Cellufine™ MAX DexS

Cellufine MAX DexS is a new chromatography resin incorporating Dextran sulfate polymer surface modification. This non-animal derived pseudo affinity ligand can be used instead of immobilized Heparin for blood protein fractionation and viral capture.

JNC offers two different Cellufine DexS resins, surface modified with different lengths of Dextran Sulfate polymer; a) DexS-HbP developed for purification of **h**eparin **b**inding **p**roteins. and b) DexS-VirS, for purifying **virus** and virus like particles especially having affinity for heparin. Both resins have been developed to improve the protein or virus capture efficiency of existing resins by employing a pseudo affinity mimetic polymer surface based on Dextran Sulfate. The cross-linked cellulose base bead has been optimized for high flow applications and is fully compatible up to 0.5 M NaOH for CIP. Resin properties are summarized in Table 1 below.

Table 1. Performance Characteristics of Cellufine MAX DexS resins.

Performance Characteristics		
Product name	Cellufine MAX DexS-HbP	Cellufine MAX DexS-VirS
Ligand	Dextran sulfate	
Matrix	Cross-linked cellulose beads	
Particle size	40 – 130 µm (ca. 90 µm)	
Sulfur contents (µmol/mL)	≥ 36	≥ 74
Lysozyme adsorption capacity (mg/ml)	≥ 50	≥ 56
pH stability	3 – 12	
Operating pressure	< 0.3 MPa	
Supplied	50% (v/v) suspension in 0.1M Phosphate – 20% (v/v) EtOH	

Column Packing

1. Calculate volume required for the desired bed dimension.
2. Prepare a 40 – 60% (v/v) slurry with water, 0.1 M NaCl solution or the appropriate buffer. Allow the gel to equilibrate at ambient temperature for one hour.
3. Gently stir or place under vacuum to degas.
4. With column outlet closed, carefully pour the slurry into 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 the column outlet and begin pumping elution buffer at rate 10 – 20% greater 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 of adsorption buffer before sample loading.

Operating Guidelines

General Operation

1. Equilibrate column with adsorption buffer.
2. Load sample at pH 4 – 9.
3. Wash with several bed volumes of adsorption buffer to remove non-binding contaminants.
4. Elute bound solute(s) with desorption buffer.

General operation procedure of Cellufine DexS is outlined in Table 2, below. Each condition such as pH, buffer, salt, flow rate and so on can be optimized for any purpose.

Table 2. General operating procedure for Cellufine DexS.

Step		CV	Notes/Explanation of the step
1	Equilibration	3 – 10	To ensure that the buffer pH and conductivity throughout the column are the same as the required loading conditions.
2	Loading	Variable	Introduction of the sample into the column for; a) flow through or b) bind and elute modes of chromatography.
3	Washing	5	10 mM Na Phosphate buffer, 0.15 M NaCl, pH 7.5
4	Elution	3 – 7	10 mM Na Phosphate buffer, 1.0 M NaCl, pH 7.5
5	Re-Equilibration	3 – 10	10 mM Na Phosphate buffer, 0.15 M NaCl, pH 7.5
6	CIP	3 – 10	0.1 – 0.5 M NaOH

Recommended Buffers

Adsorption/loading buffer: 10 mM Na phosphate buffer, 0.1 M NaCl, pH 7.5. Depending on the application, other buffer ions may be used. In general, adsorption strength varies inversely with pH and ionic strength. Increasing ionic strength slightly can aid in removing loosely bound contaminants. Non-ionic detergents (Tween®20、Triton® X, etc.) may also added to improve solubility.

Elution buffer: In general use mobile phase consisting of adsorption/load buffer with 1 – 2 M NaCl or KCl. The exact concentration can be determined by gradient elution. Step gradients are typically employed for preparative applications.

Sample Preparation and Load

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

Flow Rate

The recommended linear velocity range for Cellufine MAX DexS is 100 – 300 cm/h (Buffer). It should be operated with suitable flow velocity less than the maximum (0.3 MPa) pressure of the column.

Chemical and Physical Stability

pH 3 – 12, when operated at temperatures between 2 – 30 °C.

