Operating Instructions

Gel Filtration Chromatography Media

Cellufine® GH-25

DESCRIPTION

Cellufine GH-25 provides a rapid means of salt removal and buffer exchange for protein solutions. The semi-rigid spherical cellulose beads allow high flow rates with little compression of the column bed. The separation mechanism is based on differential solute access into the chromatographic bead.

Whereas large molecules (above 3 kD) are excluded from the packing and pass quickly through the column, small molecules (salts) diffuse into the bead and are thus retained longer. GH-25 can be used to remove alcohols, salts, detergents, fluorochromes, sugar, etc., from virtually any protein solution. It is compatible with most solvents and is stable from pH 1 - 14.

PHYSICAL–CHEMICAL CHARATERISTICS

	Cellufine GH-25
Support matrix	cellulose
Particle shape	spherical
Particle diameter (µm)	ca. 40 – 130
MW exclusion limit (kD)	3
pH stability range	1 - 14
Operating pressure	< 2 bar (29 psi)
Supplied	suspension in 20 % EtOH

COLUMN PACKING

1. Calculate volume required for the desired bed dimension.
2. Prepare a 40 – 60 % (v/v) slurry in appropriate exchange buffer.
3. With outlet closed, carefully pour the slurry into 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

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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 exchange buffer, or until the UV baseline has stabilized.

Sample Preparation and Load

Samples are typically loaded in the buffer which is to be exchanged. Filtration may be required to remove insoluble matter. The sample load is calculated as a function of column volume. Sample loads of 10% to 30% of total column volume are recommended. At higher loads, samples become less diluted. However, salt removal may not be absolute. Furthermore, volume loadability is inversely related to protein concentration.

Flow Rate

The recommended linear velocity range for GH-25 is 100–300 cm/h.

Elution

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

Chemical Compatibility

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 2-5 bed volumes of 0.1 - 0.5 M NaOH at a velocity of 50 –100 cm/h. Remove caustic by flushing with several bed volumes of DIW or exchange buffer. In the later case, measure the pH of the column eluate to ensure that the system has returned to

equilibrium.

Storage

For storage of opened containers, it is recommended that they be kept in a cold room (2 - 8 °C). Do not freeze.

Shelf Life:

5 years from date of manufacture

Product Ordering Information

Media type	Pack Size			
	100 ml	500 ml	5 lt	10 lt
Cellufine GH-25	670 000 327	19711	19712	670 000 335

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