Operating Instructions

Gel Filtration Chromatography Media

Cellufine GCL-2000HF

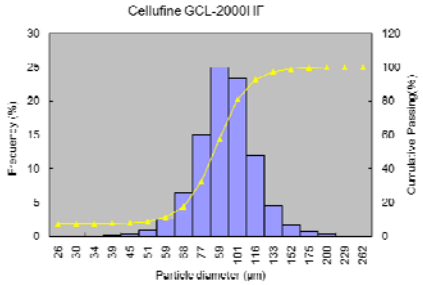
Description

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

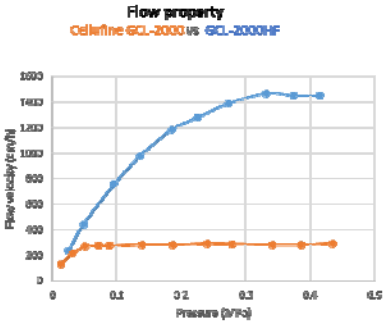
Physical-Chemical Characteristics

Support matrix	cellulose
Particle shape	spherical
Particle diameter (μm)	ca. 40 – 130 (ca 90 in average)*
pH Stability range	1 – 14
Operating pressure	< 0.2 MPa**
Supplied	suspension in 20 % EtOH

*Particle distribution of Cellufine GCL-2000HF (left) and Pressure flow property of Cellufine GCL and Cellufine GCL-2000HF (right)



Median diameter = 85 μm

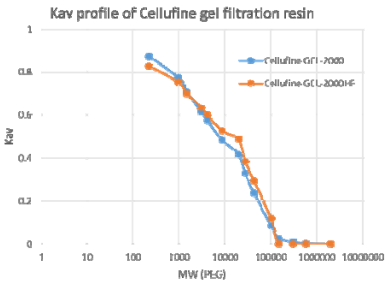


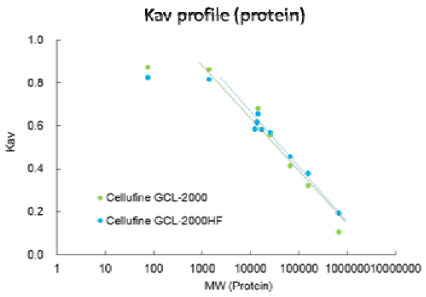
Column: 2.2 cm I.D. x 20 cm

Mobile phase: Pure water (24 ℃)

Molecular Weight Exclusion Limit (kD)

Packing	PEG	Polysaccharide	Globular Proteins
GCL-2000HF	200	-----	3,000





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

Column Packing

The following is a general procedure which can be used to pack gel filtration columns:

1. Measure bed height of a gravity settled bed before packing (with 50 ml measuring cylinder scale to know relationship between wet weight and its gravity settled bed height)
2. Prepare 40-60 % slurry concentration of resin in suitable buffer
   (Degassing is better if possible before packing)
3. Carefully pour the slurry into column and then open a bottom outlet.
4. After settling, run flow with pure water or buffer for 30 to 60 min to increase the packing at suitable pressure.
   Cellufine GCL2000HF for less than 0.2 MPa
   Otherwise
   (in the case of laboratory scale) run flow at a rate 20 – 100 % higher than the operational flow rate for 5 to 10 CV
5. Finally compress the resin based on the compression factor (Cf) ***.
   Suitable compression factor of Cellufine GCL2000 HF; 1.10 ～1.15****
6. Check and evaluate the status of packing
   As, HETP with 2 5 acetone or 0.1 M NaCl
   (injection volume: 1% of column volume)

*** Compression factor (Cf) = Settled bed height / Packing bed height

Most critical factors in column packing

Measurement of the starting media volume (Gravity settled volume after 24 hours)

Measurement of the media actually packed

**** ; For manual packing when bed height is 40 ～ 100 cm,

Packed manually on condition that Cf = 1.05

After packing, flow with pure water or buffer for 30 to 60 min at 0.2 MPa. (Please compress if gap is observed during flow.)

Operating Guidelines

General Operation

Equilibrate column with 2 – 5 volumes of buffer at the appropriate flow rates. The column is run isocratically.

Sample Preparation and Load

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

Elution

Elution occurs under isocratic conditions. If buffer exchange is desired, ensure that the column is equilibrated with the desired buffer before sample loading.

Chemical Compatibility

pH 1 – 14

Ethanol, methanol, acetone, etc.

6 M Urea

6 M Guanidine/HCl

0.1 M HCl

0.5 M NaOH

Most salts (NaCl, $(NH_4)_2SO_4$, etc.)

Most detergents (SDS, Tween®, Chap, 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 0.02 % sodium azide, 20 % ethanol, or 0.1 M NaOH. Long term storage can be conducted under identical conditions at 2 – 8 °C. Do not freeze.

Shelf Lifetime:

5 years from date of manufacture

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12. **Governing Law** - This contract shall be governed by and construed in accordance with the laws (other than those relating to conflict of laws questions) of the Commonwealth of Japan.

13. **Arbitration** - Any and all disputes or controversies arising under, out of or in connection with this contract or the sale or performance of the Products shall be resolved by final and binding arbitration in Tokyo under the rules of the Japan Arbitration Association then obtaining. The arbitrators shall have no power to add to, subtract from or modify any of the terms or conditions of this contract. Any award rendered in such arbitration may be enforced by either party in either the courts of the Commonwealth of Japan District Court for the District of Japan, to whose jurisdiction for such purposes JNC Corporation and Buyer each hereby irrevocably consents and submits.