Phenyl / Phenyl LS

Cellufine MAX Phenyl is useful for the chromatography of hydrophobic proteins. Many proteins have hydrophobic amino acid residues which will interact with the phenyl functional groups. Factors that influence this hydrophobic interaction include salt concentration, temperature, pH, organic solvents and surfactants. Protein adsorption usually occurs at high ionic strength, while elution occurs at lower salt concentrations. This is the opposite and complementary to ion exchange chromatography. This is the opposite of ion exchange chromatography and offers complementary separation benefits. Table 1 shows the characteristics of Cellufine MAX Phenyl.

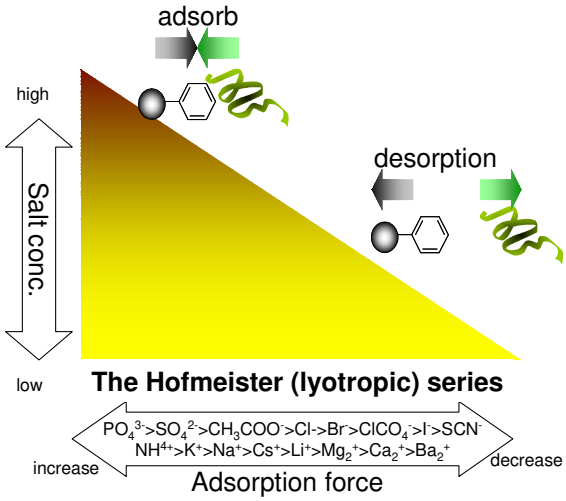
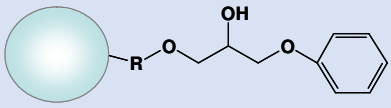
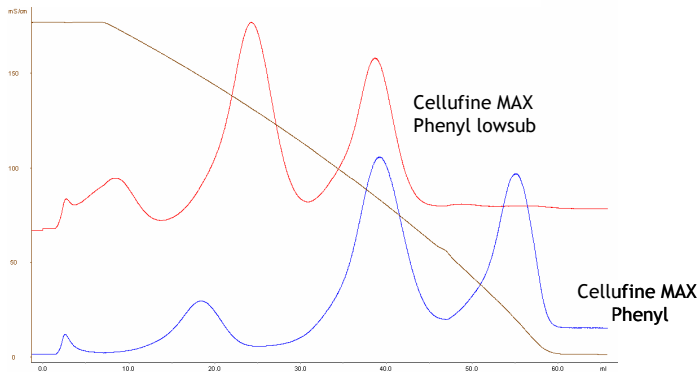


Table 1, Characteristics of Cellufine MAX Phenyl

	Cellufine MAX Pheynl	Cellufine MAX Pheynl LS
Ligand	 Phenyl	
Matrix	Highly cross-linked cellulose	
Particle size	40 - 130 µm（ca.90 µm）	
MW exclusion limit (kD)	1000	
BSA capacity (mg/mL-gel)	11	4
BSA elution efficiency (%)	40	90
Polyclonal IgG 10% DBC (mg/mL-gel)	31	19
Operating pressure	＜0.3 MPa	
pH stability range	2 - 13	
Storage	2-8 ºC in 20 % ethanol	

※Values in Table 1 are not specifications.

The figure bellow shows separation properties of Cellufine MAX Phenyl. Cellufine MAX Phenyl LS has lower ligand density than standard MAX Phenyl.

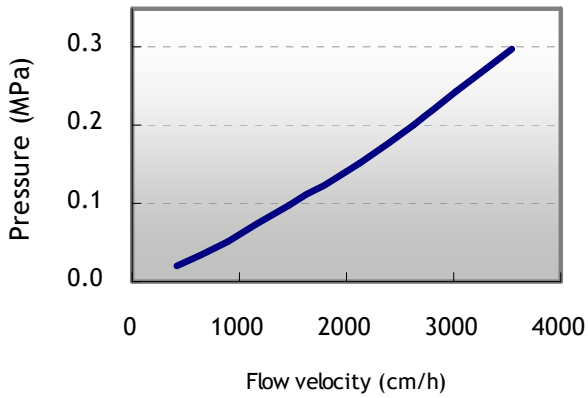


Column: 6.6mm Diam. × 5cm L

Protein: Ribonuclease A,Lysozyme, α-Chymotripsinogen A

Elution: 10 mM PB (pH7.0) 1.5 → 0 M $(NH_4)_2SO_4$ gradient

Cellufine MAX Phenyl products have superior flow-pressure properties.



