Cellufine™ GCL-2000HF

JNC offers gel-filtration media, Cellufine GCL-2000 HF which is high-flow type and has a same porosity characteristic with Cellufine GCL-2000HF.

Cellufine GCL-2000HF offers competitive flow rate and resolving power for gel filtration chromatography. The semi-rigid spherical cellulose beads exhibit good flow rates with longer bed length, even with large diameter columns, while the high pore volume allows high capacity. Furthermore, GCL-2000 is chemically stable and can be run with many buffers and solutions.

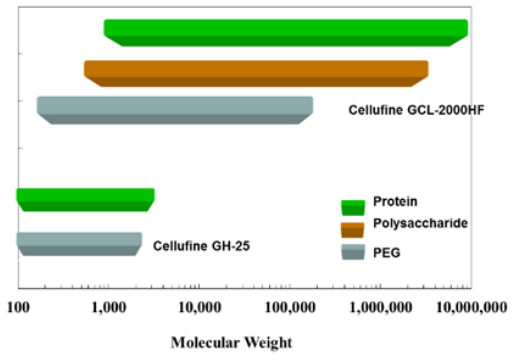


Figure1 Fractionation range

Table 1. Characteristics of Cellufine GCL-2000HF (*Values in Table 1 are not specifications.)

Matrix	Spherical cellulose particle
Particle size	40 - 130 μm（ca.90μm）
MW exclusion limit	PEG 200kD、Protein 3,000kDa
Operating pressure	＜0.2 MPa
pH stability range	1 - 14
Storage	2-8 ºC in 20 % ethanol

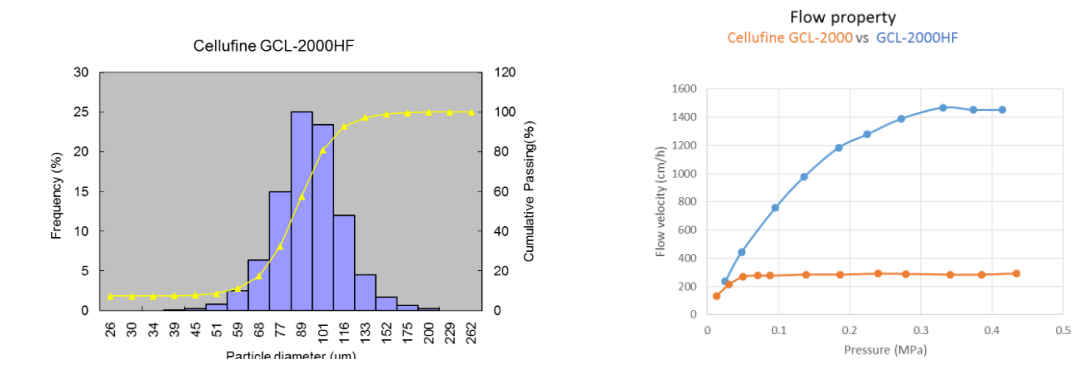
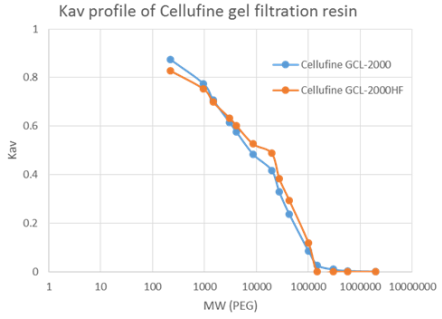
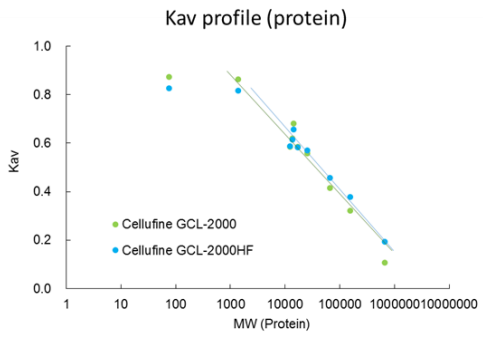


Figure 2 Particle distribution (left) and Pressure flow property(right).





