Cellufine™ S-500

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.

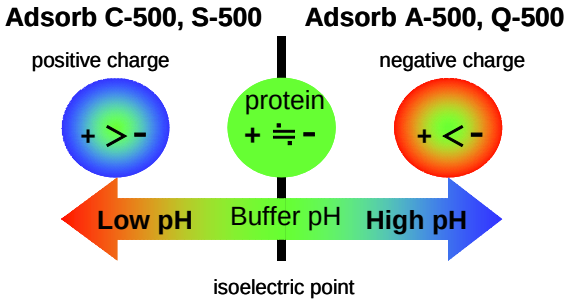
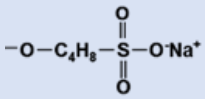


Table 1, Characteristics of Cellufine S-500

		S-500
Ligand		 sulfobutyl group
Matrix		Spherical cellulose particle
Particle size		40 - 130 μm（ca.90 μm）
MW exclusion limit (kD)		500
Ion exchange capacity (meq/mL-gel)		0.11-0.22
Operating pressure		＜0.2 MPa
Dynamic binding capacity (mg/mL-gel)	lysozyme	156
	human gamma globulin	42
Recommended CIP solution		0.5M NaOH
pH stability range		2-13
Storage		2-8 ºC in 20 % ethanol

※Values in Table 1 are not specifications.

Column Packing

Materials

- Cellufine S-500
- Lab scale column, adapter, reservoir
- Pump
- Filtration equipment (Glass filter, Buchner-Roth, aspirator, etc.)
- Graduated cylinder
- Packing solution (water, NaCl solution※, buffer※)
- Mobile phase of packed column evaluation (water, NaCl solution※, buffer※)
- Sample of packed column evaluation (1-2 %(v/v)acetone or 1M NaCl)

※NaCl solution：low salt concentration solution such as 0.1M NaCl solution
※buffer：Adsorption buffer , etc.

Preparation of slurry

1) After the bottle is warmed to room temperature, shake it several times to equalize the slurry in the bottle.
2) Aspirate through a glass filter and wash 3 times with 5 times the volume of the packing solution. Remove 20% ethanol as a preservative. If necessary, the slurry may be washed by decantation.
3) Transfer the resin to a beaker and add packing solution to make a 50-60% (v/v)slurry. Suspend the resin and remove the air under decompression for 30-40 minutes. Slow stirring with a magnetic stirrer can effectively remove the air.

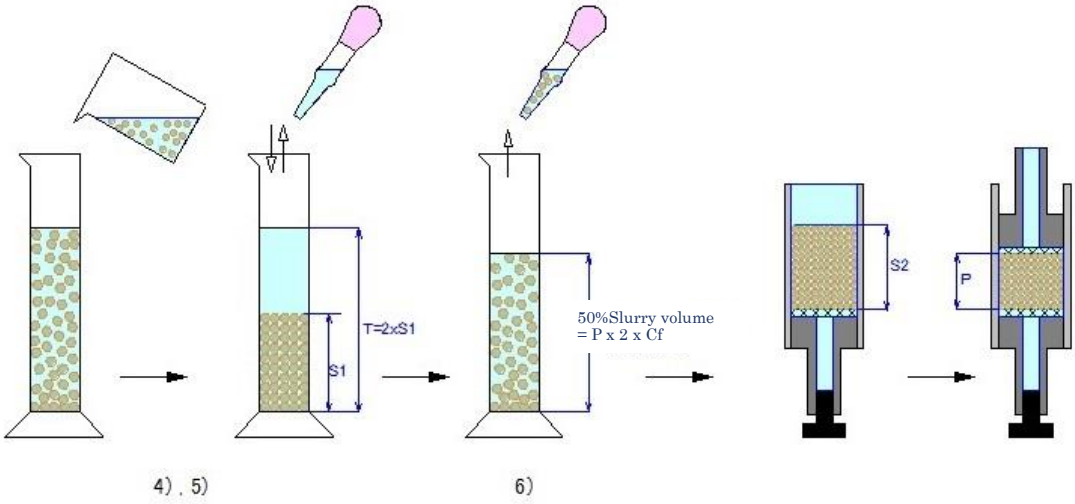


Figure2　Preparation of slurry

4) Pour the slurry into a graduated cylinder and leave it to stand for at least 4 hours. Measure bed height (volume) of a gravity settled bed and calculate the slurry concentration.

Slurry concentration (%) =
Gravity settled bed volume (S1) / Total slurry volume (T)×100

5) Adjust to a 50 % (v/v) slurry concentration of resin with packing solution.
6) Calculate the volume of slurry required to pack the column.

