# Cellufine™ Sulfate

## Cellufine sulfate can be used for purification of viruses, virus-derived antigenic proteins, serum proteins, and heparin-binding proteins.

Cellufine Sulfate can be purified and concentrated with easy operation and good reproducibility. Density gradient ultracentrifugation used in virus purification is time-consuming, difficult to reproduce, and complicated from a safety perspective, but the use of Cellufine Sulfate solves these problems. Superior purification performance in terms of cost, low ligand elution, and reproducibility compared to immobilized resins such as dextran sulfate, chondroitin sulfate, and heparin. The bound substance is easily eluted by increasing ionic strength using stepwise or gradient.

Table 1 shows the characteristics of Cellufine Sulfate.

Table 1, Characteristics of Cellufine Sulfate

| | Characteristics |
|---|---|
| **Ligand** | Sulfate ester |
| **Matrix** | Spherical cellulose particle |
| **Particle size** | 40 - 130 µm（ca.90µm） |
| **MW exclusion limit** | 3kD |
| **Sulfur content** | ＞700 µg/g dry |
| **Operating pressure** | ＜0.3 MPa （＜300 cm/h） |
| **Protein Binding Capacity** | ＞ 3 mg /ml (Lysozyme)<br>7 mg/mL (HBsAg) |
| **Recommended CIP solution** | 0.1 M NaOH |
| **pH stability range** | 3 – 12 |
| **Storage** | 2-30 ºC in 20 % ethanol |

※Values in Table 1 are not specifications.

# Column Packing

## Materials

- Cellufine Sulfate
- Lab scale column, adapter, reservoir
- Pump
- Filtration equipment (Glass filter, Buchner-Roth, aspirator, etc.)
- Graduated cylinder
- Packing solution (water, NaCl solution※, buffer※)
- Mobile phase of packed column evaluation ( water, NaCl solution※, buffer※)
- Sample of packed column evaluation ( 1-2 %(v/v)acetone or 1M NaCl )

※NaCl solution：low salt concentration solution such as 0.1M NaCl solution
※buffer：Adsorption buffer , etc.

## Preparation of slurry

1) After the bottle is warmed to room temperature, shake it several times to equalize the slurry in the bottle.
2) Aspirate through a glass filter and wash 3 times with 5 times the volume of the packing solution. Remove 20% ethanol as a preservative. If necessary, the slurry may be washed by decantation.
3) Transfer the resin to a beaker and add packing solution to make a 50-60% (v/v)slurry. Suspend the resin and remove the air under decompression for 30-40 minutes. Slow stirring with a magnetic stirrer can effectively remove the air.
4) Pour the slurry into a graduated cylinder and leave it to stand for at least 4 hours.