Protein	MW
IgM	900,000
Thyroglobulin	660,000
h-γ-globulin	155,000
BSA	66,000
Chymotrypsinogen A	25,700
α-chymotrypsin	25,200
Myoglobin	17,000
Lysozyme	14,300
RNAse	13,700
Ctrochrome C	12,400
Bacitracin	1,400
Glycine	75

Figure 3 Exclusion Limit of Cellufine GCL-2000HF

Column Packing

Materials

- Cellufine GCL-2000HF
- 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.

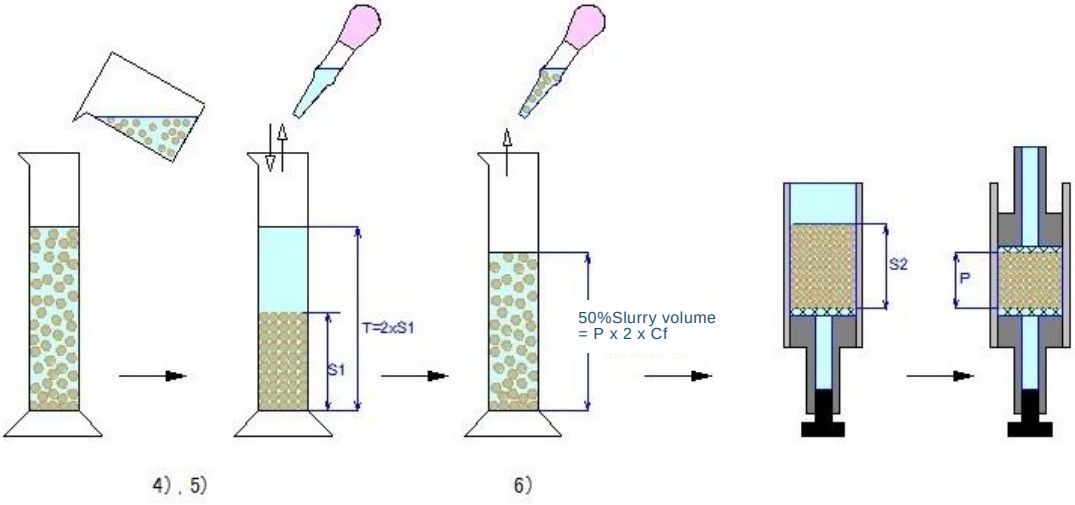


Figure4 Preparation of slurry

4) Pour the slurry into a graduated cylinder and leave it to stand for at least 4 hours. 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)×100

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) affects 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 GCL-2000HF is shown below.*** ***For manual packing when bed height is 40 -100 cm, packed manually on condition that Cf = 1.05**

Recommended Cf *
1.10 ～ 1.15

Column packing

1) Set up 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 the pump and keep the packing solution flowing at a pressure lower than the operating pressure for 30 to 60 minutes. (＜0.2MPa)

   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 to the adapter inlet with the piping filled with packing solution to exclude air. Open the column outlet and pump the packing solution. (＜0.2MPa) 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.

