

## Cellufine™ MLP DexS

### Next generation Cellufine™ with engineered large through pores optimized for purification of large biomolecules including whole virus particles

Cellufine™ MLP DexS is based on next-generation Cellufine™ MLP, and sulfated polysaccharide is immobilized as a ligand. This resin is designed for isolation and purification of inactivated virus for vaccine production.

The Cellufine™ MLP is a highly crosslinked cellulose particle that has a large continuous pore structure. In the purification of large virus and virus-like particles, MLP DexS exhibits a high binding capacity and purification capabilities because the intraparticle surface of the resin can be used as an adsorption site.

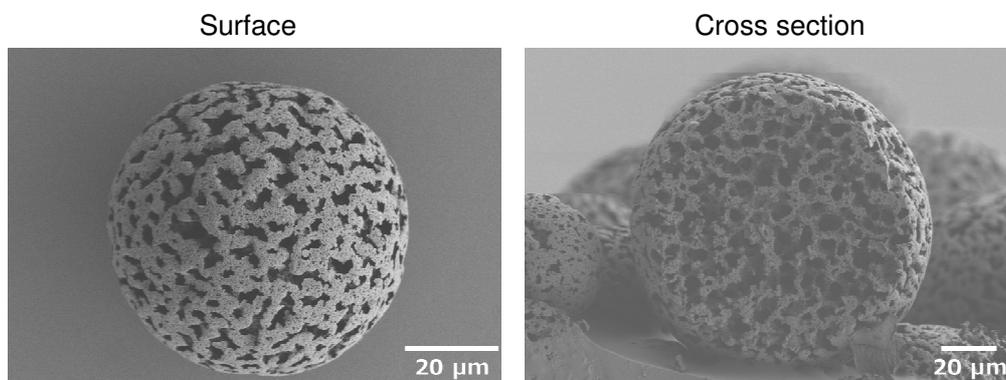


Fig.1. Cellufine™ MLP1000

### How to request products under development

Cellufine™ MLP DexS is currently under development. The quality of the Cellufine™ chromatography media is guaranteed by ISO 9001, but the quality of this product is subject to change as it is under development.

This product is NOT for sale. We can provide small volume samples for evaluation. For pharmaceutical companies, diagnostics companies or other manufacturing companies and researchers who would like to handle this product for manufacturing process, we will provide samples those who consenting to above fact.

For inquiries regarding samples, please contact the following.

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### ■ Characteristics of MLP

Cellufine™ MLP is a highly crosslinked cellulose particle with a large continuous pore structure. In the purification of large virus and virus-like particles, MLP DexS exhibits high binding capacity and purification capabilities because the intraparticle surface of the resin can be used as an adsorption site.

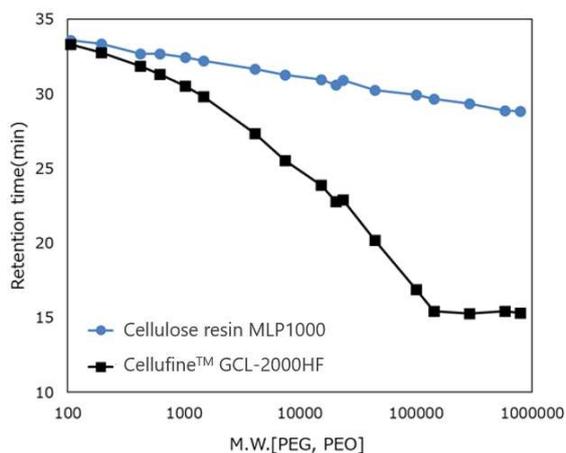


Fig. 2. Retention times of PEG and PEO in MLP

After packing the resin into 7.8 mm ID x 300 mm column, polyethylene glycol (PEG) or polyethylene oxide (PEO) with different index molecular weights was passed through the column at a flow rate of 0.4 mL/min to confirm the retention time.

Table 1. Properties of MLP DexS

Particle size	Ave. 90 μm (40 – 150 μm)
Ligand	Dextran sulfate
Base resin	Cross linked cellulose
Pressure	< 0.3MPa

### ■ Flow property

The Cellufine™ MLP DexS is a highly crosslinked cellulose particle. Despite having extremely large open pores for virus particles to enter, the resin has high pressure resistance and can be used in large columns to produce biopharmaceuticals (Fig. 3).

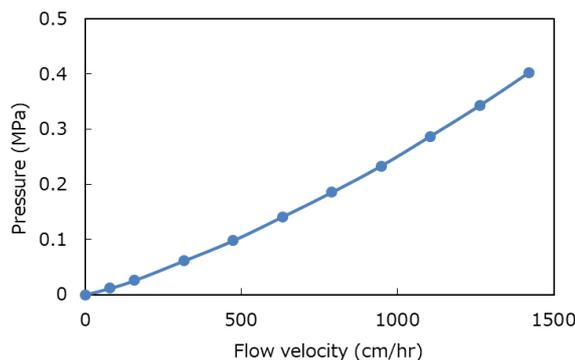


Fig. 3. Flow characteristics of MLP DexS

After packing the resin into a 2.2 cm ID x 20 cm column, pure water at 24°C ± 1°C was used as the mobile phase, and the relationship between flow rate and pressure drop across the column was evaluated.