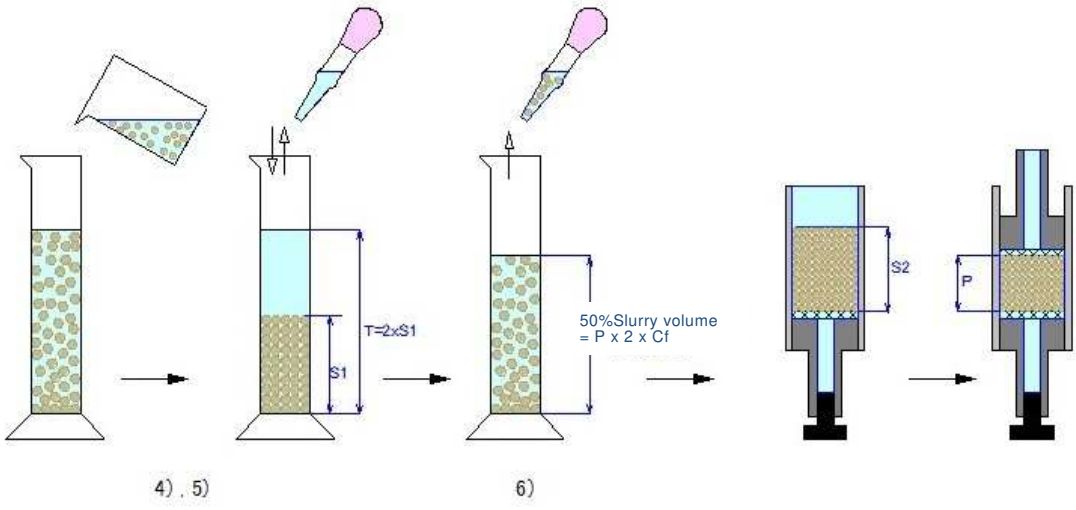



Figure1 Preparation of slurry

Measure bed height (volume) of a gravity settled bed and calculate the slurry concentration.

**Slurry concentration (%) =Gravity settled bed volume (S1) / Total slurry volume (T)**

5) Adjust to a 50 % (v/v) slurry concentration of resin with packing solution.
6) Calculate the volume of slurry required to pack the column.

**50% slurry volume required to packing = (Target packing volume (P) x 2） x Cf**

**※Cf = [gravity settled bed volume (S2) / Target packing volume (P) ]**

**Note:** *Compression factor (Cf) can impact the column packing efficiency (see Appendix 1 for details) and may need to be optimized by adjusting the axial compression on the column. The recommended Cf for Cellufine Sulfate is shown below.*

| Column size ( Diameter × Bed height) | Recommended Cf (Packing with water) |
|---|---|
| 3.2 cm× 20 cm | 1.15～1.20 |

## Column packing

1) Assemble the column. After opening the column outlet, add packing solution to remove air remaining in the filter. Leave about 1 cm of packing solution from the bottom of the column to prevent air from entering.
2) Close the column outlet and pour the slurry into the column all at once to keep air out.
3) Open the column outlet to allow the resin to settle. As the resin settles, the liquid surface becomes clear. Close the outlet when the packing solution becomes clear to 2-3 cm from the liquid surface.
4) Carefully fill the column to the top with packing solution. Keep the settling resin from floating away.
5) Set the adapter on the column to prevent air from entering the liquid surface. Close the adapter O-ring, lower the adapter, and remove the air inside.
6) Connect the column to a pump and allow the packing solution to flow at a pressure of 0.3 MPa or less for 30 to 60 minutes.

**Note:** *The flow velocity : Internal pressure at packing > Operating pressure after packing*

7) After the resin height is stabilized, stop the flow. Close the column outlet. Disconnect the inlet piping at the top of the column. Slowly lower the adapter to the surface of the resin. At this time, the packing solution in the column flows out from the column inlet.
8) Connect the piping filled with packing solution and excluded air to the adapter. Open the column outlet and pump at a pressure of 0.3 MPa or less. If the resin compresses and makes space between it and the adapter, lower the adapter to the surface of the resin.
9) Calculate column volume from column height. If the column volume is larger than the target column volume, lower the adapter to the target height. If the column volume is lower than the target column volume, the slurry concentration is low or the resin may be excessively compressed. Remove the resin from the column and pack again.