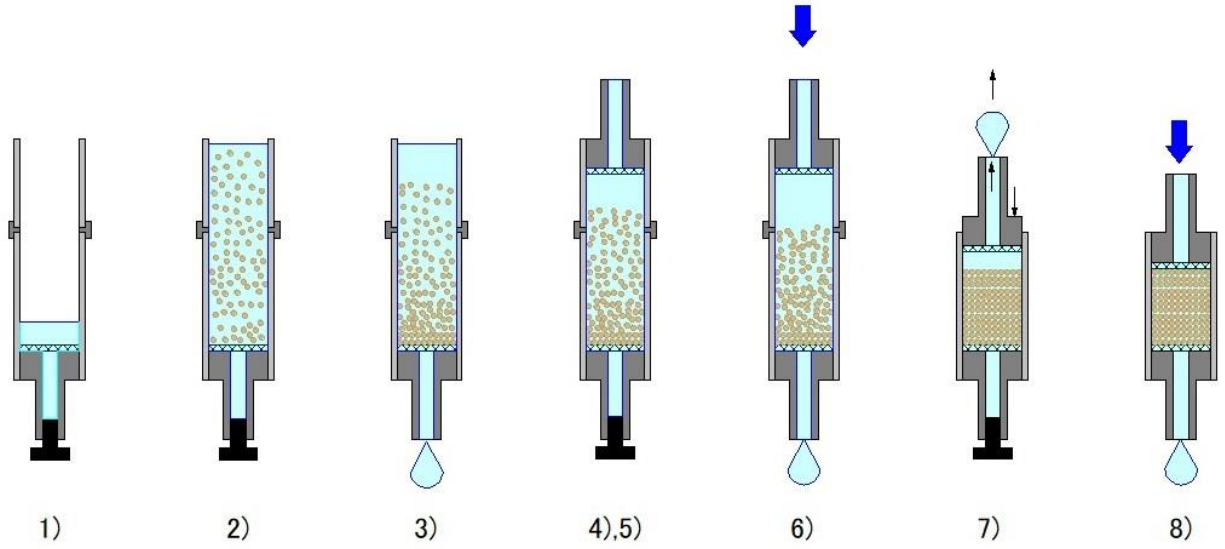


Figure 5 Process of column packing

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 equalization buffer. (3-5 column volume (CV))
2) Load the column with sample dissolved in equalization buffer.
3) Wash with equalization buffer to remove impurities. (2-3 CV)
4) Elute the target substance with equalization buffer.

Sample preparation and loading

Samples are ideally prepared in the mobile buffer. Samples can also be applied from different buffer, if buffer exchange is desired. Filtration may be necessary to remove insoluble matter. The sample load in gel filtration is a function of column volume. Sample loads of 0.1 % to 1.0 % of total column volume are used for high resolution applications,

while for general purpose preparative separations a load up to 5 % total column volume may be used. For buffer exchange or desalting, a load of 15 – 25 % of total column volume is suitable to prevent dilution of the sample. Sample protein concentrations should be between 1 – 20 mg/ml.

Recommended flow rate

5–50 cm/h. (<0.2MPa)

Elution

Elution occurs under isocratic conditions. The protein and salt/alcohol should elute at approximately 30% and 85% of the total column volume, respectively.

Stability

Stable in:

- pH 1–14
- Ethanol, methanol, acetone, etc.
- 8 M Urea, 6 M Guanidine/HCl
- 0.1 M HCl
- 0.5 M NaOH
- Most salts (NaCl, $(NH4)_2SO_4$, etc.)
- Most detergents (SDS, Tween®, Chaps, etc.)

Autoclavable: 121°C at 1 bar for 20 minutes

Regeneration

Flush the column with 5 bed volumes of 0.1 M NaOH at a velocity of 5 – 50 cm/h. Remove caustic by flushing with several bed volumes of DIW or buffer. Measure the pH of the column eluate to ensure that the system has returned to equilibrium.

Storage

Store unopened container at ambient temperature. Do not freeze.

Short term storage for bulk and column (2 weeks or less) can be stored in DIW containing, 20 % ethanol, or 0.1 M NaOH. Long term storage can be conducted under identical conditions at 2 – 8 °C. 5 years from date of manufacture.

Product Ordering Information

Description	Pack Size	Catalogue No.
Cellufine GCL-2000HF	100 mL	21400
	500 mL	21401
	5 L	21402
	10 L	21403

Purchase/Technical Support

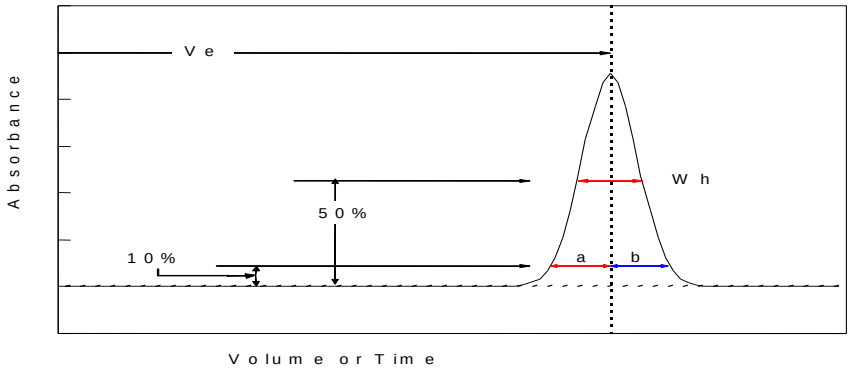
(North America & Europe)
JNC America Incorporated
555 Theodore Fremd Avenue, Suite C-206
Rye, NY 10580 USA
TEL: 914-921-5400
FAX: 914-921-8822
E-mail: cellufine@jncamericany.com

