

Operating Instructions

Ion exchange Chromatography Media Cellufine[®] MAX Q-r, Cellufine[®] MAX Q-h

Description

Cellufine MAX is the new name of 2nd generation Cellufine chromatography media.

Cellufine MAX Q strong ion exchangers are highly cross-linked, surface modified media with high dynamic binding capacities and stability at high flow velocities. These high performance optimized media offer significant opportunity for increasing downstream purification throughput.

The characteristics of Cellufine MAX “-r” sires are high recovery, high resolution and robust. The highest adsorption capacity of Cellufine MAX “-h” in present product (2010) is the characteristic.

Characteristics of Cellufine MAX

Type	Cellufine MAX Q-r	Cellufine MAX Q-h
Ion exchange type	Strong Anion / -N ⁺ (CH ₃) ₃	
Matrix	Highly cross-linked cellulose with dextran scaffold	
Particle size	ca. 40 – 130 μm	
Ion exchange capacity	0.17~0.24 m eq /ml	0.13~0.20 m eq /ml
Flow velocity	600cm/h(0.3Mpa) I.D.30cm-L20cm,pure water at 24° C	
Dynamic binding capacity	>110 mg BSA/ml	>180mg BSA/ml
pH working range	2 – 12	2 – 12
pH stability (40° C, 1week)	2 – 12	2 – 12
Chemical stability	Stable all commonly used aqueous buffers, 1M NaOH	
Storage	20% ethanol	

Packing to the column

- Calculate volume required of the desired bed dimension.
 - Packed bed volume= column cross sectional area (cm²) x bed height (cm)
 - Required sedimented gel volume=Packed bed volume x 1.1
 - The slurry concentration for bottle of Cellufine MAX Q is approximately 50% in 20% ethanol.
- Washing the gel with water or the appropriate buffer.
- Prepare a 40 – 60 % (v/v) slurry with water or the appropriate buffer (high salt). Allow to equilibrate at ambient temperature for one hour.

4. Gently stir. If required place under vacuum to degas.
5. Column
 - (a) A column is prepared according to the instruction of the column.
 - (b) A filter is dipped in a packing solution or 20% ethanol before use and extracts air.
 - (c) Pour the packing solution into the column tube and it checks that the solution comes out from a column exit certainly. A stop plug is shut when approximately 0.5 to 1cm height of the solution remains.
6. Carefully pour the slurry into the column without air bubbles. Depending on the volume, a filler tube may be necessary.
7. Mount the top adapter on the top of column. (Don't put in air)
8. Open the column outlet and begin pumping elution buffer for 10 min at 1000cm/h or 0.3Mpa. Caution: do not exceed the operation pressure limit for using column.
9. Mark the gel bed height. Stop the pumping and stop plug in column outlet.
10. Disconnect the top adapter line from the pump. The seal is loosened and move the top adapter to mark of the gel bed hit at packing.
11. After the bed stabilizes, lock the adapter and set the line from the pump. Equilibrate with 10 column volumes of adsorption buffer before sample loading.

Packing to the fixed length column

1. Calculate volume required of the desired bed dimension.
 - (a) Packed bed volume= column cross sectional area (cm²) x bed height (cm)
 - (b) Required sedimented gel volume=Packed bed volume x 1.1
 - (c) Note: When using a packing connector, the amount of excesses can be used from required sedimented gel volume.
 - (d) The slurry concentration for bottle of Cellufine MAX Q is approximately 50% in 20% ethanol.
2. Washing the gel with water or the appropriate buffer.
3. Prepare a 40 – 60 % (v/v) slurry with water or the appropriate buffer (high salt). Allow to equilibrate at ambient temperature for one hour.
4. Gently stir. If required place under vacuum to degas.
5. Column
 - (a) A column is prepared according to the instruction of a using column.
 - (b) A filter is dipped in a packing solution or 20% ethanol before use and extracts air.
 - (c) Pour the packing solution into the column tube and it checks that the solution comes out from a column exit certainly. A stop plug is shut when approximately 0.5 to 1cm high of the solution remains.
6. Carefully pour the slurry into the column and packing connector without air bubbles. Depending on the volume, a filler tube may be necessary.

7. Mount the top adapter on the top of packing connector.
8. Open the column outlet and begin pumping elution buffer for 10 min at 1000cm/h or 0.3Mpa. Caution: do not exceed the operation pressure limit for using column.
9. Stop the pumping and stop plug in column outlet.
10. Disconnect the top adapter line from the pump. Remove the packing connector. Before remove excess medium from the packing connector, if necessary.
11. Mount the top adapter, lock the adapter and set the line from the pump. Equilibrate with 10 column volumes of adsorption buffer before sample loading.

Evaluation of packing

See appendix 1

Operating Guidelines

General Operation

Typically, adsorption to Cellufine MAX Anion Exchange medium occurs in relatively low ionic strength (e.g., below 0.1 M NaCl) in the pH range from 6 – 9 under the *pI* of the target protein.

The binding capacity is strongly affected by pH and conductivity. Under these conditions, 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.

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 comparable conditions of ionic strength and pH. If necessary, exchange sample buffer using dialysis, diafiltration or desalting chromatography.

Recommended Buffers

Adsorption buffer: 0.01 – 0.05 M phosphate or Tris-HCl (pH 6 to 9)

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.

Regeneration and equilibration

After separation, wash the binding material with over 5-bed volume of high ionic strength solution (1-2M NaCl). After washing the column, feed the over 5-bed of the adsorption buffer, or until the column eluate will be stable pH and conductivity values.