Column: 2.2cm Diam. x 20 cm L, Temperature: 24±1 ºC, Mobile phase: water

Column Packing

Materials

- Cellufine resin
- Lab scale column, adapter, reservoir
- Pump
- Filtration equipment (Glass filter, Buchner-Roth, aspirator, etc.)

- Graduated cylinder
- Packing solution (water , buffer)
- Mobile phase of packed column evaluation (water, NaCl solution, buffer)
- Sample of packed column evaluation (1-2 %(v/v)acetone or 1M NaCl)

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.

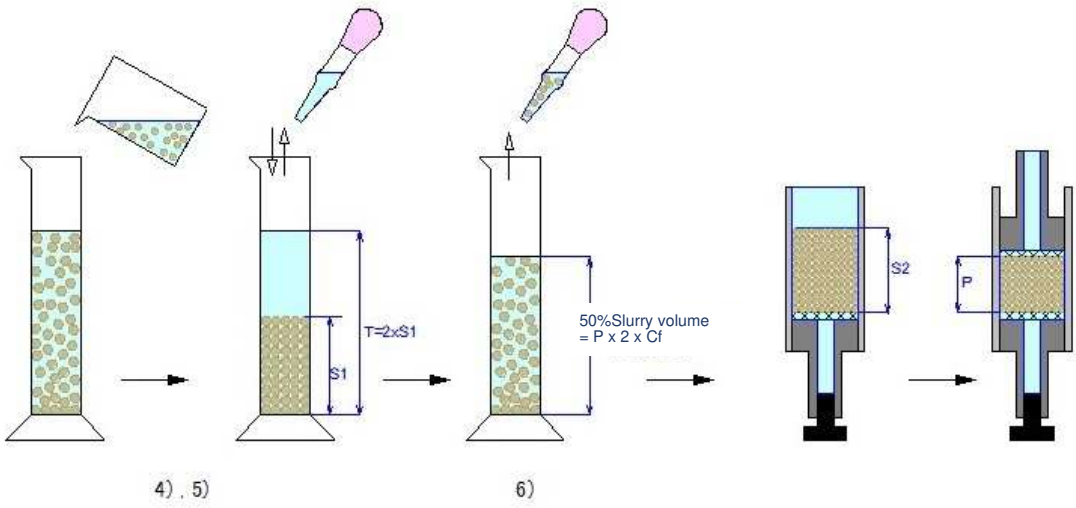


Figure1 Preparation of slurry

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 size（ Diameter × Bed height）	Recommended Cf (approximately) MAX Pheynl or MAX Phenyl LS
10.0 cm× 20 cm	1.15～1.25

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. (＜ 0.3MPa)

   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. (＜ 0.3MPa)
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.

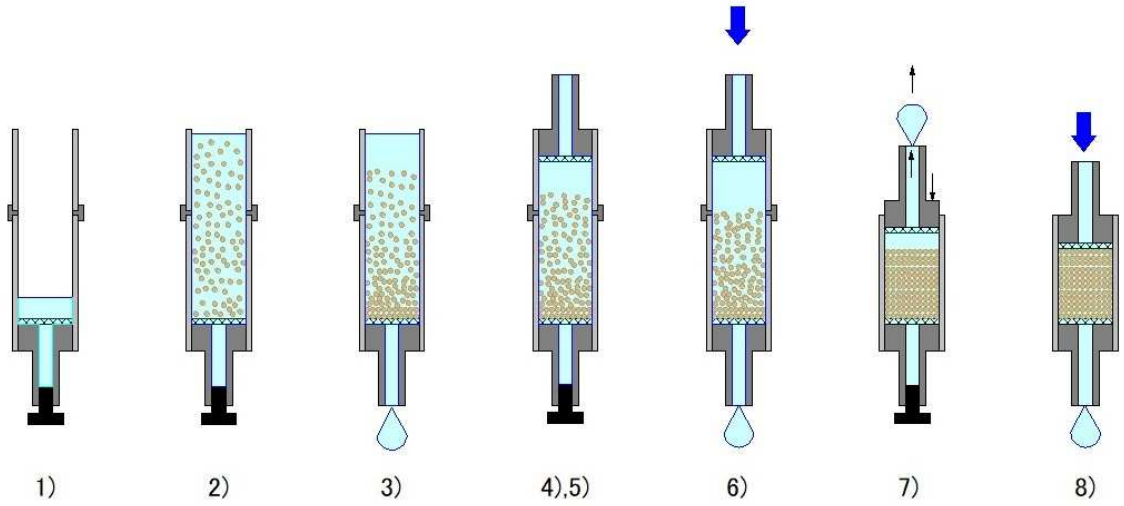


Figure 2 Process of column packing

Evaluation of column packing

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

Operating Guidelines

How to use

1) Equilibrate column with 2 – 5 volumes of elution buffer (low salt concentration), then wash with the same amount of loading buffer.
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.