(Asia & Others)
JNC Corporation
Life Chemicals Division
〒100-8105
2-1, Otemachi 2-Chome, Chiyoda-ku Tokyo 100-8105 Japan
Tel: +81-3-3243-6150
Fax: +81-3-3243-6219
E-mail: cellufine@jnc-corp.co.jp

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

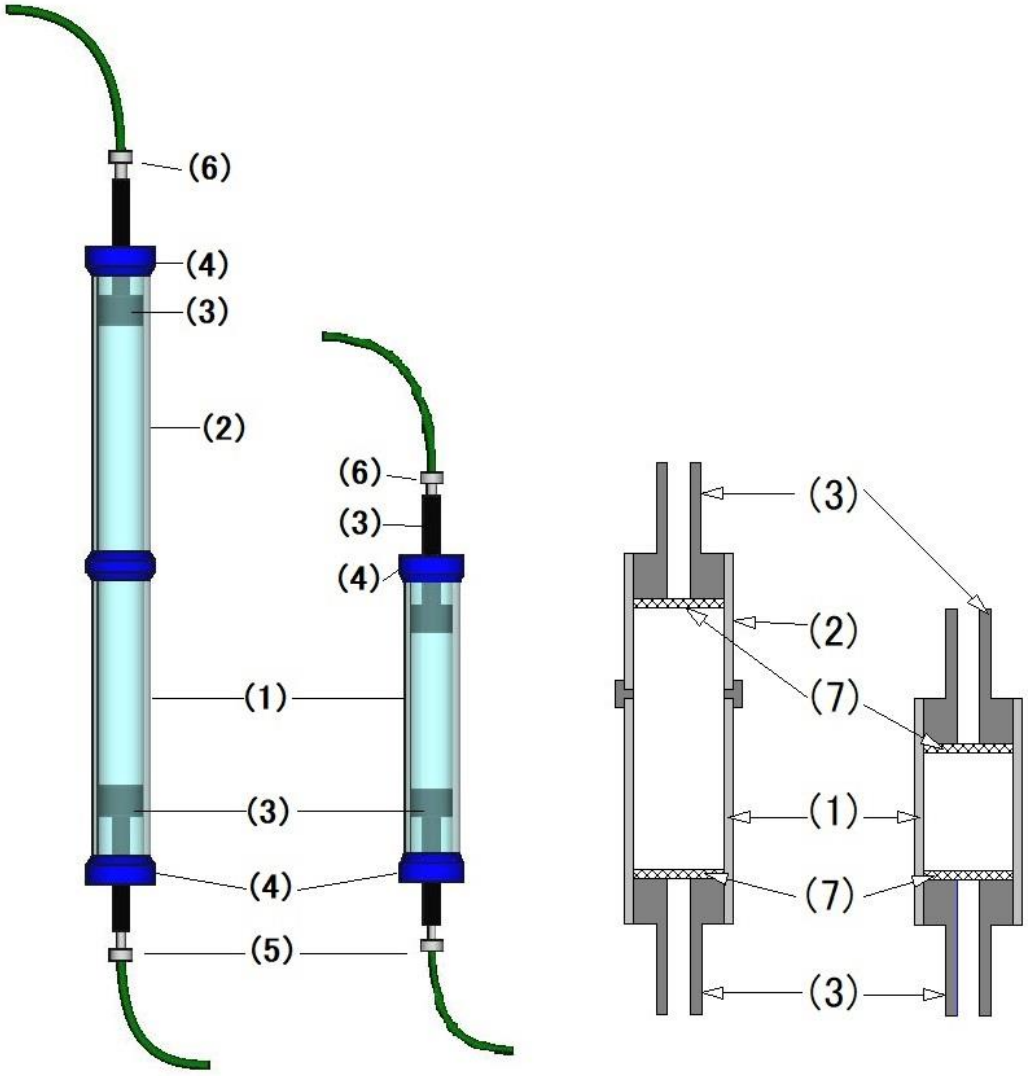


L	Column length [cm or m]
V_e	Elution time or volume
W_h	Half of width of peak
a, b	Peak width of 10% peak hight (a) front (b) rear
Note	Ve,Wh and a, b should have same dimensional units

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.

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		