### ■ Purification of influenza virus

Chromatography purification was performed using allantoic fluid containing influenza A virus H1N1. The Cellufine™ MLP DexS was packed into the column and the allantoic fluid was directly loaded. Virus in the load, flow through, wash and elution fractions was measured using an HA assay. Figure 4 shows a comparison between MLP DexS and a commercially available resin A (dextran sulfate modified resin). MLP DexS continued to adsorb the virus without a 10% break-through until 20 mL loading, while commercially available resin A showed a 10% break-through when about 4 mL of liquid was passed through the column.

In the case of general chromatography resin, the pore size is small and virus particles cannot enter inside the particle. For this reason, the virus is adsorbed on only the surface of the resin, and the binding sites are limited. In contrast, the Cellufine™ MLP DexS has extremely large through-pores. This allows virus particles to enter inside the resin. In this experiment, it did not show a 10% break-through even if the load sample exceeded 20 mL, which is more than five times 10% DBC of a commercially available resin A.

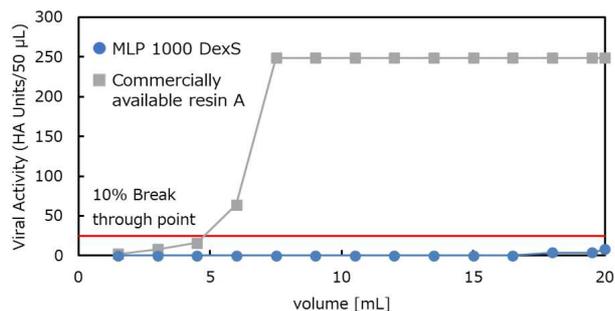


Fig. 4. Dynamic binding capacity of Inactivated Influenza Virus

Experimental conditions

Resin: Cellufine™ MLP DexS,

Commercially available resin A

Column: I.D. 5.0 x 15 mm (0.3 mL)

Load sample: Allantoic fluid containing inactivated influenza A virus

Virus strain: A/Hyogo/YS/2011(H1N1)

Flow rate: 0.5 ml/min (150 cm/h, R.T. 0.6 min)

Equilibration: 10 mM Na phosphate, 120 mM NaCl, pH 7.4

Wash: 10 mM Na phosphate, 120 mM NaCl, pH 7.4

Elution: 10 mM Na phosphate, 2 M NaCl, pH 7.4

The recovery rate and purity of inactivated influenza A virus are summarized in Table 2. With chromatography using the Cellufine™ MLP DexS, the liquid volume was reduced from 20 mL to 1.5 mL and the virus could be concentrated up to 13-fold. The amount of protein impurities was reduced from 10,100 µg to 975 µg, resulting in a 90.3% reduction. It was also able to reduce the amount of dsDNA to 22% in the viral elution fraction, achieving a final virus recovery rate of 93%. The Cellufine™ MLP DexS shows a high binding capacity and excellent product recovery under high flow conditions.

Table 2. Summary of recovery and purity of inactivated influenza virus

1) Cellufine™ MLP DexS

Process	Volume ml	Virus activity HA activity	Proteins µg	dsDNA µg	Recovery %
Allantoic fluid	20	99,600	10,100	6.4	100
Flow through	20	400	9,220	3.6	1
Wash	6	1,560	786	0.3	2
Elution	1.5	92,880	975	1.4	93

2) Commercially available resin A

Process	Volume ml	Virus activity HA activity	Proteins µg	dsDNA µg	Recovery %
Allantoic fluid	20	99,600	10,100	6.4	100
Flow through	20	73,600	7,520	4.6	74
Wash	6	6,720	780	0.18	7
Elution	1.5	12,900	330	0.7	13

**Purification of Human coronavirus OC43**

Human coronavirus OC43 (hCoV OC43) is a common cold-causing virus of approximately 100 nm in size belonging to the same beta coronavirus genus as SARS-CoV-2 that causes COVID-19 infection. Following the example of the purification of influenza virus, we present an example of the purification of human coronavirus OC43 cultured in Vero cells by chromatography.

The chromatographic purification was performed using cell culture supernatant fluid containing hCoV OC43. The Cellufine™ MLP DexS was packed into the column and the cell culture supernatant fluid was directly loaded. The virus in the load, flow through, wash and elution fractions was measured using an HA assay. Figure 5 shows that the MLP DexS adsorbs a larger amount of the virus than the commercially available resin A (dextran sulfate modified resin). While the commercially available resin A showed a 10% break-through when about 18 mL loading, the MLP DexS continued to adsorb the virus without a 10% break-through even after 34.5 mL was loaded.

