

# Cellufine™ MAX DexS-VirS

A new chromatography resin incorporating a dextran sulfate polymeric ligand, acting as a Heparin mimetic for capture and purification of viral and VLP particles

Dextran sulfate is a synthetic derivative of the natural polysaccharide dextran and is reported to have similar bioactivity as heparin. For example, dextran sulfate selectively inhibits HIV-1 replication *in vivo* or rapidly binds heparin cofactor II. Dextran sulfate is also used to prepare cation exchange chromatography resins. JNC has developed a new chromatography resin, Cellufine MAX DexS-VirS incorporating high molecular weight dextran sulfate polymer for viral and VLP capture and purification. This new resin is built on a crystalline highly cross-linked stable cellulose base bead ideally suited for large scale bio-pharma manufacturing processes. The new bead structure is resistant to base CIP and can be operated under high flow modes with minimal back pressure. Table 1 summarizes the properties of this new viral and VLP capture affinity resin.

Table 1. Properties of Cellufine MAX DexS-VirS.

Properties	
Base bead matrix	Highly cross-linked cellulose
Particle size	90 µm average (40-130 µm)
Ligand	High MWt. Dextran sulfate polymer
CIP	0.5 M NaOH
Lysozyme adsorption capacity (mg/ml)	≥ 56 mg/ml
Operating pressure	< 0.3 MPa

## Model Protein adsorption to Cellufine MAX DexS-VirS resin

A series of model protein binding to this Heparin mimetic resin were investigated to characterize the resin comparing to Cellufine Sulfate and Dextran sulfate coated cross-linked agarose beads. The results are summarized in Figure 1 as a contour plot from a DOE experiment, and Figure 2 comparing 10% DBC with different resins.

Figure 1. Contour Plot Analysis of Lactoferrin binding to Cellufine MAX DexS-VirS.

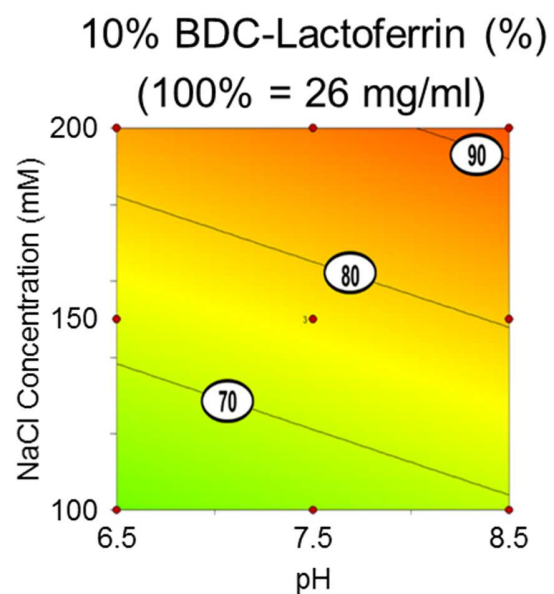
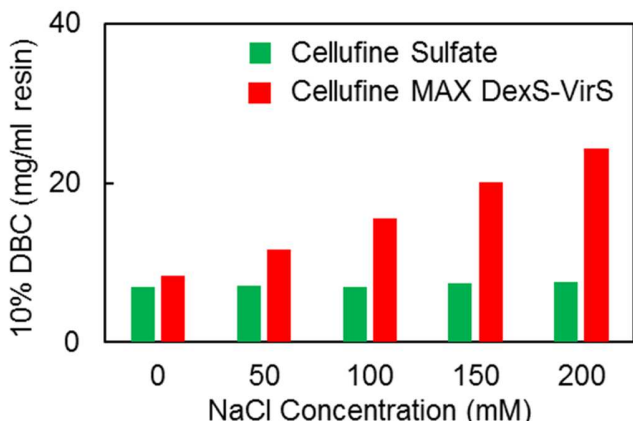


