

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

	Cellufine A-200	Cellufine A-500	Cellufine 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
Swelling degree (ml / g dry media)	6-9	8-10	12-16
pH stability range	1 - 13		
Tapped volume (ml / g succession media)	1.3 – 1.5	1.3 – 1.5	1.4-1.6
Operating pressure	< 2 bar (29 psi)		
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 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, $(\text{NH}_4)_2\text{SO}_4$, etc.)

Most detergents (SDS, Tween®, Chaps, etc.)

< 0.5 M 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 M 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 M 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					
	Mini-Column 1ml x 5	Mini-Column 5ml x 5	100ml	500ml	5 lt	10 lt
Cellufine A-200	19611-51		676 980 327	19611	19612	676 980 335
Cellufine A-500	19805-51	19805-55	675 980 327	19805	19806	675 980 335
Cellufine A-800	19865-51	19865-55	673 980 327	19800	19801	673 980 335
Cellufine Q-500	19907-51	19907-55	675 982 327	19907	19908	675 982 335
Cellufine C-500	19800-51	19800-55	675 983 327	19865	19866	675 983 335

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>

TERMS AND CONDITIONS OF SALE

1. **Acceptance**-Buyer's placement of this order shall create a contract subject to and expressly limited by these terms and conditions. Acceptance may only be made on the exact terms and conditions hereof and if additional or different terms are proposed by Buyer, such response shall constitute a counteroffer. **THE TERMS OF THIS CONTRACT SHALL SUPERSEDE ANY CONFLICTING TERMS CONTAINED ON BUYERS PURCHASE ORDER OR ANY DOCUMENT OR INSTRUMENT SUBMITTED BY BUYER.**

2. **Prices, Taxes and Payment** - All prices are firm unless otherwise agreed to in writing. JNC Corporation reserves the right to change the prices and specifications of its Products at any time without notice. Any tax, duty, custom or other fee of any nature imposed upon this transaction by any federal, state or local governmental authority shall be paid by Buyer in addition to the price quoted or invoiced. In the event JNC Corporation is required to prepay any such tax, Buyer will reimburse JNC Corporation. Payment terms shall be net 30 days after shipment by JNC Corporation. An interest charge equal to 1½% per month (18% per year) will be added to invoices outstanding beyond 30 days after shipment. In addition JNC Corporation reserves the right to require C.O.D. payment terms from any Buyer whose account is overdue for a period of more than 60 days or who has an unsatisfactory credit or payment record. JNC Corporation may also refuse to sell to any person until overdue accounts are paid in full.

3. **Delivery and shipment** - JNC Corporation will make every effort to ship the Products or provide the services hereunder in accordance with the requested delivery date, provided, that JNC Corporation accepts no liability for any losses or for general, special or consequential damages arising out of delays in delivery. Shipment of all Products shall be F.O.B. point of distribution by JNC Corporation; identification of the Products shall occur when they leave JNC Corporation's point of distribution, at which time title and risk of loss shall pass to Buyer. All shipment costs shall be paid by Buyer and if prepaid by JNC Corporation the amount thereof shall be reimbursed to JNC Corporation.

4. **Inspection** - Buyer shall be responsible for inspecting all Products shipped hereunder prior to acceptance, provided, that if, Buyer shall not have given JNC Corporation written notice of rejection within 30 days following shipment to Buyer, the Products shall be deemed to have been accepted by Buyer.

5. **Disclaimer of Express and Implied Warranties** - The Products shall be covered by the applicable JNC Corporation standard warranty. **NO OTHER EXPRESS OR IMPLIED WARRANTY IS MADE WITH RESPECT TO THE PRODUCTS. JNC CORPORATION EXPRESSLY EXCLUDES THE IMPLIED WARRANTIES OF MERCHANTABILITY AND OF FITNESS FOR A PARTICULAR PURPOSE.** Any model or sample furnished to the Buyer is merely illustrative of the general type and quality of goods and does not represent that the Products will conform to the model or sample. Buyer's remedies under JNC Corporation's warranty shall be limited to repair or replacement of the Product or component which failed to conform to JNC Corporation's warranty. **JNC Corporation shall not be liable for any consequential damages or economic loss or property damage incurred by Buyer.**

6. **Returned Goods** - No Products shipped under this contract may be returned without the express prior authorization of JNC Corporation. All returns of Products are subject to a restocking charge. No returns will be authorized after 120 days following shipment to Buyer.

7. **Technical Advice** - JNC Corporation may, at Buyer's request, furnish technical assistance, advice and information with respect to the Products if and to the extent that such advice, assistance and information is conveniently available. It is expressly agreed that there is no obligation to provide such information which is provided without charge at the Buyer's risk and which **is provided subject to the**

disclaimers set forth in paragraph 5 above.

8. **Agents, etc.** - No agent, employee or other representative has the right to modify or expand JNC Corporation's standard warranty applicable to the Products or to make any representations as to the Products other than those set forth in JNC Corporation's product literature and any such affirmation, representation or warranty, if made, should not be relied upon by Buyer and shall not form a part of this contract.

9. **Fair Labor Standards** - JNC Corporation represents that the Products or services provided hereunder were produced and/or performed in compliance with the requirements of all sections of the Fair Labor Standard Act of 1938, as amended.

10. **Equal Employment Opportunity** - JNC Corporation is an Equal Opportunity Employer. It does not discriminate in any phase of the employment process against any person because of race, color, creed religion, national origin, sex, age, veteran or handicapped status. The JNC Corporation Equal Opportunity Certificate, which is mailed annually to all vendors and vendees, is incorporated into this contract by reference.

11. **Modifications, Waiver, Termination** - This contract may be modified and any breach hereunder may be waived only by a writing signed by the party against whom enforcement thereof is sought.

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 Corporation and Buyer each hereby irrevocably consents and submits.