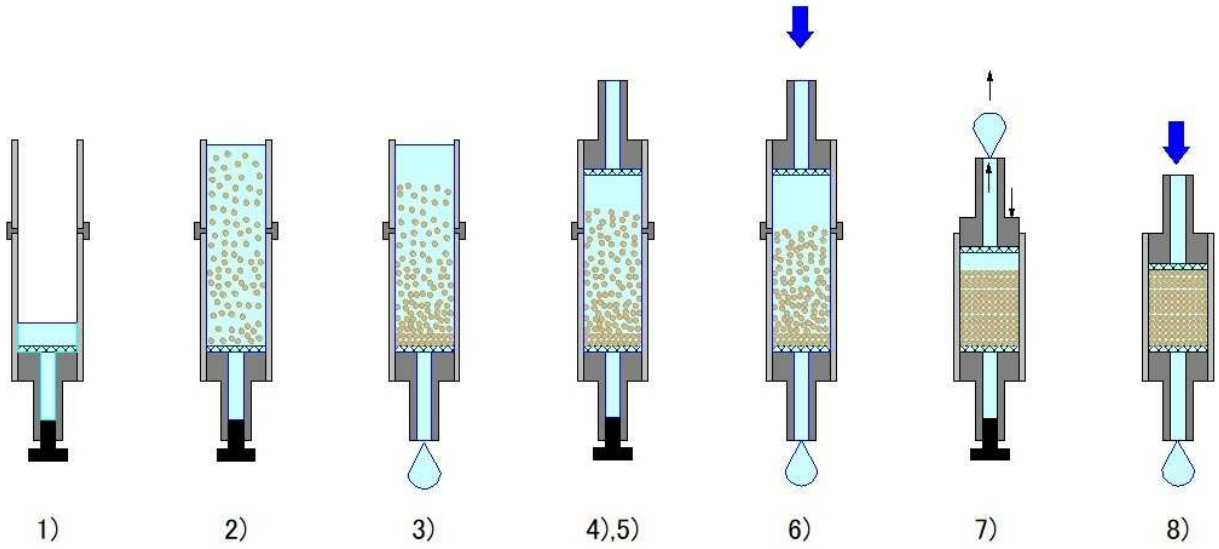



Figure 2 Process of column packing

## Evaluation of column packing

Column packing efficiency is evaluated by checking HETP and asymmetry (As). (Appendix 1)

## Operating Guidelines

**How to use**

1) Equilibrate the column with adsorption buffer. (3-5 column volume (CV))
2) Load the column with sample dissolved in adsorption buffer. (1-20mg/mL)
3) Wash with adsorption buffer to remove impurities. (2-3 CV)

4) Elute the adsorbed target substance with elution buffer.

## Recommended buffer

**Adsorption buffer**：0.01 M sodium phosphate + 0.1 M NaCl pH 7.5

Other buffers may be used depending on the application. In general, the strength of protein adsorption depends on pH and ionic strength. The ionic strength of the buffer can be increased slightly to weaken the binding of impurities. Nonionic surfactants (Tween®20, Triton® X, etc.) promote elution of impurities.

**Elution buffer**：Add 1-2 M NaCl or KCl to the adsorption buffer.

The optimal salt concentration is determined by testing with gradient elution. Stepwise elution is commonly used in preparative chromatography.

## Sample preparation and loading

Samples are dissolved in adsorption buffer to 1-20 mg/ml.

Insoluble material is removed by centrifugation or filtering. Buffer may be exchanged with a desalting filter, dialysis, or a desalting column such as Cellufine GH-25.

## Recommended flow velocity

＜300 cm/h in a 45 cm diameter column with 23 cm bed height at <0.3 MPa.

## Regeneration and Depyrogenation

Wash the column with 2-3 M NaCl-containing buffer. If inadequate, wash with 3-10 column volumes of 0.05-0.15 M NaOH and wash with 2-3 M NaCl-containing buffer to pH 9. Wash with adsorption buffer for next operation.

## Cleaning in Place

Wash ionically adsorbed material by the same operation as regeneration, then wash with 0.1 M NaOH in 5 column volumes（Recommended flow velocity :30-60cm/h）.Equilibrate with adsorption buffer for next operation.

## Stability

pH range of 3 to 12 and operating temperature of 2 to 30°C are recommended.

Autoclavable in neutral buffer solution at 121°C for 30 minutes.

## Storage

Store unused resin in its container at a temperature of 2 to 30°C. Equilibrate opened

resin and packed column in 20% ethanol and store at 2 to 30°C. Do not freeze.

## Application

This is an application for adsorption test of influenza virus using a lab-scale column. Table 2 and Figures 1 and 2 show the results of loading influenza virus cultures onto the Cellufine Sulfate column. The recovery rate was 86%, and the sample could be concentrated to about 40 times its original volume. Host cell protein and DNA were removed to about 10%.