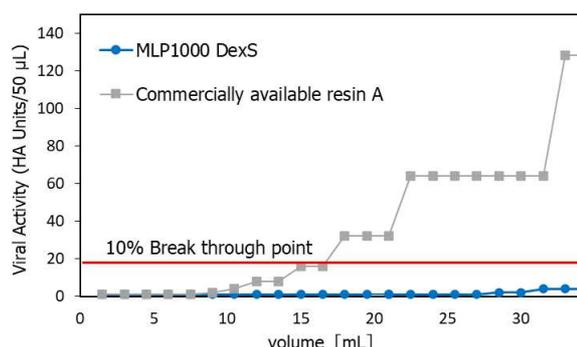


Fig. 5. Dynamic binding capacity of Inactivated Human coronavirus OC43

Experimental conditions

- Resin: Cellufine™ MLP DexS, Commercially available resin A
- Column: I.D. 5.0 x 15 mm (0.3 mL)
- Load sample: cell culture supernatant fluid containing inactivated hCoV OC43
- Flow rate: 0.3 ml/min (90 cm/h, R.T. 1.0 min)
- Equilibration: 10 mM Na phosphate, 150 mM NaCl, pH 7.4
- Wash: 10 mM Na phosphate, 150 mM NaCl, pH 7.4
- Elution: 10 mM Na phosphate, 2 M NaCl, pH 7.4

In this human coronavirus adsorption experiment, the Cellufine™ MLP DexS showed the extremely high adsorption capacity with less than a 3% break-through, when a 10 % break-through was observed with the commercially available resin.

The recovery rate and purity of the chromatographically purified inactivated human coronaviruses are summarized in Table 3. With the chromatographic purification using the Cellufine™ MLP DexS, the amount of protein impurities was reduced from 6394 µg to 170 µg, resulting in a 97.3% reduction. It was also able to reduce the amount of dsDNA to 9% in the viral elution fraction, achieving a final virus recovery rate of 91%, which showed a high recovery performance.

It was shown that MLP DexS enables more efficient purification of the human coronavirus OC43 belonging to the same beta coronavirus genus as SARS-Cov-2 as well as the influenza virus.

Table 3. Summary of recovery and purity of inactivated human coronavirus OC43

3) Cellufine™ MLP DexS

Process	Volume ml	Virus activity HA activity	Proteins µg	dsDNA µg	Recovery %
supernatant fluid	34.5	120186	6394	52.79	100
Flow through	34.5	1020	4376	55.53	0.8
Wash	4.5	90	57	2.25	0.1
Elution (gradient, isocratic)	10.5	108990	170	4.95	91

4) Commercially available resin A

Process	Volume ml	Virus activity HA activity	Proteins µg	dsDNA µg	Recovery %
Allantoic fluid	34.5	120186	6960	45.48	100
Flow through	34.5	25770	5479	50.3	21
Wash	4.5	600	59	2.4	0.5
Elution (gradient, isocratic)	10.5	83550	164	8.94	70

## ■ Conclusion

Cellufine™ MLP is a next-generation chromatography resin with large pores that overturns conventional wisdom. Its huge through-pores allow giant biomolecules such as virus to diffuse into the particle inside. Conventional chromatography resins have poor pressure resistance when their pores are large enough to allow entry of whole virus particles, making it difficult to use at large process scale. In contrast, the Cellufine™ MLP has extremely high-pressure resistance and can be used in large bioprocess columns.

Recently there has been growing increasing trend in the use large biomolecules as pharmaceuticals. For example, adeno-associated virus (AAV) is used in gene therapy, and herpes simplex virus (HSV) is used in antitumor virotherapy. In addition, exosomes, which are particles of about 100 nm, have been vigorously studied in the field of regenerative medicine. The mRNA based COVID-19 vaccines are also large molecules. Such biomolecules have difficulty entering inside the pores of conventional chromatography resin, limiting adsorption performance.

Cellufine™ MLP has an extremely large pore size that allows virus particles to diffuse within the inside structure. As a result, the virus can access the intraparticle surface, leading to an extremely high dynamic binding capacity. The bottleneck of conventional chromatography media can be eliminated.

JNC Corporation is working on the development of this new chromatography resin in response to the demand for next-generation modality purification. We will continue to contribute to the development of medical care by providing new purification processes for large biopharmaceuticals.

## ■ Technical information: Reference

K. Kadoi, E. Iwamoto, T. Nakama, Fabrication and characterization of a cellulose monolith-like particle for virus purification, *Biochem Eng J.* 192 (2023) 108849.  
<https://doi.org/10.1016/j.bej.2023.108849>.

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