Regeneration and Depyrogenation

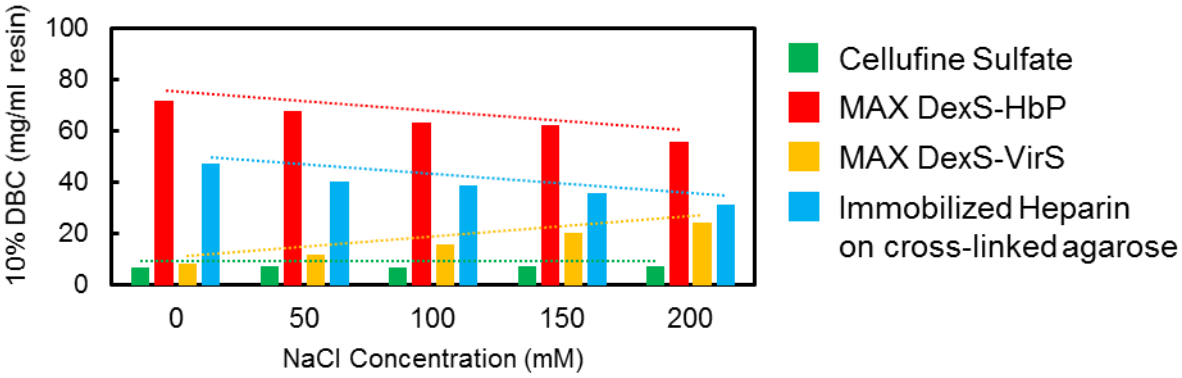
Cellufine DexS is typically regenerated with high ionic strength (1 – 3 M) NaCl. If this is not sufficient, regenerate more aggressively with 3 – 10 column volumes of 0.05 – 0.15 M NaOH at 2 – 10 °C, then wash with 1 – 3 M NaCl until pH drops below 9. Wash gel again with starting buffer until re-equilibrated.

Application Example

Dextran sulfate is a synthetic polysaccharide derived from natural non-animal derived polysaccharide. Dextran sulfate is reported to have similar bioactivity as heparin, showing selective inhibition of HIV-1 replication *in vivo* or binds rapidly with heparin cofactor II. Dextran sulfate is also well-known as a cation exchange ligand in IEX chromatography. Due to these properties, the adsorption/desorption performance of the resin is affected by pH and conductivity (ionic strength). Operation conditions should be carefully optimized. An example of lactoferrin adsorption properties with Cellufine MAX DexS resins is shown in Figure1,

below. For more information, please refer to our technical note(s) available on-line at http://www.jnc-corp.co.jp/fine/en/cellufine/guide/index.html.

Figure 1. Lactoferrin binding to DexS resin(s), Cellufine Sulfate and Immobilized Heparin.



A 5 mmID x 2.5 cmL column was packed with the above resins and equilibrated with 10 mM Na Phosphate buffer pH 7.5 + 0, 50, 100 and 150 mM NaCl. Lactoferrin (2 mg/ml) was prepared in the above buffers and loaded on to the above columns at a flow rate of 0.125 ml/min for a residence time of 4 min. 10% DBC (mg/ml resin volume) was estimated from the protein breakthrough curves at each buffer condition. In between loading the column was eluted with the above buffer + 1.0 M NaCl.

Cellufine MAX DexS-HbP and immobilized heparin showed a salt dependent decrease in retention of lactoferrin. This is typical of resin(s) acting in cation exchange mode of chromatography. In contrast, Cellufine MAX DexS-VirS showed an increase in lactoferrin binding in higher salt suggestive of a different mechanism of action. As expected, Cellufine sulfate did not show any appreciable retention of lactoferrin.

Storage

Store unopened container at 2 – 8 °C . Do not freeze.
Short term (2 weeks or less), bulk and column can be stored in 1 M NaCl in neutral buffer at 2 – 8 °C. Longer term storage can be conducted under identical conditions; however, a preservative such as 20% (v/v) ethanol or 2% (v/v) benzyl alcohol should be added to the buffer.

Shelf Life

5 years from date of manufacture.

Description	Quantity	Catalogue No.
Cellufine MAX DexS-HbP	5 x 1 mLmini column	21 700-51
	1 x 5 mL mini column	21 700-15
	10 mL	21 700
	50 mL	21 701
	500 mL	21 702
	5 L	21 703
	10 L	21 704
Cellufine MAX DexS-VirS	5 x 1 mL mini column	21 800-51
	1 x 5 mL mini column	21 800-15
	10 mL	21 800
	50 mL	21 801
	500 mL	21 802
	5 L	21 803
	10 L	21 804

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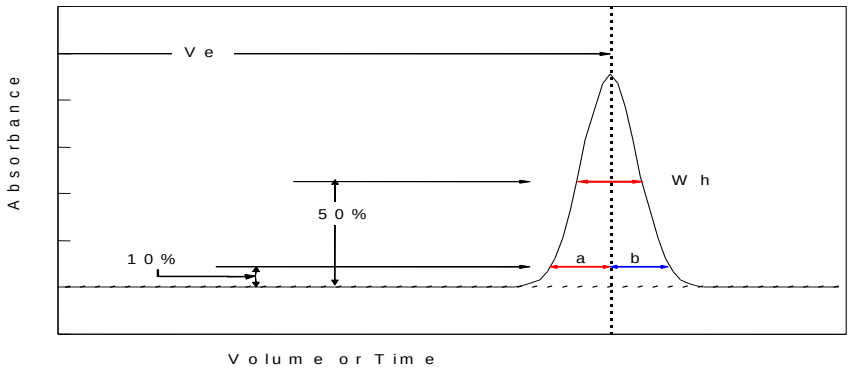
Appendix 1: Evaluation of column packing

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 **$N = 5.54 \times (Ve/Wh)^2$** **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.

