Cellufine™ MAX Q-hv

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 and high adsorption capacity.

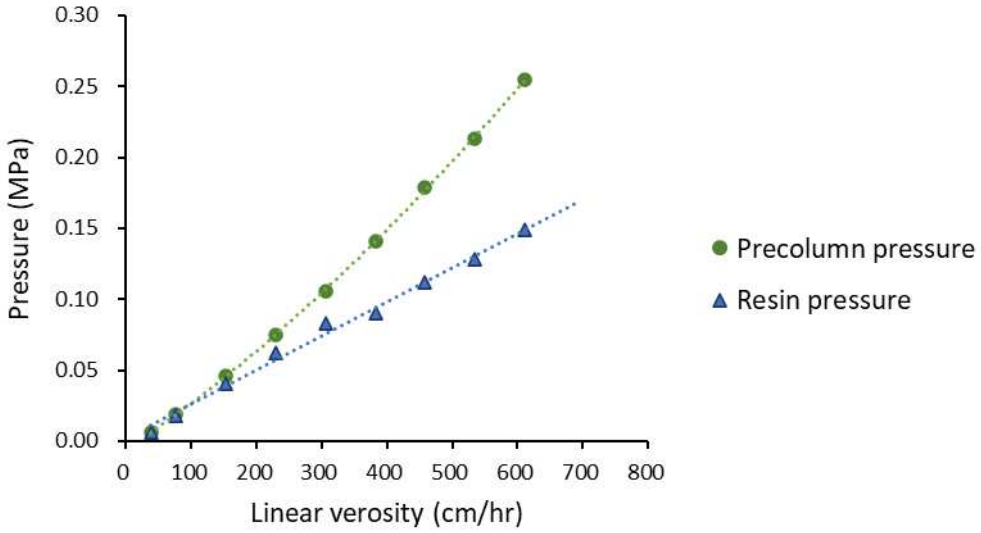
The Cellufine MAX Q series includes Cellufine MAX Q-r, which has excellent dissolution properties, and Cellufine MAX Q-h, which has high adsorption performance. **Cellufine MAX Q-hv** has a ligand design aimed at improving the elution of target substances such as highly hydrophobic polysaccharide vaccines and virus particles while maintaining high adsorption capacity. Therefore, separation behavior different from conventional MAX Q-r and MAX Q-h can be expected.

Table 1 Features of Cellufine MAX Q-hv

Product	Cellufine MAX Q-r	Cellufine MAX Q-h	**Cellufine 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	0.10～0.20 meq /ml	0.13～0.22 meq /ml	0.04～0.07 meq /ml
Flow velocity	600 cm/h(0.3 MPa), I.D.30 cm-L20 cm, pure water at 24 ºC		
Dynamic binding capacity	110 mg BSA/ml	180 mg BSA/ml	120 mg BSA/ml
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		0.5 M NaOH
Storage	20 % ethanol		

Flow pressure property

Cellufine MAX Q-hv has superior flow-pressure properties.



Column: I.D. 10 cm x H 20 cm
Compression Factor: 1.30
Temp.: 24±1 °C
Mobile Phase: Pure water

Packing Method

1. Calculate volume required of the desired bed dimension.
   (a) Packed column volume = column cross-sectional area (cm2) x column height (cm)
   (b) Required column settling volume = packed column volume x 1.08-1.12
2. Washing the gel with water or the appropriate buffer.
3. Prepare a 40-60 % (v/v) slurry with water, 0.1 M NaCl solution or the appropriate buffer.
4. Gently stir. If required place under vacuum to degas for 1 hour at room temperature.
5. With the column outlet closed, carefully pour the slurry into the column. Depending on column packing volume, a reservoir may be required.
6. While removing the air by opening the upper adaptor, insert the movable adaptor to the surface of the slurry to fix it.
7. Open the column outlet and begin pumping elution buffer 20-30% faster than the operating flow rate.
8. Once the bed has stabilized, close the column outlet. Then, with the top adaptor open, lower the top adaptor to the surface of the bed. Equilibrate with 10 CV (column volume) of adsorption buffer before loading the sample.

