Operating Instructions

Hydrophobic Interaction Chromatography Media

Cellufine MAX Butyl

Description

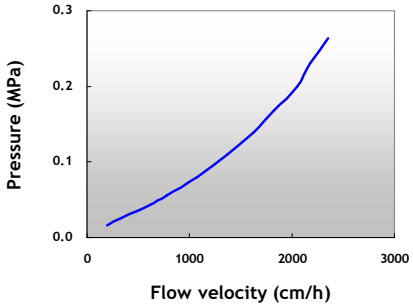
Cellufine MAX Butyl is useful for the chromatography of hydrophobic proteins. Many proteins have hydrophobic amino acid residues which will interact with the butyl functional groups. Factors that influence this hydrophobic interaction include salt concentration, temperature, pH, organic solvents and surfactants. Protein adsorption usually occurs at high ionic strength, while elution occurs at lower salt concentrations. This is the opposite and complementary to ion exchange chromatography. This is the opposite of ion exchange chromatography and offers complementary separation benefits.

Physical-Chemical Characteristics

	Cellufine MAX Butyl
Support matrix	Highly cross-linked cellulose
Particle shape	Spherical
Particle diameter (μm)	ca. 40 – 130
Ligand type	Butyl
BSA capacity (mg/ml)	≧ 9
BSA elution efficiency (%)	70
Polyclonal IgG 10% DBC (mg/ml)	17
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 products have superior flow-pressure properties.



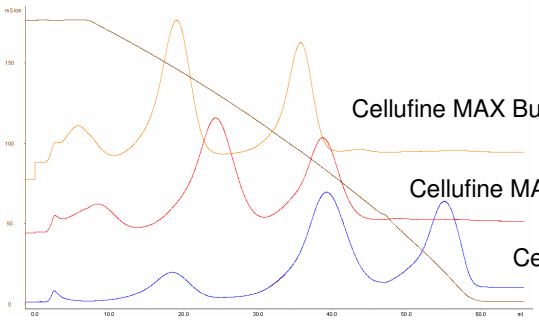
Column: 2.2cm Diam. x 20 cm L

Temperature: 24±1 ºC

Mobile phase: water

Retention of model proteins

The figure bellow shows separation properties of Cellufine MAX butyl in comparison with Cellufine MAX Phenyl and Cellufine MAX Phenyl LS. Protein separation studies show that relative binding strengths are MAX Phenyl > MAX Phenyl LS > MAX Butyl.



Column: 6.6mm Diam. × 5cm L
Protein: Ribonuclease A,Lysozyme,
α-Chymotripsinogen A
Elution: 10 mM PB (pH7.0) 1.5 →
0 M $(NH_4)_2SO_4$ gradient

Column Packing

1. Calculate volume required for the desired bed dimension.
2. Prepare a 40 – 60 % (v/v) slurry in 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 affix the top adjuster assembly at the slurry interface.
6. Open column outlet and begin pumping buffer at a rate 20-30 % higher than the operational flow rate.
7. After the bed stabilizes, close the column outlet. Then with the inlet open, reposition the end cell on top of the bed. Equilibrate with 10 column volumes adsorption buffer before sampling.

Operating Guidelines

General Operation

Equilibrate column with 2 – 5 volumes of elution buffer (low salt concentration), then wash with the same amount of loading 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. Adsorption strength is a function of salt concentration, pH and temperature. In general, high concentration promotes adsorption. Desorption (elution) is then accomplished by lowering the salt concentration. Fore more information, see References.

Sample Preparation and Load

Samples are ideally prepared in loading buffer. Filtration may be required to remove insoluble matter. If necessary, buffer exchange may be accomplished using diafiltration or desalting chromatography. Protein adsorption and recovery will vary with each packing. Usually, in terms of binding strength, Cellufine MAX Phenyl > MAX Phenyl LS > MAX Butyl. The sample (prepared in the loading buffer) is applied after washing of column with the loading buffer. After loading of sample, flush with 5 column volumes of loading buffer to remove unbound material. Subsequently, bound product can be eluted.

Flow Rate and Elution

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.

Desorption of bound material is accomplished by step or gradient elution with low concentration of salt (e.g., less than 0.5 M). The use of chaotropic agents (e.g., KSCN), surfactants (e.g., Octyl Glucoside, CHAPS, Triton X, Chaps or Tween), denaturating agent (e.g., guanidine hydrochloride, urea, ethanol) will improve the recovery of tightly adsorbed proteins.

Chemicals and Physical Stability

pH 2 – 13, when operated 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 using 0.5 N NaOH.

Autoclavable in suspension at neutral pH for 20 minutes at 121 ºC.

Regeneration

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

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 0.02 % sodium azide or 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

Product Ordering Information (Catalogue No.)

Media type	Pack Size					
	Mini-column 1 ml x 5	Mini-column 5 ml x 5	100ml	500ml	5 lt	10 lt
Cellufine MAX Butyl	21100-51	21100-55	21100	21101	21102	21103

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