

# Purification of Human Coronavirus hOC43 with Cellufine Sulfate

An affinity chromatography resin for concentration and purification of viral particles from the  $\beta$  Coronavirus genus

Advances in vaccines and clinical diagnostics have created an increasing demand for large volumes of highly purified and concentrated viral particles. Cellufine Sulfate (CS) eliminates cumbersome, time-consuming, and potentially unsafe classical ultra-centrifugation and density gradient methods. It can also provide a significant improvement in concentration and purity. Elution of the bound product is affected through simple stepwise or gradient increases in ionic strength.

CS is based on a low porosity cellulose bead whose surface (see Figure 1) has been modified with sulfate groups. This bead structure was optimized for improved purification of large (> 100 nm) viral particles while minimizing non-specific protein binding from the egg or cell culture derived sample matrices. SEM analyses of beads (see Figure 2) with adsorbed viral particles show a dense surface coating.

Human coronavirus OC43 (hCoV OC43) is a common cold causative virus. In this report, we will introduce an example of purification of hCoV OC43 with Cellufine Sulfate, which has a well-known experience in virus purification.  $\beta$ coronavirus hCoV OC43 was highly purified with Cellufine Sulfate. Cellufine Sulfate is a chromatography resin effective in the purification of  $\beta$ coronavirus.

Figure 1, Partial Structure of resin affinity ligand

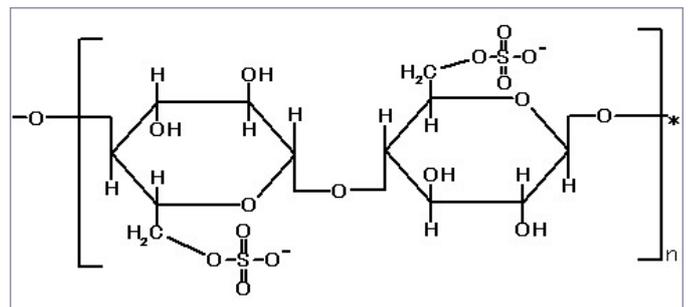
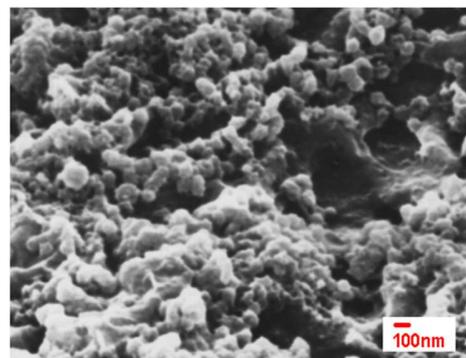


Figure 2, SEM Analysis of the Surface of Cellufine Sulfate Loaded with Influenza Viral Particles



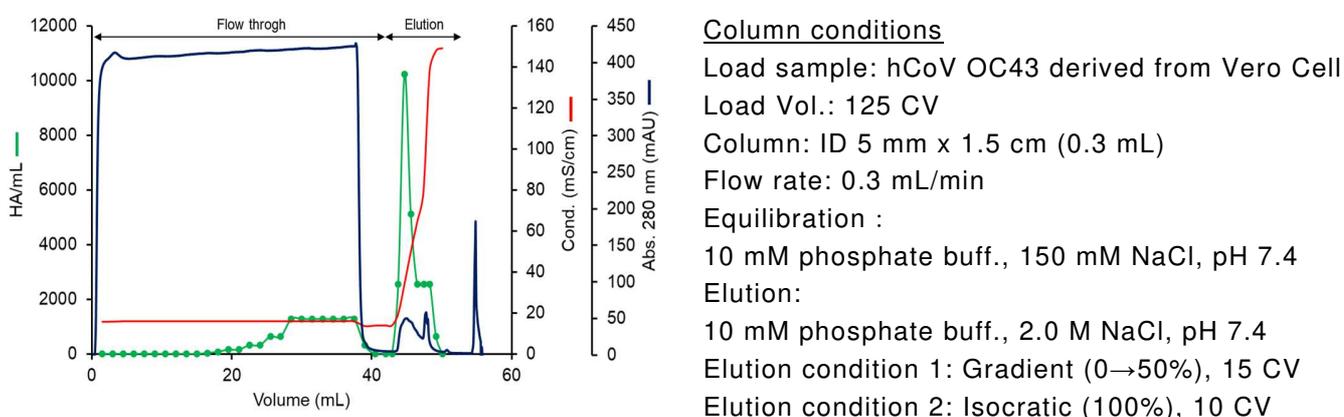
Virus Strain: A/duck/Hokkaido /Vac-2/04(H7N7)

## 1. Purification of hCoV OC43 Viral Particles

Human coronavirus OC43 (hCoV OC43) was cultured in Vero cells was inactivated with  $\beta$ -propiolactone prior to purification on CS by pseudo affinity chromatography.