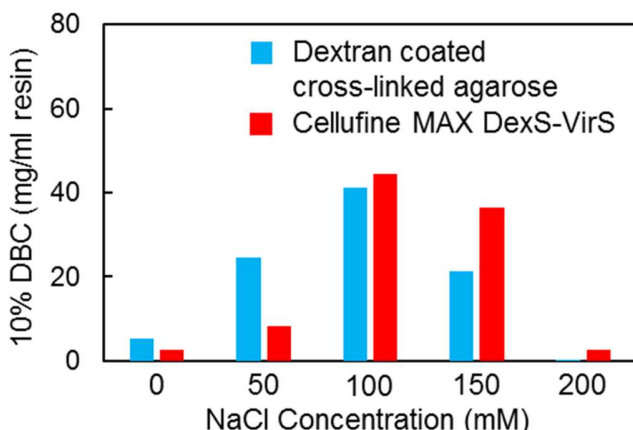
Figure 2. DBC of Lactoferrin with Cellufine MAX DexS-VirS and Cellufine Sulfate.



DBC was measured in a 5 mmID x 2.5 cmL (volume 0.49 ml) flow packed column at a flow rate of 0.125 ml/min for a residence time of 4 min. Lactoferrin at 2 mg/ml was loaded in a 50 mM Tris HCl buffer pH 7.5 + 0, 50, 100, 150 and 200 mM NaCl.

Polyclonal immunoglobulin (IgG) binding was investigated over a range of salt concentrations at pH 5.0 in Acetate buffer is summarized in Figure 3, below.

Figure 3. DBC of Polyclonal IgG binding to Cellufine DexS-VirS and Dextran sulfate coated on cross-linked agarose.



DBC was measured in a 5 mmID x 2.5 cmL (volume 0.49 ml) flow packed column at a flow rate of 0.125 ml/min for a residence time of 4

min. Polyclonal IgG at 2 mg/ml was loaded in a 10 mM Na Acetate buffer pH 5.0 + 0, 50, 100, 150 and 200 mM NaCl.

**Capture of Inactivated Influenza Virus with Cellufine MAX DexS-VirS**

Dextran sulfate affinity on cross-linked agarose has been used to capture and purify inactivated influenza virus from allantoic and cell culture samples (see Ref 1) with a high degree of purity.

Ref. 1, Data file 28-9616-49 AA. Capto DeVirS, GE Healthcare Bio-Sciences AB

Inactivated influenza virus (A/duck/Hokkaido /Vac-2/2007 H7N7) was captured and eluted in a purification workflow to measure 10% DBC. The study was carried out with a 5 mmID x 2.5 cmL (0.49 mL volume) column in 10 mM Na Phosphate, 120 mM NaCl buffer pH 7.4 at a flow rate of 0.2 ml/min (60 cm/h for a residence time of 2.5 min). Viral adsorption breakthrough was estimated by measuring HA viral activity in collected fractions. The results are summarized in Figure 4 and Table 2 below.

Figure 4. 10% DBC of Inactivated Influenza Virus with Cellufine MAX DexS-VirS.

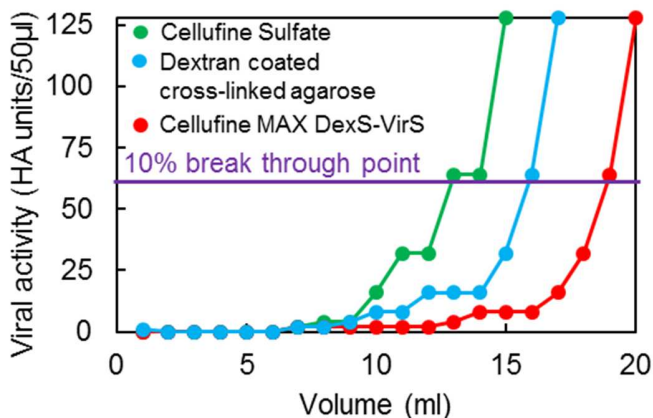


Table 2. Recovery of Inactivated Influenza Flu Viral particles with Cellufine MAX DexS-VirS.