Recommended Buffers

Adsorption buffer : The typical loading buffer is 50 mM of sodium phosphate, pH 7.0 containing 0.5 – 2.5 M Na_2SO_4, $(NH_4)_2SO_4$ or NaCl. Adsorption strength is a function of salt concentration, pH and temperature. In general, high concentration promotes adsorption.

Elution buffer : Desorption of bound material is accomplished by step or gradient elution with low concentration of salt (e.g., less than 0.5 M). The use of chaotropic agents (e.g., KSCN), surfactants (e.g., Octyl Glucoside, CHAPS, Triton X, Chaps or Tween), denaturating agent (e.g., guanidine hydrochloride, urea, ethanol) will improve the recovery of tightly adsorbed proteins.

Sample Preparation and Load

Prepare samples at a concentration of 1 – 20 mg/ml, in adsorption buffer. Remove insoluble material by centrifugation or microfiltration. If necessary, exchange sample buffer using dialysis, diafiltration or desalting chromatography.

Protein adsorption and recovery will vary with each packing. Usually, in terms of binding strength, Cellufine MAX Phenyl > MAX Phenyl LS > Butyl. The sample (prepared in the loading buffer) is applied after washing of column with the loading buffer. After loading of sample, flush with 5 column volumes of loading buffer to remove unbound material. Subsequently, bound product can be eluted.

Flow Rate

MAX Phenyl ≦800cm/h（＜0.3MPa）
MAX Phenyl LS ≦1200cm/h（＜0.3MPa）

The recommended flow pressure for hydrophobic Cellufine MAX media is less than 0.3MPa. Generally, low flow rates are used for binding and elution, while higher flow rates are acceptable for washing and regeneration steps.

Regeneration and Depyrogenation

Flush the column with 2 - 5 bed volumes of 0.5M NaOH. In some cases, an additional flush with 2 - 5 bed volumes of 70 % EtOH/30 % DIW /0.1 M AcOH followed by distilled water may be required to remove adsorbed lipids.

Stability

pH 2 – 13, when operated at room temperature. Stable in most salts (NaCl, $(NH_4)_2SO_4$, etc.) and most detergents (SDS, Tween etc) and other chemicals (70% ethanol, 30% isopropyl alcohol, 6M Guanidine hydrochloride and Urea). The products can be cleaned using 0.5 M NaOH.
Autoclavable in suspension at water for 20 minutes at 121 ºC.

Storage

Store unused resin in its container at a temperature of 2 to 8°C. Short term (2 weeks or less), bulk and column can be stored at room temperature with 2 M $(NH_4)_2SO_4$ or 0.05 N NaOH. Long term storage should be in neutral buffer containing 0.02 % sodium azide or 20 % EtOH at 25 ºC or lower temperature. Do not freeze.Shelf Lifetime is 5 years from manufacture.

Refernces

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.
Cellufine MAX Phenyl	1 mL x 5 (Mini-Column)	20700-51
	5 mL x 5 (Mini-Column)	20700-55
	100 mL	19524
	500 mL	19545
	5 L	19546
	10 L	684 987 330
Cellufine MAX Phenyl LS	1 mL x 5 (Mini-Column)	20800-51
	5 mL x 5 (Mini-Column)	20800-55
	100 mL	20800
	500 mL	20801
	5 L	20802
	10 L	20803

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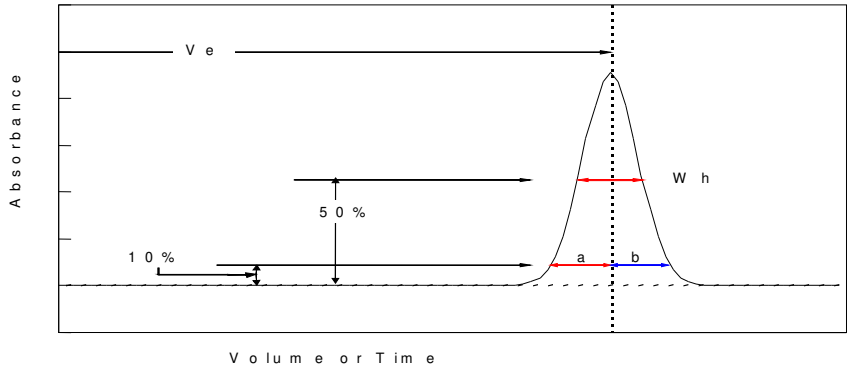
E-mail : cellufine@jnc-corp.co.jp
http://www.jnc-corp.co.jp/fine/en/cellufine