Evaluation of packing

See appendix 1

Operating Guidelines

General Operation

Typically, adsorption to Cellufine MAX Anion Exchange medium occurs in relatively low ionic strength (e.g., below 0.1 M NaCl) in the pH range from 6 – 9 under the pI of the target protein. The binding capacity is strongly affected by pH and conductivity. Under these conditions, proteins with neutral or net negative charge will bind. Bound components are then eluted by stepwise elution with buffers containing progressively higher salt concentration or by elution with a linear salt gradient.

1. Equilibrate the column with adsorption buffer.
2. Load a sample.
3. Wash 5 CV with adsorption buffer to remove non-adsorbed impurities.
4. Elute the adsorbed target molecules with elution buffer.

A general procedure is outlined in Table 2 below. Conditions such as pH, buffers, salts and flow rates can be optimized according to the purpose.

Table 2 General procedure of Cellufine MAX Q-hv

Process	CV	Explanation of each process
Equilibration	3 – 10	Ensure that the pH and conductivity of the column buffer is the same as the required sample load buffer.
Sample load	-	Flow-through or bind and elute mode cam be used.
Wash	5	Use adsorption buffer.
Elution	3 – 7	Use elution buffer.
CIP	3 – 10	0.5 M NaOH

Recommend buffers

Adsorption buffer: 10-50 mM Na phosphate (pH 6-8) or Tris-HCl (pH 6-9)
Sample load: Prepare a sample that has been replaced with adsorption buffer
Elution buffer: Adsorption buffer with 0.1 - 2.0 M sodium chloride

Sample preparation and sample-loading

Replace the sample with adsorption buffer. Adjust sample concentration to 1 – 20 mg/ml. If necessary, remove insoluble matter with a filter. The sample is desalted with a desalting filter or desalting column and adjusted to the desired ionic strength. After equilibrating the column with adsorption buffer, load the sample. Wash with 5 CV of adsorption buffer after sample loading to remove unadsorbed impurities.

Operating flow rate

Cellufine MAX Q-hv is based on highly crosslinked cellulose particles. Therefore, it can be used stably even at high flow velocities. A column of 2.2 cm ID x 20 cm height can be run at a flow rate of 1,000 cm/h at an operating pressure of 0.3 MPa or less. A column with a diameter of 30 cm or more and a height of 20 cm can be used at a flow rate of 500 cm/h or more at an operating pressure of 0.3 MPa or less.

Chemical Stability

The resin can be used stably under pH 2-12 conditions at room temperature. Stable in most salts (NaCl, $(NH_4)_2SO_4$, etc.) and most detergents (SDS, Tween etc) and other chemicals (70 % ethanol, 30 % isopropyl alcohol, 6M Guanidine hydrochloride and Urea). The products can be cleaned with 0.2 N NaOH.

Cleaning in place (CIP)

Stability in 0.5 M NaOH exposure for 15 minutes showed no performance degradation after at least 100 repeated exposures.

Depyrogen

Wash the column with 5 CV of 0.2 M NaOH in water, let stand for 16 hours, then wash with endotoxin-free water or equilibration buffer. 0.2 M NaOH-20% EtOH is effective for endotoxin removal. Furthermore, 0.2 M NaOH-90% EtOH can reduce endotoxins in 2 hours.

Application: Purification of polysaccharide vaccine

1. Culture

   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.

2. Lysis and recovery

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).

3. Ammonium sulfate precipitation

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 to about 1,000 µg/mL polysaccharide. Ammonium sulfate corresponding to 50% of the saturated solubility was added to this concentrate, and the solution was filtered through a 0.2 µm membrane filter to obtain a load sample.

4. Hydrophobic Interaction Chromatography with Cellufine MAX Butyl HS

For the operation method, refer to the instruction manual for Cellufine MAX Butyl HS.

