

## Operating Instructions

### Strong cation exchange Chromatography Media Cellufine™ MAX S-r, Cellufine™ MAX S-h

#### Description

Cellufine MAX is the new name of 2<sup>nd</sup> generation Cellufine chromatography media. Cellufine MAX S strong ion exchangers are highly cross-linked, surface modified media with high dynamic binding capacities and stable at high flow velocities. These high performances of the optimized media offer significant opportunity for increasing downstream purification throughput.

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

#### Characteristics of Cellufine MAX

Type	S-r	S-h
Ion exchange type	Strong cation / -SO <sub>3</sub> <sup>-</sup>	
Matrix	Highly cross-linked cellulose with dextran scaffold	
Particle size	ca. 40 – 130 μm	
Ion exchange capacity	0.09 ~ 0.21 meq /ml	0.10 ~ 0.22 meq /ml
Flow velocity	600 cm/h(0.3 MPa) I.D.30 cm-L 20 cm, pure water at 24°C	
Dynamic binding capacity	> 130 mg IgG /ml gel	> 180 mg IgG /ml gel
pH working range	2 – 13	3 - 14
pH stability (40°C, 1week)	2 – 14	3 - 14
Chemical stability	Stable all commonly used aqueous buffers, 1 M NaOH	
Storage	20 % ethanol	

#### Packing to the column

1. Calculate volume required for the desired bed dimension.
  - (a) Packed bed volume = column cross sectional area (cm<sup>2</sup>) x bed height (cm)
  - (b) Required sedimented gel volume ÷ Packed bed volume x 1.2
  - (c) The slurry concentration in the bottle of Cellufine MAX S is approximately 50% in 20 % ethanol.

2. Wash the gel with water or the appropriate buffer.
3. Prepare a 40 – 60 % (v/v) slurry with water, 0.1 M NaCl solution or the appropriate buffer. 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 instructions from the column supplier.
  - (b) The bed support should be wetted in a packing solution or 20 % ethanol before use to remove air.
  - (c) Pour the packing solution into the column tube and it check that solution flows out from the column exit. Shut the exit valve when approximately 0.5 to 1cm height of the solution remains.
6. Carefully pour the slurry into the column without creating air bubbles. Depending on the volume, a filler tube may be necessary.
7. Mount the top adapter on the top of column. (Be careful to not entrain air)
8. Open the column outlet and begin pumping elution buffer for 10 min at 1000cm/h or 0.3Mpa. Caution: do not excess the operation pressure limit for the selected column.
9. Mark the gel bed height. Stop the pump and shut the column outlet valve.
10. Disconnect the top adapter line from the pump. Loosen the adaptor seal and move the top adapter down to mark the gel bed reached at packing.
11. After the bed stabilizes, lock the adapter and reconnect 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 (cm<sup>2</sup>) x bed height (cm)
  - (b) Required sedimented gel volume=Packed bed volume x 1.15
  - (c) Note: When using a packing connector, the amount of excess gel corresponds to the required sedimented gel volume.
  - (d) The slurry concentration in the bottle of Cellufine MAX S is approximately 50 % in 20 % ethanol.
2. Wash the gel with water, 0.1 M NaCl solution or the appropriate buffer.
3. Prepare a 40 – 60 % (v/v) slurry with water, 0.1 M NaCl solution or the appropriate buffer. 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 instructions from the column supplier.
  - (b) The bed support wetted in a packing solution or 20% ethanol before use to remove air.
  - (c) Pour the packing solution into the column tube and it check that solution flows out from the column exit. Shut the exit valve when approximately 0.5 to 1 cm high of the solution remains.
6. Carefully pour the slurry into the column and packing connector without creating 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 0.3 Mpa. Caution: do not excess the operation pressure limit for the selected column.
9. Stop the pump and shut the column outlet valve.
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 reconnect 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 Cation Exchange medium occurs in relatively low ionic strength (e.g., below 0.1 M NaCl) in the pH range from 4.0 – 9.0 below the *pI* of the target protein.

The binding capacity is strongly affected by pH and conductivity. The binding capacity vs. pH and NaCl concentration in matrix is show below with typical proteins. As thus examples, it should be necessary to find the optimum condition by screening tests in the target protein. 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.