50% slurry volume required to packing = (Target packing volume (P) x 2）x Cf

※Cf = [gravity settled bed volume (S2) / Target packing volume (P)]

Note: *Compression factor (Cf) affects the column packing efficiency (see Appendix 1 for details) and may need to be optimized by adjusting the axial compression on the column.*

Column packing

1) Set up the column. After opening the column outlet, add packing solution to remove air remaining in the filter. Leave about 1 cm of packing solution from the bottom of the column to prevent air from entering.
2) Close the column outlet and pour the slurry into the column all at once to keep air out.
3) Open the column outlet to allow the resin to settle. As the resin settles, the liquid surface becomes clear. Close the outlet when the packing solution becomes clear to 2-3 cm from the liquid surface.
4) Carefully fill the column to the top with packing solution. Keep the settling resin from floating away.
5) Set the adapter on the column to prevent air from entering the liquid surface. Close the adapter O-ring, lower the adapter, and remove the air inside.
6) Connect the column to the pump and keep the packing solution flowing at a pressure lower than the operating pressure for 30 to 60 minutes. (＜ 0.2MPa)

Note: *The flow velocity : Internal pressure at packing ＞ Operating pressure after packing*

7) After the resin height is stabilized, stop the flow. Close the column outlet. Disconnect the inlet piping at the top of the column. Slowly lower the adapter to the surface of the resin. At this time, the packing solution in the column flows out from the column inlet.
8) Connect to the adapter inlet with the piping filled with packing solution to exclude air. Open the column outlet and pump the packing solution. (＜ 0.2MPa)
9) If the resin compresses and makes space between it and the adapter, lower the adapter to the surface of the resin.
10) Calculate column volume from column height. If the column volume is larger than the target column volume, lower the adapter to the target height. If the column volume is lower than the target column volume, the slurry concentration is low, or the resin may be excessively compressed. Remove the resin from the column and pack again.

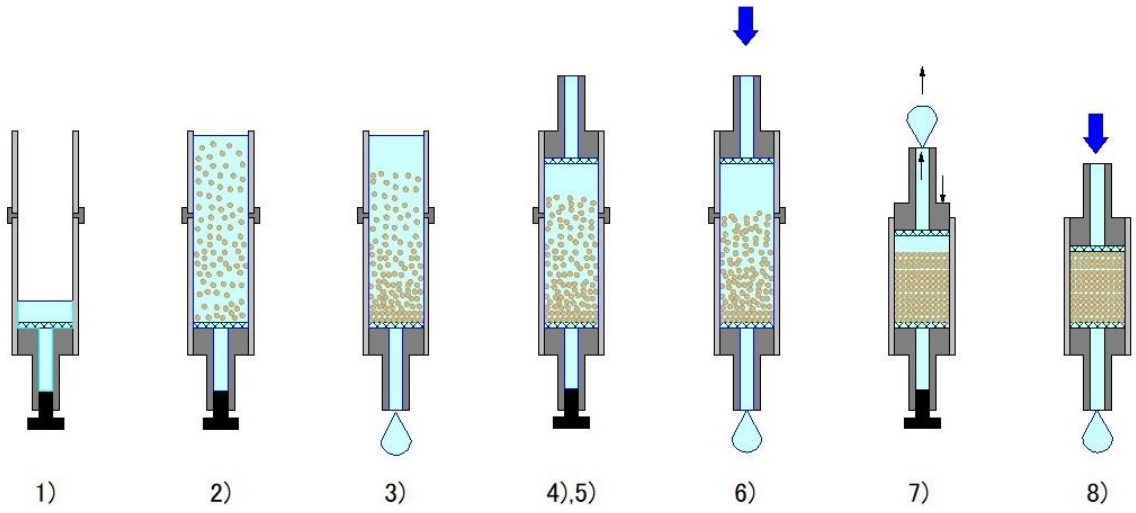


Figure 3　Process of column packing

Evaluation of column packing

Column packing efficiency is evaluated by checking HETP and asymmetry (As). (Appendix 1)

Operating Guidelines

How to use

1) Equilibrate the column with adsorption buffer. (3-5 column volume (CV))
2) Load the column with sample dissolved in adsorption buffer. (1-20mg/mL)
3) Wash with adsorption buffer to remove impurities. (2-3 CV)
4) Elute the adsorbed target substance with elution buffer.

※There is also a method that allows impurities to be adsorbed and the target substance to pass through.