- column size：ID22 mm×185 mm ⇒70mL
- Buffer
  Equilibrate：0.01M phosphate buffer,pH7.4
  Wash 1、2：0.01M phosphate buffer,pH7.2
  Elute：0.01M phosphate buffer,3 M NaCl,pH7.0
- Flow velocity：1 mL/min
- influenza virus strain：Vac-2
- Sample volume：940mL

Table 2 Result of influenza virus purification

| | Volume ml | Total HA-Titer HA (recovery %) | Total Protein μg (recovery %) | DNA ng (recovery %) |
|---|---|---|---|---|
| Load | 940 | 9,625,600 (100) | 184,494 (100) | 611,560 (100) |
| Pass | 1213 | 194,000 ( 2) | 157,259 (85) | 490,340 (80) |
| Elute | 25 | 8,257,536 (86) | 14,855 (8) | 62,800 (10) |

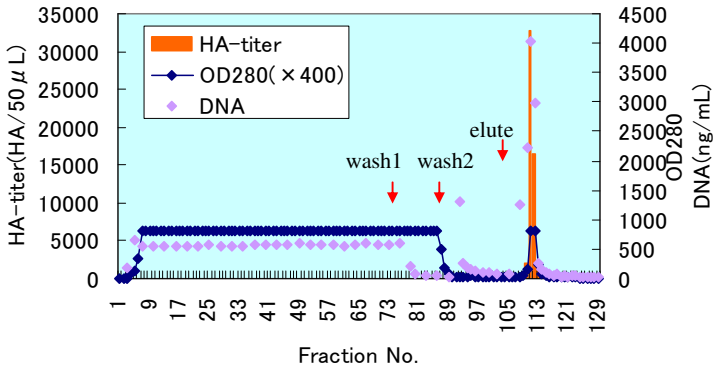
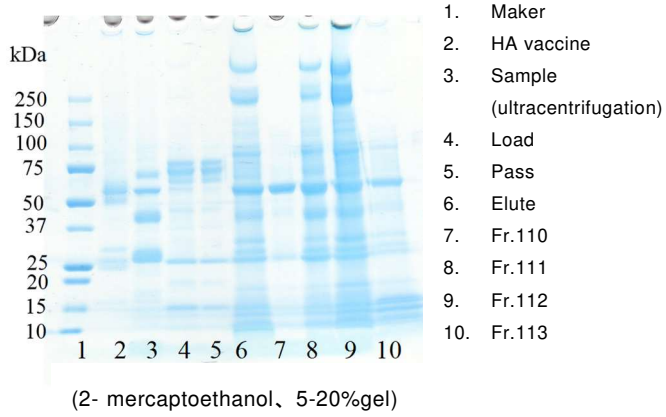



Figure 3 Chromatogram




(2- mercaptoethanol、5-20%gel)

Figure 4 Evaluation of purification by SDS-PAGE

## Product Ordering Information

| Description | Quantity | Catalogue No. |
|---|---|---|
| **Cellufine Sulfate** | 5 x 1 mL (Mini-column) | 19845-51 |
| | 1 x 5 mL (Mini-column) | 19845-15 |
| | 10 mL | 676 943 324 |
| | 50 mL | 19845 |
| | 500 mL | 19846 |
| | 5 L | 19847 |
| | 10 L | 19849 |

## Purchase/Technical Support

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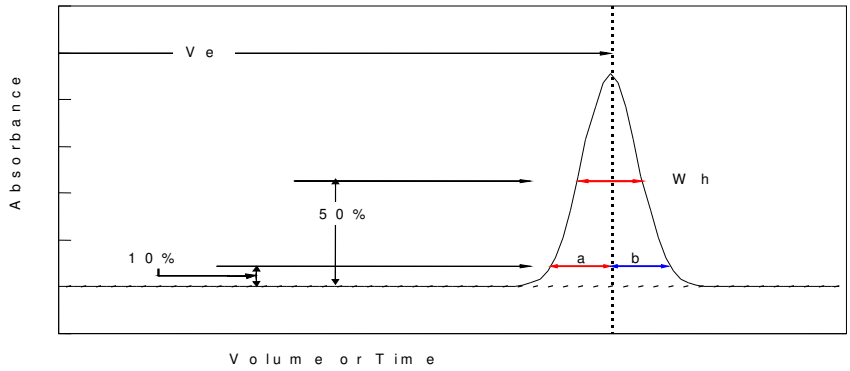
## Appendix 1: Evaluation of column packing

