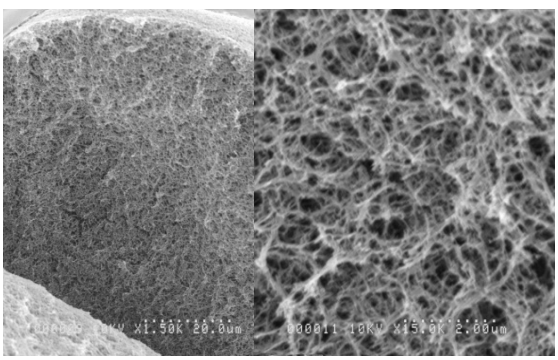

Fig 1. SEM analysis of Cellufine MAX base resin

Ligand structure of Cellufine MAX Q-hv

Ligand structure for Cellufine MAX Q-hv is described in Fig.2.

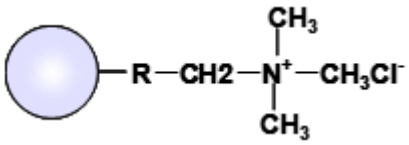

Fig.2 Ligand Structure of Cellufine MAX Q-hv

Characteristics of Cellufine MAX Q-hv

Basic characteristics are shown in Table 1. All Cellufine MAX ion-exchange chromatography resins are based on highly crosslinked cellulose particles with an average particle size of 90um and modified with dextran. The pore size and ion exchange capacity of the Cellufine MAX Q-hv has adjusted to optimize purification of polysaccharides and virus particles, which are the main components of vaccines.

	MAX Q-r	MAX Q-h	**MAX Q-hv**
Ion exchange type	Strong Anion / -$N^+(CH_3)_3$		
Matrix	Highly cross-linked cellulose with dextran scaffold		
Particle size	ca. 40 – 130 μm (Average 90)		
Ion exchange capacity meq/ml	0.10～0.20	0.13～0.22	0.04～0.07
Flow velocity	600 cm/h(0.3 MPa), I.D.30 cm-L20 cm, pure water at 24 ºC		
DBC BSA g/ml	110	180	120
pH working range	2－12	2－12	2－12
pH stability (30ºC, 1week)	2－12	2－12	2－12
Chemical stability	Stable all commonly used aqueous buffers		
CIP	1 M NaOH		0.5M NaOH
Storage	20 % ethanol		

Table 1 Features of Cellufine MAX Q-hv

High dynamic binding capacity and recovery

Cellufine MAX Q-hv is a strong anion exchange chromatography resin that is effective for the purification of polysaccharides. Cellufine has a lineup of several types of strong anion exchange chromatography resins, but Cellufine MAX Q-hv shows high dynamic binding capacity and good recovery in the purification of polysaccharides (Table 2).

	DBC [mg/mL]	Recovery [%]
MAX Q-hv	22	109
MAX Q-h	17	72
MAX Q-r	9	95

Table 2 Adsorption performance of polysaccharides derived from *Streptococcus pneumoniae*

Dynamic binding capacity (DBC) and recovery were determined using partially purified polysaccharide derived from *Streptococcus pneumoniae*. The amount of DBC is the amount of adsorption at the 20% breakthrough point.

Chromatography conditions

Column: 6.7 mm ID x 30 mmH (1.06 mL)

Flow rate: 0.212 mL/min (RT 5 min, 36 cm/h)

Adsorption buffer: 50 mM Na phosphate, pH 6.0

Elution buffer: 50 mM Na phosphate + 1 M NaCl, pH 6.0

Chemical stability and cleaning in place

Usable chemicals, etc.

✓Ethanol (70%)
✓Isopropanol (30%)
✓Guanidine hydrochloride (6M)
✓Urea (6M)
✓NaOH (0.5M)
✓Surfactant

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 NaOH aqueous solution can be used for cleaning in place (CIP) of Cellufine MAX Q-hv. After use, the resin should be washed and stored at 2-25°C in 20% ethanol.

Stability with cleaning-in-place solutions

Cellufine MAX Q-hv is stable to 0.5 M NaOH, which is commonly used for cleaning in place. The performance was maintained even after 7 days of immersion (Table 2). From this result, it can be seen that when the exposure time is set to 15 minutes during cleaning in place, it is stable after 672 cleanings in place.

Storage days	BSA adsorption [mg/mL]	IEX capacity [meq/mL]	N Contents [%]
0	155	0.06	0.9
7	146	0.05	0.9

Table 2 Stability after immersion in 0.5 M NaOH

Application: Purification of polysaccharide vaccine

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

1. First-step purification with MAX Butyl HS
   For the operation method, refer to the instruction manual for Cellufine MAX Butyl HS.
2. Second-step purification with MAX Q-hv
   Chromatography was performed according to the following method with Cellufine MAX Q-hv

Process	Solution	Volume
Equilibration	Buffer A	5 CV
Sample loading	サンプル溶液	-
Wash	Buffer A	15 CV
Elution	Buffer B	10 CV
Wash	Buffer A	10 CV
CIP	0.5M NaOH 溶液	10 CV
Equilibration	Pure water	20 CV

Column: 6.7 mmID×30 mmH (1.06 mL)
Flow rate: 0.12 mL/min (RT 2.5 min, 36 cm/hr)
Buffer A: 50 mM Sodium phosphate, pH7.0
Buffer B: 50 mM Sodium phosphate, 1.0 M Sodium chloride, pH 6.0

3. 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.

	10% DBC µg/mL	Ps Recovery %	Ps Purity%	Pr µg/mL	NA µg/mL
Load sample	-	-	76	5.1	162
After column	6.4	98	99	N.D.	4

Ordering Information

Product	Pack size	Catalogue No.
Cellufine MAX Q-hv	5 x 1 mL (Mini-Column)	22100-51
	1 x 5 mL (Mini-Column)	22100-15
	100 mL	22100
	500 mL	22101
	5 L	22102
	10 L	22103

Purchase / Technical Support Request

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