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

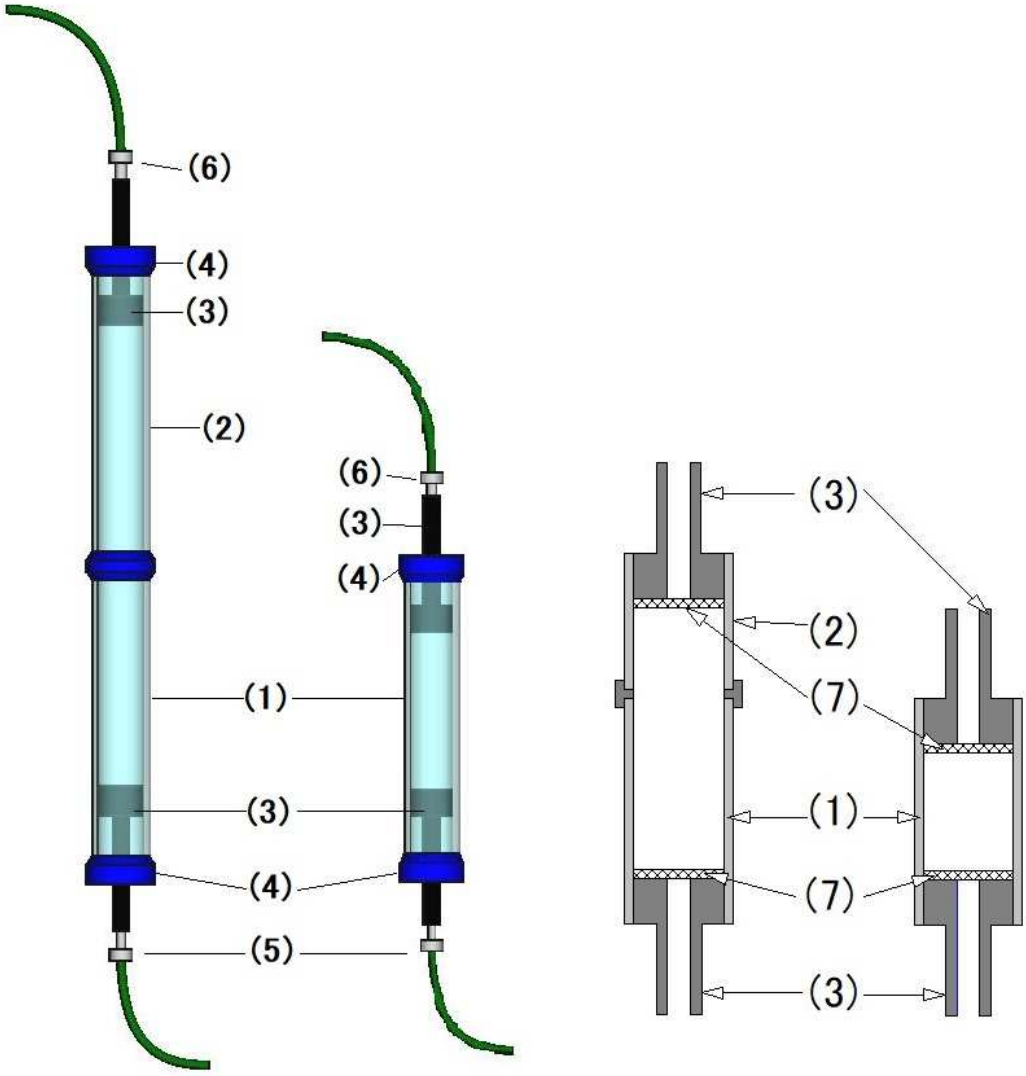


L	Column length [cm or m]
V_e	Elution time or volume
W_h	Half of width of peak
a, b	Peak width of 10% peak hight (a) front (b) rear
Note	Ve,Wh and a, b should have same dimensional units

$HETP = L/N$ $N = 5.54 \times (Ve/Wh)^2$ $As = b/a$

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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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
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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.