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： 0.1-0.3M NaCl aq） |
| Flow rate (cm/h) | 30 cm/h |
| Detector | UV, Conductivity |

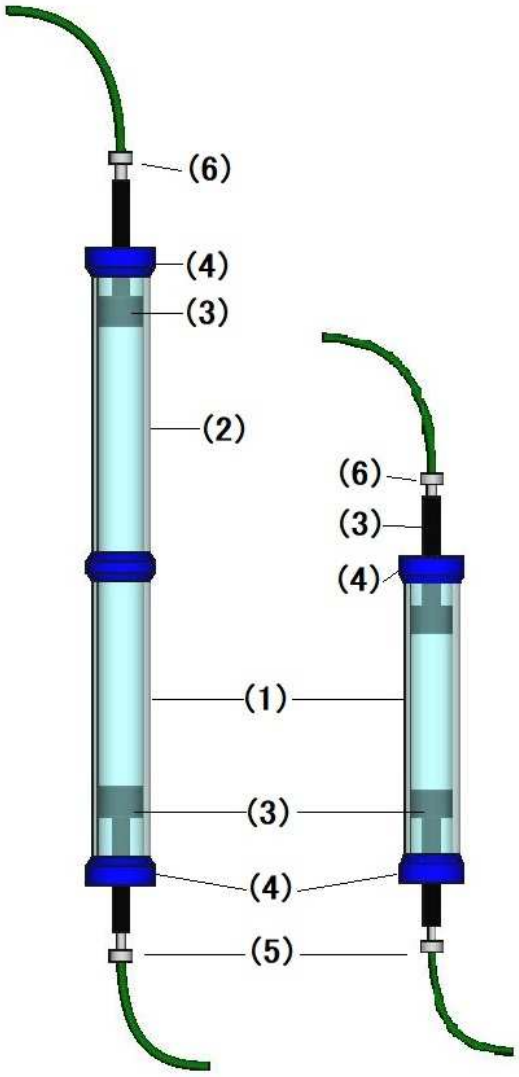


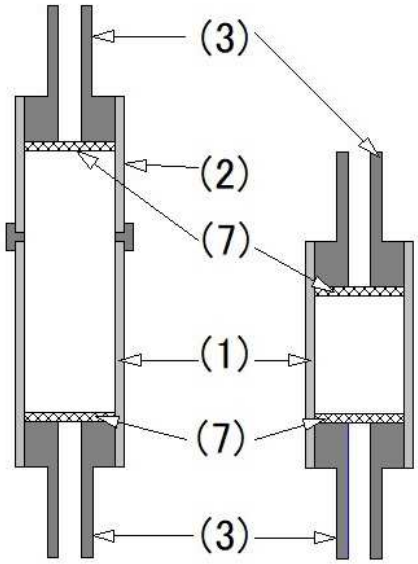

| | |
|---|---|
| L | Column length [cm or m] |
| $V_e$ | Elution time or volume |
| $W_h$ | Half of width of peak |
| a, b | Peak width of 10% peak hight<br>(a) front<br>(b) rear |
| Note | Ve,Wh and a, b should have same dimensional units |

| | | |
|---|---|---|
| **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.

## Appendix 2 : Figure of columns




The Operating Instructions use a simple cross-sectional figure of the column, as shown on the right.

| | | | |
|---|---|---|---|
| (1) | Column tube | (4) | Column end |
| (2) | Reservoir | (5) | Column outlet |
| (3) | Adapter | (6) | Column inlet |
| (7) | Filter | | |