Recommended Buffers

In general, adsorption on Cellufine cation exchangers occurs at relatively low ionic strength (e.g., below 0.1 M NaCl) in the pH range 5.0 to 7.0. For cation exchange, the adsorption buffer should have a pH value at least 1 pH lower than the isoelectric point of the target sample. Bound samples are separated by stepwise elution with a high salt concentration buffer or by elution with a linear salt concentration gradient.

Adsorption buffer： 0.02 – 0.05 M sodium acetate (pH 5.5)
Elution buffer：Use an adsorption buffer with 0.1-2.0 M NaCl.

In a volume of 5-10 CV, increase the NaCl concentration by gradient to a final concentration of about 0.5 M. If the target sample is not eluted, further increase the NaCl concentration or change the pH. Stepwise elution is commonly used in preparative chromatography, and the optimum salt concentration for elution should be determined by a preliminary gradient elution study.

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.

Flow Rate

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

Regeneration and Depyrogenation

Regenerate with 5 CV of 1-3 M NaCl-containing buffer. If inadequate, wash with 3-10 CV of 0.1 N HCl followed by 1-3 M NaCl-containing buffer. Then equilibrate with adsorption buffer and prepare for the next operation.

If a pyrogen-free column is required, wash the column with 5 CV of 0.2 M NaOH solution and keep it for 16 hours or overnight (3-5 hours for 0.2 M NaOH-20% EtOH or 1 hour for 0.2 M NaOH-90% EtOH). After washing with pyrogen-free elution buffer, check the endotoxin concentration and equilibrate with the adsorption buffer to be used.

Cleaning in Place

After washing ionically adsorbed substances by the same operation as for regeneration, flow 5 CV of 0.2 M NaOH (recommended flow rate: 30-60 cm/h). If contamination is significant, flow 5 CV of 0.5 M NaOH. Then equilibrate with adsorption buffer.

To wash strongly hydrophobic materials, wash with 70% EtOH or 30% IPA. In this case, avoid air bubbles in the column by varying the concentration of the solvent in the gradient.

※When using organic solvents, bubbles are more likely to form in the column. If bubbles are generated, the column will need to be packed again.

Stability

pH range of 2 to 13 and operating temperature of 2 to 30°C are recommended.

Storage

Store unused resin in its container at a temperature of 2 to 8°C. Equilibrate opened resin and packed column in 20% ethanol and store at 2 to 8°C. Do not freeze. Shelf Lifetime is 5 years from 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.)

	Pack Size	Catalogue No.
S-500	1 mL x 5 （mini-column）	21200-51
	5 mL x 5 （mini-column）	21200-55
	100 mL	21200
	500 mL	21201
	5 L	21202
	10 L	201203

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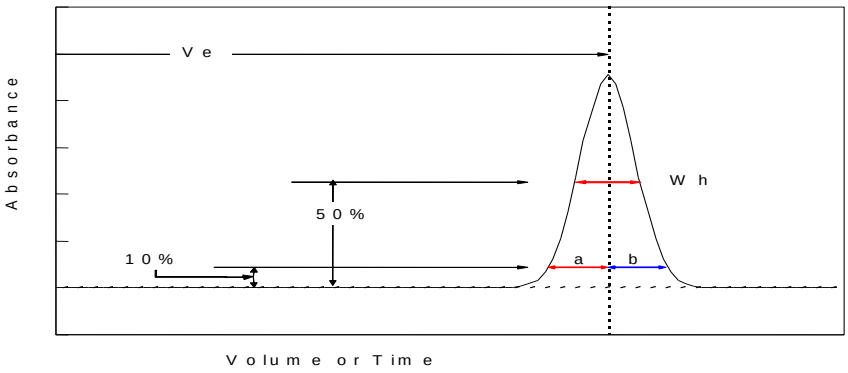
Appendix 1: Evaluation of column packing

The packing condition of the column is evaluated using indices such as theoretical number of stage plates (N), theoretical stage equivalent height (HETP), and asymmetry (As). These indices are affected by the measurement conditions. For example, these indices vary with column diameter/height differences, piping, solvent sample volume, flow rate, temperature, etc. Therefore, it is necessary to use the same measurement conditions each time. A flow velocity of 30 cm/h is recommended, but higher velocities are possible. However, the higher the flow velocity, the smaller the theoretical number of plates (N). The same conditions (flow rate, column size, mobile phase, and sample) must be used each time to evaluate the column.

