Cellufine

Operating Instructions

Anion Exchange Chromatography Media

Cellufine A-500

Description

Cellufine A-200, A-500 and A-800 media are designed for the anion exchange chromatography of proteins, peptides and other biomolecules. The media are comprised of beaded spherical cellulose, functionalized with DEAE (diethylaminoethyl).

The pore size and structure of each packing determines its respective applications. Cellufine A-200 medium is ideal for the chromatography of low molecular weight peptides or proteins (< 30kD), A-500 for proteins up to 500 kD and A-800 for use with larger biomolecules up to 1000 kD. The superior rigidity of Cellufine media allows for high flow rates, and thus, rapid processing times.

Physical-Chemical Characteristics

	A-200	**A-500**	A-800
Support matrix	cellulose		
Particle shape	spherical		
Particle diameter (µm)	ca. 40 – 130		
Ion capacity (meq/g dry)	0.9	**1.3**	0.8
MW exclusion limit (kD)	30	**500**	1000
pH stability range	1 - 13		
Operating pressure	< 2 bar (29 psi)		
Supplied	suspension in 20 % EtOH		

Column Packing

1. Slurry the appropriate volume in 2 – 3 volumes of elution buffer (high salt) and allow to equilibrate at ambient temperature for one hour.
2. Gently stir or place under vacuum to degas.
3. With column outlet closed, carefully pour the slurry into the column. If necessary, fit column with a filler or extension tube to accommodate the entire slurry volume.
4. Attach upper end cell to column, then pump 10 column volumes of elution buffer at a flow rate 20 % – 50 % greater than the operational flow rate.
5. After flushing, remove filter tube and reattach end cell to the column tube.
6. Equilibrate with 5 –10 column volumes of adsorption buffer in preparation for sample loading.

Operating Guidelines

General Operation

Typically, adsorption to Cellufine Anion Exchange medium occurs in relatively low ionic strength (e.g., below 0.1 M NaCl) in the pH range from 6.0 – 8.5. Under these conditions, most proteins with neutral or net negative charge will bind. Bound components are then resolved by stepwise elution with buffers containing progressively higher salt concentration or by elution with a linear salt gradient.

Recommended Buffers

Adsorption buffer: 0.02 – 0.05 M sodium phosphate (pH 7.5) or Tris-HCl (pH 8.0).

Elution buffer: 0.1 – 2.0 M sodium chloride in adsorption buffer.

Other common buffer systems may be used. For additional information on chromatographic methods of protein purification, see References.

Sample Preparation and Load

Prepare sample by centrifugation or microfiltration to remove insoluble material. Samples should be prepared to a concentration of 1 – 20 mg/ml, in adsorption buffer or at a comparable conditions of ionic strength and pH. If necessary, exchange sample buffer using dialysis, diafiltration or desalting chromatography.

Flow Rate

The recommended linear velocity range for Cellufine A-200, A-500 and A-800 media is 50 – 200 cm/h.

Chemical and Physical Stability

Stable in:

- Most salts (NaCl, $(NH_4)_2SO_4$, etc.)
- Most detergents (SDS, Tween®, Chaps, etc.)
- < 0.5 N NaOH

Autoclavable: 121 °C at 1 bar (14.5 psi) for 20 minutes

Regeneration and Depyrogenation

To regenerate a column, flush bed with 2 - 5 column volumes of 0.5 N NaOH, followed by several volumes of elution buffer. Then equilibrate as usual. If the column needs to be pyrogen free, wash the column with 2 - 5 column volumes of 0.5 N NaOH followed by several column volumes of pyrogen free elution buffer. Monitor the pyrogen levels in the column eluate during a blank gradient

elution prior to reusing the column.

Storage

Store unopened container at ambient temperature. Do not freeze.

Short term storage for bulk and column (2 weeks or less) can be at a room temperature with 0.05 M NaOH. Longer storage should be in neutral buffer containing 0.02 % sodium azide or 20 % ethanol, at 2 – 8 °C. 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				
	MC* 1ml x 5	100ml	500ml	5 lt	10 lt
Cellufine A-200		676 980 327	19611	19612	676 980 335
Cellufine A-500	**19805-51**	**675 980 327**	**19805**	**19806**	**675 980 335**
Cellufine A-800		673 980 327	19800	19801	673 980 335
Cellufine Q-500	19907-51	675 982 327	19907	19908	675 982 335
Cellufine C-500	19800-51	675 983 327	19865	19866	675 983 335

MC = Mini-Column