

## Operating Instructions

### Hydrophobic Interaction Chromatography Media Cellufine MAX Phenyl / MAX Phenyl LS

#### Description

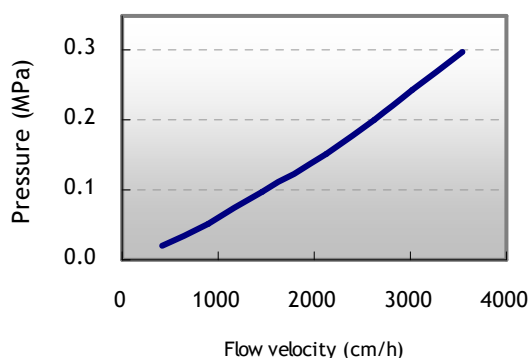
Cellufine MAX Phenyl is useful for the chromatography of hydrophobic proteins. Many proteins have hydrophobic amino acid residues which will interact with the phenyl functional groups. Factors that influence this hydrophobic interaction include salt concentration, temperature, pH, organic solvents and 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.

#### Physical-Chemical Characteristics

|                                | Cellufine MAX Phenyl          | Cellufine MAX Phenyl LS |
|--------------------------------|-------------------------------|-------------------------|
| Support matrix                 | Highly cross-linked cellulose |                         |
| Particle shape                 | Spherical                     |                         |
| Particle diameter (µm)         | ca. 40 – 130                  |                         |
| Ligand type                    | Phenyl                        |                         |
| BSA capacity (mg/ml)           | 14                            | 6                       |
| BSA elution efficiency (%)     | 40                            | 90                      |
| Polyclonal IgG 10% DBC (mg/ml) | 31                            | 19                      |
| MW exclusion limit (kD)        | 1,000                         |                         |
| pH stability range             | 2 – 13                        |                         |
| Operating pressure             | < 0.3 MPa                     |                         |
| Supplied                       | suspension in 20 % EtOH       |                         |

#### Flow pressure property

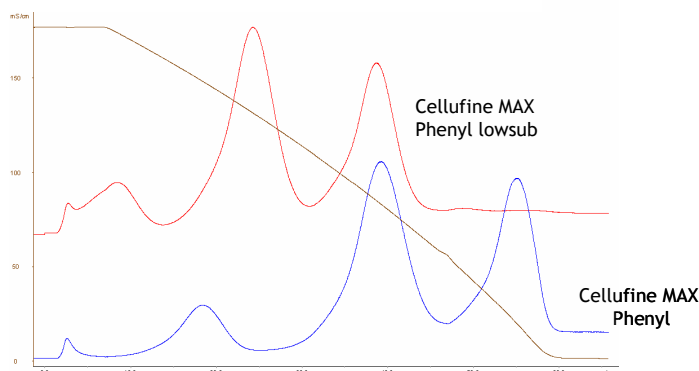
Cellufine MAX Phenyl products have superior flow-pressure properties.



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

### Retention of model proteins

The figure below 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-Chymotrypsinogen A  
Elution: 10 mM PB (pH7.0) 1.5 →  
0 M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> gradient

### Column Packing

1. Calculate volume required for the desired bed dimension.
2. Prepare a 40 – 60 % (v/v) slurry in 50 mM sodium phosphate, 1 M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, at pH 7.0.
3. Gently stir or place under vacuum to degas.
4. With column outlet closed, carefully pour the slurry into the column. Depending on the volume, a filler tube may be necessary.
5. With the inlet open to release air, insert and affix the top adjuster assembly at the slurry interface.
6. Open column outlet and begin pumping buffer at a rate 20-30 % higher than the operational flow rate.
7. After the bed stabilizes, close the column outlet. Then with the inlet open, reposition the end cell on top of the bed. Equilibrate with 10 column volumes adsorption buffer before sampling.

### Operating Guidelines

#### General Operation

Equilibrate column with 2 – 5 volumes of elution buffer (low salt concentration), then wash with the same amount of loading buffer. The typical loading buffer is 50 mM of sodium phosphate, pH 7.0 containing 0.5 – 2.5 M Na<sub>2</sub>SO<sub>4</sub>, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> or NaCl. Adsorption strength is a function of salt concentration, pH and temperature. In general, high concentration promotes adsorption. Desorption (elution) is then accomplished by lowering the salt concentration. For more information, see References.

### **Sample Preparation and Load**

Samples are ideally prepared in loading buffer. Filtration may be required to remove insoluble matter. If necessary, buffer exchange may be accomplished using 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 and Elution**

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.

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), denaturing agent (e.g., guanidine hydrochloride, urea, ethanol) will improve the recovery of tightly adsorbed proteins.

### **Chemicals and Physical Stability**

pH 2 – 13, when operated at room temperature. Stable in most salts (NaCl,  $(\text{NH}_4)_2\text{SO}_4$ , 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 N NaOH.

Autoclavable in suspension at neutral pH for 20 minutes at 121 °C.

### **Regeneration**

Flush the column with 2 - 5 bed volumes of 0.5N 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.

### **Storage**

Short term (2 weeks or less), bulk and column can be stored at room temperature with 2 M  $(\text{NH}_4)_2\text{SO}_4$  or 0.05 N NaOH. Long term storage should be in neutral buffer containing 0.02 % sodium azide or 20 % EtOH at 25 °C or lower temperature. 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               |                         |       |       |       |       |
|-------------------------|-------------------------|-------------------------|-------|-------|-------|-------|
|                         | Mini-column<br>1 ml x 5 | Mini-column<br>5 ml x 5 | 100ml | 500ml | 5 lt  | 10 lt |
| Cellufine MAX Phenyl    | 20700-51                | 20700-55                | 20700 | 20701 | 20702 | 20703 |
| Cellufine MAX Phenyl LS | 20800-51                | 20800-55                | 20800 | 20801 | 20802 | 20803 |

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