A column (ID 0.5 mm x H 1.5 cm) was packed with 0.3 mL of Cellufine Sulfate and equilibrated with load buffer (10 mM Na Phosphate, 150 mM NaCl pH 7.4) at a flow rate of 0.3 mL/min. 125 CV of clarified Virus culture supernatant (0.45  $\mu$ M PVDF filter) was loaded on to the column to adsorb the hCoV OC43 viral particles. After loading, the column was washed with 10 CV of load buffer to wash away weakly bound impurities. The hCoV OC43 adsorbed on the column was then eluted with 10 mM phosphate buffer, 2.0 M sodium chloride, pH 7.4. The elution sample was then analyzed for recovery of the viral particles, residual host cellular DNA and protein to assess the degree of purification. A typical chromatogram showing this purification of hCoV OC43 with Cellufine Sulfate is shown (Fig.3).

Figure 3, Purification of hCoV OC43 with Cellufine Sulfate



## 2. Viral Recovery by Hemagglutination (HA) Titer

Virus recovery in the elution fraction was quantified by the hemagglutination titer (HA titer). By gradient elution with NaCl, hCoV OC43 was eluted with a conductivity of around 30 mS/cm, and the recovery rate was 77% (Table 1). Dynamic binding capacity (DBC) at the 10 % breakthrough point was estimated to be 76,200 HA/mL-resin at a flow rate of 0.3 mL/min (92 cm/h with a residence time of 0.95 min)

Table 1, Recovery and DBC of hCoV OC43 Purified by Cellufine Sulfate

Recovery (%)	Dynamic Binding Capacity (HA/mL resin) *
77	76,200

\* Measured at 10% breakthrough point

### 3. Residual Host Cell DNA and Protein Recovery

The recovery of host-derived DNA and protein in the elution fraction was evaluated by PicoGreen® and protein assay to assess removal of impurities. Data is summarized in Tables 2 and 3.

Table 2, Host Cell DNA Recovery

Fraction	DNA (µg)	DNA Recovery (%)	Residual DNA (pg/HA)
Load	84	100	1758
Flow through	75	89	4433
Elution	7	9	312

DNA derived from a host cell has a negative charge derived from a phosphate group. Cellufine Sulfate also has a negative charge because it uses a sulfate ester group as a ligand. Therefore, DNA is accumulated in the flow-through fraction without being adsorbed on Cellufine Sulfate. In this study, 89% of DNA was accumulated in the flow-through fraction.

Table 3, Residual Host Cell Protein (HCP) after Purification

Fraction	Protein (mg)	Protein Recovery (%)	Residual Protein (ng/HA)
Load	1.96	100	41
Flow through	0.49	25	29
Elution	0.38	20	16

Since these protein fractions may contains a virus-derived proteins, evaluation by Western Blotting will be required to confirm purity of the final elution fraction. In this study, >80% of the protein was removed from the starting load sample.

## Conclusion

Cellufine Sulfate is a pseudo affinity chromatography resin widely used for the purification of several viruses and virus like particles. This cellulose bead-based resin can be used at physiological pH and conductivity washing buffers to eliminate many non-specific weakly bound contaminants. In addition, a neutral pH high-salt buffer can be used for virus elution, suppressing viral inactivation. These characteristics make it ideal for the purification of several enveloped viruses such as Japanese encephalitis, rabies, and influenza virus. Cellufine Sulfate have been widely used by many large-scale viral vaccine manufacturers around the world.

In this study, human coronavirus OC43 was purified with high recovery and purity using a single pseudo affinity chromatography step. In this study, 92% of host cell DNA and 80% of host cell protein were removed from the starting Vero cell culture sample with 77% recovery of the viral HA activity in the elution fraction. These results show that hCoV OC43 can be easily purified from the culture supernatant with Cellufine Sulfate. This resin will contribute to the rapid development of a vaccine for the genus  $\beta$ coronavirus.

## On-line Links to more Cellufine Information

Cellufine Sulfate

<https://www.jnc-corp.co.jp/fine/en/cellufine/grade/grade-1-sulfate/>

Operation instruction and technical information are available on-line at;

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

## Other JNC Chromatography Products Useful in this Application

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

**ET Clean S** (2,000 MWt. cut-off pore size)

**ET Clean L** (> 2 x 10<sup>6</sup> MWt. cut-off pore size)

[https://www.jnc-corp.co.jp/fine/pdf/en/techdata/td\\_ETclean\\_DF027\\_EN\\_20110324.pdf](https://www.jnc-corp.co.jp/fine/pdf/en/techdata/td_ETclean_DF027_EN_20110324.pdf)

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

[https://www.jnc-corp.co.jp/fine/en/cellufine/guide/pdf/gel/TD\\_GH-25\\_N1\\_V4\\_E.pdf](https://www.jnc-corp.co.jp/fine/en/cellufine/guide/pdf/gel/TD_GH-25_N1_V4_E.pdf)

## Ordering Information

Description	Quantity	Catalogue No.
Cellufine Sulfate	5 x 1 ml cartridge	19845-51
	1 x 5 mL cartridge	19845-15
	10 mL	676943324
	50 mL	19845
	500 mL	19846
	5 L	19847
	10 L	19849

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