Depyrogenation

The column wash with 5-bed volume of 0.2 M NaOH let stand for 16 hours, and then wash with endotoxin-free water or equilibration buffer.

- 0.2 M NaOH-20% EtOH is more effective for endotoxin-free. Moreover 0.2M NaOH-90% EtOH can rapidly decrease LPS, at least contact for 2 hours.

Chemical and Physical Stability

Stable in:

Most salts (NaCl, $(\text{NH}_4)_2\text{SO}_4$, etc.), Alcohol (30 % (v/v) IPA, 70 % (v/v) EtOH),

Urea (6M) and Guanidine-HCl (6M)

pH 2 to pH12 (MAX Q-r & MAX Q-h) at 40 °C, 1week

0.1M NaOH at less than 20°C, 4weeks (MAX Q-r & Q-h)

Cleaning-in-place (CIP)

Cellufine MAX Q performance remains constantly at least 300 CIP operating cycles with 0.5M NaOH.

Flow Rate

Cellufine MAX Q-r and MAX Q-h are based on highly cross-linked cellulose gel, and have stability at high flow velocities.

1,000cm/h in a 2.2cm diameter column with 20cm bed height at <0.3Mpa.

Over 500cm/h 30cm diameter column with 20cm bed height at <0.3Mpa.

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 pH 2 to pH 13(MAX Q-r and MAX Q-h). It is possible to store under alkaline condition at less than 20 °C as recommendation.

0.1M NaOH ; MAX S-h for 2 months, MAX S-r for 2 months

0.5M NaOH ; MAX S-h for less than 1 week, MAX S-r for 2 week

Longer storage should be in neutral buffer containing 20 % ethanol, at 2 – 8 °C. Do not freeze.

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	MC 5ml x 5	100ml	500ml	5L	10L
Cellufine MAX Q-r	20500-51	20500-55	20500	20501	20502	20503
Cellufine MAX Q-h	20600-51	20600-55	20600	20601	20602	20603

MC = Mini-Column

JNC CORPORATION

Life Chemicals Sales Department

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

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

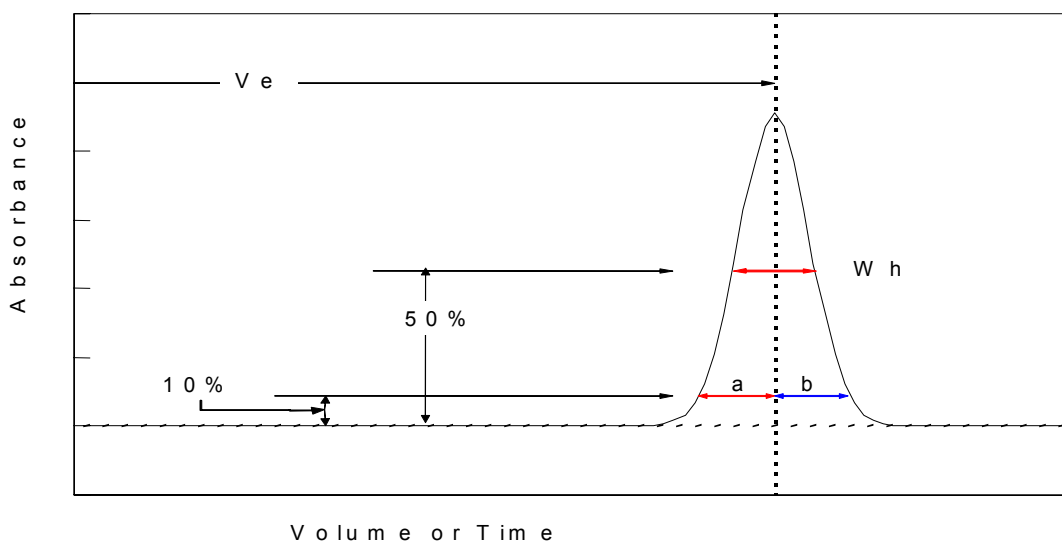
E-mail: cellufine@jnc-corp.co.jp<http://www.jnc-corp.co.jp/fine/en/cellufine>

Appendix 1: Evaluation of column packing of Cellufine

Evaluation methods for the packing status of a column can employ indices, such as the number of (theoretical) plates (N), the height equivalents to a theoretical plate (HETP), and a symmetry factor (As).

The selected evaluation indices are affected by the measurement condition. For example, they vary with changes in the difference between the diameter/height of a column, the difference in piping, solvent sample volume, the flow velocity, temperature, etc. Therefore, it is necessary to use the same measurement conditions each time.

Conditions	
Sample volume	1% (MAX 2.5%) of column bed volume
Sample concentration	1-2 % (V/V) acetone or 1M NaCl
Flow rate	~30 cm/h (X mL/hr/column cross section)
Detector	UV, conductivity



Formula
$HETP = L/N$
$N = 5.54 \times (Ve/Wh)^2$

L	Column length [cm or m]
Ve	Elution time or volume
Wh	Half of width of peak
a,b	Peak width of 10% peak height (a)front, (b)rear
Note	Ve,Wh and a, b should have same dimensional units

(Note)

Generally, a larger value of N is good. (Likewise a smaller value of HETP is good.)

The asymmetry factor value (As) should be close to 1. Generally, acceptable symmetry values range from 0.8-1.6.

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.