Cellufine MAX S-r 10%DBC of polyclonal		pH			Cellufine MAX S-h 10%DBC of polyclonal IgG(mg/mL)		pH		
		4.3	5.0	5.5			4.3	5.0	5.5
NaCl mM (in 10mM acetate buffer)	10	133	192	158	NaCl mM (in 10mM acetate buffer)	10	207	228	238
	50	159	107	3		50	188	199	2
	100	121	1	-		100	150	1	-
Cellufine MAX S-r 10%DBC of Lysozyme(mg/mL)		pH			Cellufine MAX S-h 10%DBC of Lysozyme(mg/mL)		pH		
		7.8	8.5	9.5			7.8	8.5	9.5
NaCl mM (in 50mM Tris-HCl buffer)	10	124	143	160	NaCl mM (in 50mM Tris-HCl buffer)	10	156	167	198
	50	93	119	134		50	104	134	156
	100	59	69	-		100	63	74	-

Table. 10%DBC vs. pH and NaCl concentration

### 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 sodium acetate (pH 4 to 6), and 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 volumes of high ionic strength solution (1-2 M NaCl). After washing the column, pump an additional 5-bed volume of adsorption buffer, or until the column eluate will be stable pH and conductivity values.

### Depyrogenation

Wash the column with 5-bed volumes 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 effective for endotoxin removal. Moreover 0.2 M NaOH-90 % EtOH can rapidly decrease LPS, with contact for at least 2 hours.

### Chemical and Physical Stability

Stable in:

Most salts (NaCl, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, etc.), Alcohol ( 30 % (v/v) IPA, 70 % (v/v) EtOH),  
Urea (6 M) and Guanidine-HCl (6 M)  
pH 2 to pH14 (MAX S-r) , pH3 to pH14 (MAX S-h) at 40 °C, 1week  
0.1 M NaOH at less than 20°C, 3 months (MAX S-r & S-h)

### Cleaning-in-place (CIP)

Cellufine MAX S performance remains constant for at least 100 CIP operating cycles employing 0.5 M NaOH.

### Flow Rate

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

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

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

### 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 14(MAX S-r) and pH 3 to pH 14(MAX S-h). It is possible to store under alkaline condition at less than 20 °C as recommendation.

0.1M NaOH ; MAX S-h for 2-4 months, MAX S-r for 2-6 months

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

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*. 2<sup>nd</sup> 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	100 mL	500 mL	5 L	10 L
Cellufine MAX™ S-r	20300-51	20300-55	20300	20301	20302	20303
Cellufine MAX™ S-h	20400-51	20400-55	20400	20401	20402	20403

MC = Mini-Column

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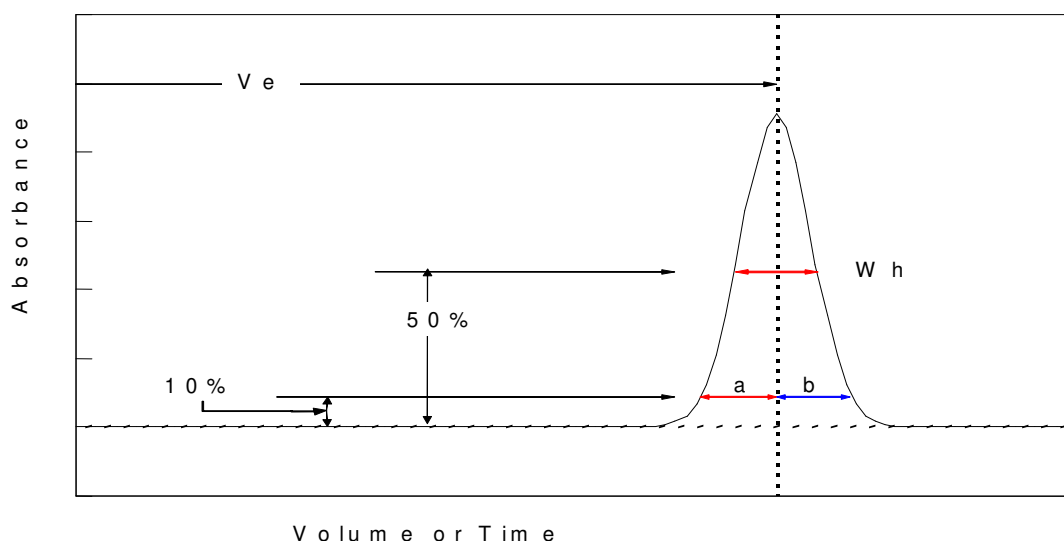
<http://www.jnc-corp.co.jp/fine/en/cellufine>

## 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% (MAX2.5%) of column bed volume
Sample concentration	1-2%(V/V)acetone or 1M NaCl
Flow rate	~30cm/h ( X mL/hr/column cross section )
Detector	UV, conductivity



<b>Formula</b>
<b>HETP = L/N</b>
<b>N = 5.54 x (Ve/Wh)<sup>2</sup></b>

L	Column length [ cm or m ]
Ve	Elution time or volume
Wh	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

(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.

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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.