Operating Instructions

Cation Exchange Chromatography Media

Cellufine™ S-500

Description

Cellufine S-500 media manufactured by JNC are designed for the cation exchange chromatography of basic proteins and other biomolecules. The resins are comprised of beaded spherical cellulose, functionalized with sulfobutyl as ligand. Cellufine S-500 medium is ideal for the chromatography of proteins molecular weight up to 500 kD. The superior rigidity of Cellufine gels allows for high flow rates, and thus rapid processing times, even in large diameter process scale columns.

Physical-Chemical Characteristics

	Cellufine S-500
Support matrix	Cellulose
Particle shape	Spherical
Particle diameter (μm)	ca. 40 – 130 (average 90)
Ion capacity (meq/mL)	0.11 – 0.22
MW exclusion limit (kD)	500
pH stability range	2 – 13
Operating pressure	< 0.2 MPa
Supplied	suspension in 20 % EtOH

Column Packing

1. Prepare a 40 – 60 % (v/v) slurry with water, 0.1 M NaCl solution or the appropriate buffer 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 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 10 – 15 column volumes of adsorption buffer in preparation for sample loading.

Operating Guidelines

General Operation

Typically, adsorption to Cellufine Cation Exchange medium occurs in relatively low ionic strength (e.g., below 0.1 M NaCl) in the pH range from 5.0 – 7.0. Under these conditions, proteins with neutral or net positive 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.

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 binding buffer or at a comparable condition of ionic strength and pH. If necessary, exchange sample buffer using dialysis, diafiltration or desalting chromatography.

Recommended Buffers

Adsorption buffer: 0.02 – 0.05 M sodium acetate (pH 5.5)
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.

Chemical and Physical Stability

Stable in:

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

Flow Rate

The recommended linear velocity range for Cellufine S-500 media is 50 – 200 cm/h.

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 to 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

Short term (1 week or less), bulk and column can be stored at a room temperature with 0.05 N NaOH. Longer storage should be in neutral buffer containing 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					
	Mini-Column 1 mL x 5	Mini-Column 5 mL x 5	100 mL	500 mL	5 L	10 L
Cellufine™ S-500	21200-51	21200-55	21200	21201	21202	201203

JNC CORPORATION

Life Chemicals Division

2-1, Otemachi 2-Chome, Chiyoda-ku, Tokyo 100-8105, Japan

Phone +81-3-3243-6150, Fax+81-3-3234-6219

E-mail : cellufine@jnc-corp.co.jp

http://www.jnc-corp.co.jp/fine/en/cellufine

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provide such information which is provided without charge at the Buyer's risk and which **is provided subject to the**

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12. **Governing Law** - This contract shall be governed by and construed in accordance with the laws (other than those relating to conflict of laws questions) of the Commonwealth of Japan.

13. **Arbitration** - Any and all disputes or controversies arising under, out of or in connection with this contract or the sale or performance of the Products shall be resolved by final and binding arbitration in Tokyo under the rules of the Japan Arbitration Association then obtaining. The arbitrators shall have no power to add to, subtract from or modify any of the terms or conditions of this contract. Any award rendered in such arbitration may be enforced by either party in either the courts of the Commonwealth of Japan District Court for the District of Japan, to whose jurisdiction for such purposes JNC or Buyer each hereby irrevocably consents and submits.