Cellufine™ MAX Butyl HS

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.

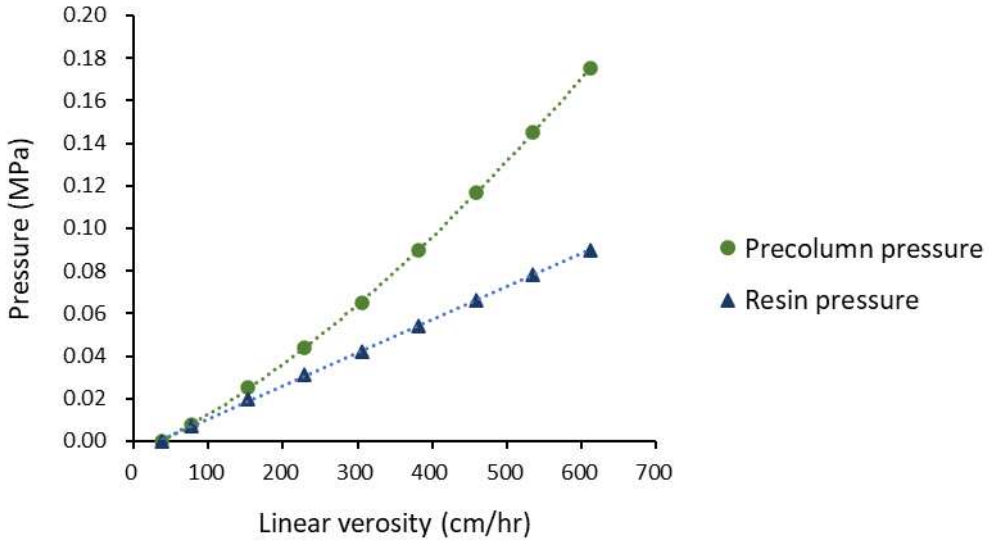
Cellufine MAX Butyl HS is used for the chromatography purification of hydrophobic proteins. Many proteins have hydrophobic amino acid residues. These amino acids and the butyl group of the resin are adsorbed through hydrophobic interaction. Factors that affect hydrophobic interactions include salt concentration, temperature, pH, organic solvents, and surfactants. Protein adsorption is typically performed at high ionic strength. On the other hand, it elutes proteins at low salt concentrations. Under such conditions, the adsorption mechanism is opposite to that of ion-exchange chromatography resin. Therefore, it has the advantage of different separation behavior from ion-exchange chromatography. Cellufine MAX Butyl HS is modified with a high concentration of butyl groups. This design makes it particularly effective for the purification of polysaccharide vaccines.

Table 1 Features of Cellufine MAX Butyl HS

Features	
Product	Cellufine MAX Butyl HS
Ligand	Butyl group
Base resin	Highly cross-linked cellulose
Particle diameter	40 – 130 μm (ca. 90 μm)
BSA capacity (mg/ml)	≧ 13
BSA elution efficiency (%)	≧ 36
MW exclusion limit (kD)	1,000
pH stability range	2 – 13
Operating pressure	< 0.3 MPa
Supplied	suspension in 20 % EtOH

Flow pressure property

Cellufine MAX Butyl HS has superior flow-pressure properties.



Column: I.D. 10 cm x H 20 cm

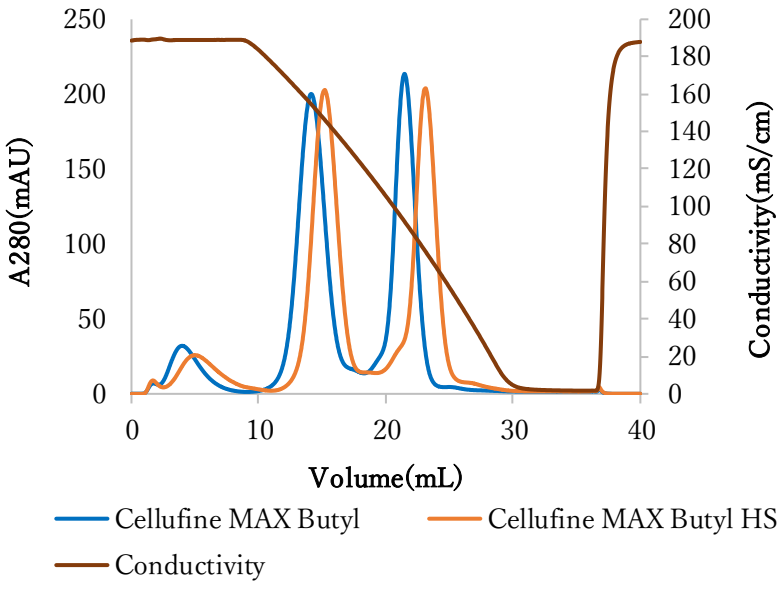
Compression Factor: 1.30

Temp.: 24±1 °C

Mobile Phase: Pure water

Hydrophobic strength

The figure below shows the separation characteristics of model proteins of Cellufine MAX Butyl HS. From this result, the hydrophobic strength is in the order of MAX Butyl HS > MAX Butyl.