Panel A. Cellufine Sulfate.

Step	HA		Total protein			DNA		
	HAU	%	mg	%	ng/HAU	mg	%	pg/HAU
Load	153,600	100	4.8	100	31.3	18.5	100	120.6
Flow-through	9,080	5.9	3.2	67.2	356.5	—	—	—
Elute 1	69,920	45.5	0.2	4.1	2.8	2.0	10.7	28.3
Elute 2	35,120	22.9	0.09	1.8	2.5	0.8	4.1	21.8
<b>Total recovery</b>	<b>114,120</b>	<b>74.3</b>	<b>3.5</b>	<b>73.1</b>	<b>30.8</b>	<b>—</b>	<b>—</b>	<b>—</b>

Panel B. Dextran coated cross-linked agarose.

Step	HA		Total protein			DNA		
	HAU	%	mg	%	ng/HAU	mg	%	pg/HAU
Load	204,800	100	7.6	100	17.1	24.9	100	121.4
Flow-through	21,620	10.6	4.2	54.5	192.1	—	—	—
Elute 1	122,560	59.8	0.4	5.0	3.1	1.6	6.2	12.7
Elute 2	37,280	18.2	0.12	1.5	3.1	1.5	6.1	41
<b>Total recovery</b>	<b>181,460</b>	<b>88.6</b>	<b>4.7</b>	<b>61.0</b>	<b>25.6</b>	<b>—</b>	<b>—</b>	<b>—</b>

Panel C. Cellufine MAX DexS-VirS.

Step	HA		Total protein			DNA		
	HAU	%	mg	%	ng/HAU	mg	%	pg/HAU
Load	204,800	100	6.4	100	31.3	24.7	100	120.6
Flow-through	7,760	3.8	4.1	64.2	530.7	—	—	—
Elute 1	139,520	68.1	0.4	5.9	2.7	3.0	12.0	21.3
Elute 2	64,160	31.3	0.1	1.5	1.5	3.4	13.9	53
<b>Total recovery</b>	<b>211,440</b>	<b>103.2</b>	<b>4.6</b>	<b>71.6</b>	<b>21.7</b>	<b>—</b>	<b>—</b>	<b>—</b>

## Discussion

Lactoferrin showed 10% DBC binding to Cellufine MAX DexS-VirS > 20 mg/ml at up to 200 mM NaCl while loading at up to pH 8.5. Under the same conditions Cellufine Sulfate showed only < 10 mg/ml capacity. In both case their capacities declined as the [NaCl] increased. Polyclonal IgG showed salt dependent binding at pH 5.0 for the two Dextran sulfate coated resins. Inactivated influenza viral particles showed increased (+55%) retention over Cellufine Sulfate and the Dextran coated agarose.

In summary, the dextran sulfate coated Cellufine MAX DexS-VirS showed significantly higher binding capacity for viral particles compared to the industry standard Cellufine Sulfate.

### Additional Cellufine Products used during Viral and VLP Capture and Purification

**Cellufine ET clean (poly(ε-lysine))** - can remove endotoxin from a cellular product solution at physiological pH, ionic strength of  $\mu = 0.02 - 1.0$ , and  $0 - 25$  °C.

- ET Clean S** (2,000 MWt. cut-off pore size)
- ET Clean L** (>  $2 \times 10^6$  MWt. cut-off pore size)

**Cellufine GH-25 desalting media** - based on porous, spherical, highly crosslinked cellulose particles. The sharp 3 kDa exclusion limit allows proteins to pass through the column in the void volume while retarding smaller molecular weight solutes in the internal pores.

Description	Quantity	Catalogue No.
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 lt	21 803
	10 lt	21 804

## Purchase/Technical Support

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