5. Anion Exchange Chromatography with Cellufine MAX Q-hv

Chromatography was performed according to the following method.

Process	Solution	Volume
Equilibration	Buffer A	5 CV
Sample loading	Sample solution	-
Wash	Buffer A	15 CV
Elution	Buffer B	10 CV
Wash	Buffer A	10 CV
CIP	0.5M NaOH	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

6. 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 mg/mL-resin	Ps Recovery%	Ps Purity%	Pr µg/mL	NA µg/mL	Pr/Ps %	NA/Ps %
Load sample	-	-	75.6	5.1	161.7	1	31
After column	6.4	97.8	98.7	Not detected	4.3	0	1

Storage

For short periods of up to 2 weeks, it can be stored at room temperature under pH 2 - 12 conditions. Long term storage should be in neutral buffer containing 20 % EtOH at 25 ºC or lower temperature. Do not freeze.

Shelf Lifetime

5 years from date of manufacture.

References

1. Harris, E.L.V. and Angal, S., *Protein Purification Methods: A practical Approach*. New York: Oxford University Press, 1989.
2. Janson, J.-C. and Ryden, L., *Protein Purification: Principles, High Resolution Methods, and Applications.* 2nd ed. New York: John Wiley & Sons, Inc., 1998

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	22 03

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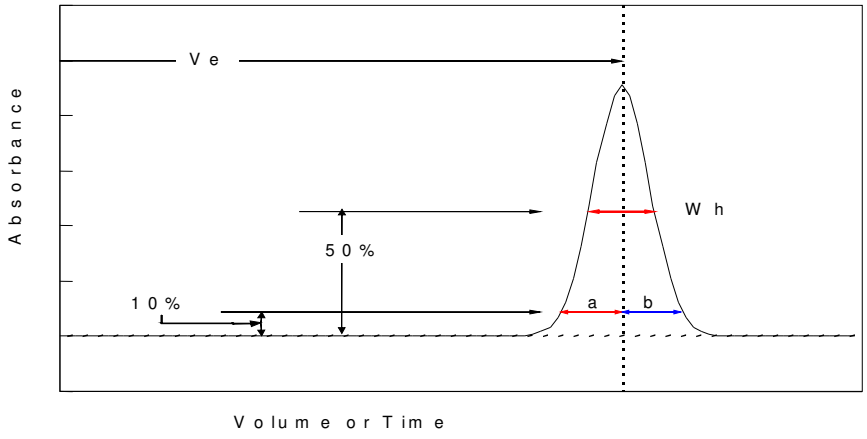
Appendix 1: Evaluation of column packing of Cellufine

Evaluation methods for the packing status of a column can employ indices, such as the number of (theoretical) plates (N), the height equivalents to a theoretical plate (HETP), and asymmetry factor (As). The selected evaluation indices are affected by the measurement condition. For example, they vary with changes in the difference between the diameter/height of a column, the difference in piping, solvent sample volume, the flow velocity, temperature, etc. Therefore, it is necessary to use the same measurement conditions each time.

Conditions	
Sample volume	1% (MAX 2.5 %) of column bed volume
Sample concentration	1-2 % (V/V) acetone (mobile phase: water)
	1M NaCl (mobile phase: 0.1-0.4 M NaCl aq.)
Flow rate	~30 cm/h (X mL/hr/column cross section)
Detector	OD 280nm (For acetone) Conductivity (For NaCl)



Formula

HETP = L/N

N = 5.54 x (Ve/Wh)^2

As = b/a

L	Column length [cm or m]
V_e	Elution time or volume
W_h	Half of width of peak
a, b	Peak width of 10% peak height (a) Peak width in front half of center (b) Peak width in the latter half of the center
Note	Ve,Wh and a, b should have same dimensional units

Generally, number of theoretical plates (N) is good if it is over 3000 N/m. Acceptable asymmetry factor (As) values range from 0.7-1.5.