Column: I.D. 6.6mm × H 5cm

Pritens: Ribonuclease A, Lysozyme, α-chymotrypsinogen A

Elution: 10 mM PB (pH7.0), Gradient

Column Packing

1. Calculate volume required for 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 1.25-1.33
2. Prepare a 40 – 60 % (v/v) slurry with 50 mM sodium phosphate, 1 M $(NH_4)_2SO_4$, at pH 7.0.
3. Gently stir or place under vacuum to degas.
4. With column outlet closed, carefully pour the slurry into the column. Depending on the volume, a filler tube may be necessary.
5. With the inlet open to release air, insert and fix the top adapter at the slurry surface.
6. Open column outlet and begin pumping buffer at a rate 20-30 % higher than the operational flow rate.
7. After the bed stabilizes, stop pump and close the column outlet. Then with the inlet open, reposition the top adaptor on top of the bed. Equilibrate with 10 column volumes adsorption buffer before sample loading.

Operating Guidelines

General Operation

1. Equilibrate the column with adsorption buffer. The typical loading buffer is 50 mM of sodium phosphate, pH 7.0 containing 0.5 - 2.5 M Na_2SO_4, $(NH_4)_2SO_4$ or NaCl.
2. Load the sample.
3. Wash 5 CV with adsorption buffer to remove non-adsorbed impurities.
4. Elute the adsorbed molecules with elution buffer.

A general operating 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 for using Cellufine MAX Butyl HS

Process		CV	Explanation of each process
1	Equilibration	3 – 10	Ensure that the pH and conductivity of the column buffer is the same as the required sample load buffer. 50 mM Na phosphate, pH 7.0 + 0.5-2.5 M sodium sulfate
2	Sample load	-	Flow-through or bind and elute mode cam be used.
3	Wash	5	Use adsorption buffer.
4	Elution	3 – 7	10 mM Na phosphate buffer, pH 7.0
5	CIP	3 – 10	0.5 M NaOH

Recommend buffers

Adsorption/Sample Loading Buffer: Common adsorption buffers are 50 mM Na Phosphate, pH 7.0 + 0.5-2.5 M Sodium Sulfate or Ammonium Sulfate or Sodium Chloride. The strength of adsorption is affected by salt concentration, pH and temperature. In general, using a salt with a high salt concentration result in a high adsorption capacity.

Elution buffer: Elution is performed by decreasing the salt concentration. Elution is performed stepwise elution or gradient elution using a low-salt buffer (0.5 M concentration or less). Chaotropic reagents (KSCN), detergents (Octyl glycoside, CHAPS, Triton X, Chaps, Tween), and denaturants (guanidine hydrochloride, urea, ethanol) may improve the recovery of strongly adsorbed proteins.

Sample preparation and sample-loading

Replace the sample with adsorption buffer. 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

The recommended flow pressure for hydrophobic Cellufine MAX media is less than 0.3MPa. Generally, low flow rates are used for binding and elution, while higher flow rates are acceptable for washing and regeneration steps.

Chemical Stability

The resin can be used stably under pH 2-13 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 0.5 M NaOH. Autoclavable in suspension at neutral pH for 20 minutes at 121 ºC.

Regeneration and depyrogenation

Wash the column with 2 - 5 bed volumes of 0.5 N NaOH. In some cases, an additional wash with 2 - 5 bed volumes of 70 % EtOH/ 30 % distilled water / 0.1 M AcOH followed by distilled water may be required to remove adsorbed lipids.

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

Chromatography was performed according to the following method.

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

Column: 6.7 mmID x 30 mm (1.06 mL)

Flow rate: 0.212 mL/min (RT 5 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

5. 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	Pr/Ps %	NA/Ps%
Load sample	726	-	46	73	772	10	106
After column	518	89	54	N.D.	436	0	84

Storage

Short term (2 weeks or less), bulk and column can be stored at room temperature with 2 M $(NH_4)_2SO_4$ or 0.05 N NaOH. 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 Butyl HS	5 x 1 mL Mini-column	22200-51
	5 x 5 mL Mini-column	22200-55
	100 mL	22200
	500 mL	22201
	5 L	22202
	10 L	22203

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