Parameter	Condition
Sample volume	1 -2.5% of column volume (CV)
Sample concentration	1-2 %(v/v) acetone (mobile phase：water or adsorption buffer)
	1M NaCl （mobile phase： 0.1～0.4M NaCl aq）
Flow rate (cm/h)	30 cm/h
Detector	UV, Conductivity

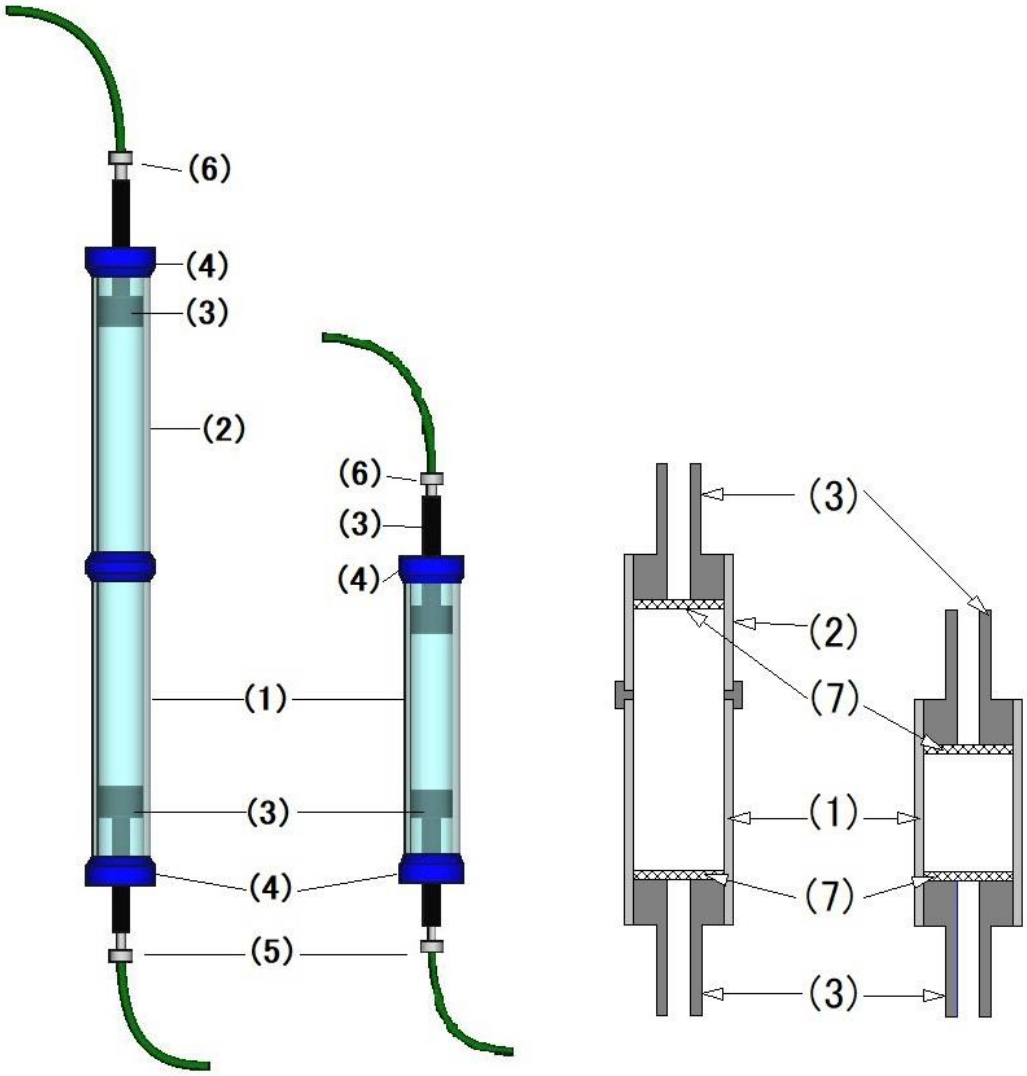


L	Column length [cm or m]
V_e	Elution time or volume
W_h	Half of width of peak
a, b	Peak width of 10% peak hight (a) front (b) rear
Note	Ve,Wh and a, b should have same dimensional units

HETP = L/N　　N = 5.54 x (Ve/Wh)^2　　As = b/a

Generally, number of (theoretical) plates (N) is good if it is over 3000. Acceptable asymmetry factor (As) values range from 0.7-1.5.

Appendix 2 : Figure of columns



The Operating Instructions use a simple cross-sectional figure of the column, as shown on the right.

(1)	Column tube	(4)	Column end
(2)	Reservoir	(5)	Column outlet
(3)	Adapter	(6)	Column inlet
(7)	Filter		

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6. **Returned Goods** - No Products shipped under this contract may be returned without the express prior authorization of JNC. 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 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's standard warranty applicable to the Products or to make any representations as to the Products other than those set forth in JNC'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 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 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 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 or Buyer each hereby irrevocably consents and submits.