IN017EN Ver1.3

Operating Instructions

Cellufine **Gel Filtration Chromatography Media**

Cellufine GCL-2000

Description

Cellufine GCL-2000 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
pH Stability range	1 – 14
Operating pressure	< 1 bar (14.5 psi)
Supplied	suspension in 20 % EtOH

Molecular Weight Exclusion Limit (kD)

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

Column Packing

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

1. Calculate volume required for the desired bed height 60 – 80 cm for gel filtration, 15 – 30 cm for desalting / buffer exchange.
2. Prepare a 40 – 60 % (v/v) slurry in buffer (eg. 0.1 M NaH_2PO_4, pH 7)
3. With column outlet closed, carefully pour the slurry into the column. Depending on the volume, a filler tube may be necessary.
4. With the inlet open to release air, insert and affix the top adjuster assembly at the slurry interface.
5. Open the column outlet and begin pumping buffer at a rate 10 – 20 % higher than the operational flow rate.

6. After the bed stabilizes, close the column outlet. Then with the inlet open, reposition the end cell on top of the bed.

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

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

Ordering Information (Catalogue No.)

Media type	Pack Size			
	100ml	500ml	5L	10L
Cellufine GCL-2000	**672 000 327**	**19791**	**19792**	**672 000 335**
Cellufine GH-25	670 000 327	19711	19712	670 000 335

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