instructions

CM Sepharose CL-6B

CM Sepharose™ CL-6B is a weak cation exchanger with excellent flow properties and high capacity for proteins of all pI values. The ion exchange group is a carboxy methyl group which remains charged and maintains consistently high capacity over the entire working range, pH 6–12.

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Amersham Biosciences

Table 1. Medium characteristics

Type of ion exchanger:	Weak cation	
Total ionic capacity:	0.10–0.14 mmol H^+/ml medium	
Available capacity*:	IgG (MW 160 000)	9.5 mg/ml Bovine
	Bovine COHb (MW 69 000)	75 mg/ml
	Ribonuclease (MW 13 700)	120 mg/ml
Bead structure:	6% cross-linked agarose	
Bead size range:	45–165 µm	
Mean particle size:	90 µm	
Max. linear flow rate**:	150 cm/h at 25 °C, HR 16/10 column, 5 cm bed height	
pH working range:	6–12	
pH stability***		
long term:	3–12	
cleaning-in-place:	2–14	
Chemical stability:	All commonly used aqueous buffers, 1.0 M acetic acid, 1.0 M NaOH, 8 M Urea, 8 M guanidine hydrochloride, ethanol, methanol etc.	
Physical stability:	Negligible volume variations due to changes in pH or ionic strength	
Autoclavable:	In 0.1 M sodium acetate at 121 °C for 30 min	

* The available capacity was determined in a 0.5 × 5 cm column at a linear flow rate of 300 cm/h. Binding buffer used was 0.1 M acetate, pH 5.0. Elution buffer contained 2 M NaCl.

** Linear flow rate = $\frac{\text{volumetric flow rate (cm}^3\text{/h)}}{\text{column cross-sectional area (cm}^2\text{)}}$

*** The ranges given are estmated based on our knowledge and experience. Please note the following:
pH stability, long term, refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subseqent chromatographic performance.
pH stabilty, short term, refers to the pH interval for regeneration, cleaning-in-place and sanitization procedures, see later.

Preparation of the medium

CM Sepharose CL-6B is supplied pre-swollen in 20% ethanol. Prepare a slurry by decanting the 20% ethanol solution and replace it with binding buffer in a ratio of 75% settled medium to 25% buffer. The binding buffer should not contain agents which significantly increase the viscosity. The column may be equilibrated with viscous buffers at reduced flow rates after packing is completed.

Packing Sepharose CL-6B

1. Equilibrate all material to the temperature at which the chromatography will be performed.
2. De-gas the medium slurry.
3. Eliminate air from the column dead spaces by flushing the end pieces with buffer. Make sure no air has been trapped under the column net. Close the column outlet with a few centimeters of buffer remaining in the column.
4. Pour the slurry into the column in one continuous motion. Pouring the slurry down a glass rod held against the wall of the column will minimize the introduction of air bubbles.
5. Immediately fill the remainder of the column with buffer, mount the column top piece onto the column and connect the column to a pump.
6. Open the bottom outlet of the column and set the pump to run at the desired flow rate. This should be at least 133% of the flow rate to be used during subsequent chromatographic procedures. However, the maximum flow rate, see Table 1, is typically employed during packing.

   Note: If you have packed at the maximum flow rate, do not exceed 75% of this value in subsequent chromatographic procedures.

7. Maintain the packing flow rate for 3 bed volumes after a constant bed height is reached.

Use of an adaptor

Adaptors should be fitted as follows:

1. After the medium has been packed as described above, close the column outlet and remove the top piece from the column. Carefully fill the rest of the column with buffer to form an upward meniscus at the top.
2. Insert the adaptor at an angle into the column, ensuring that no air is trapped under the net.
3. Make all tubing connections at this stage. There must be a bubble-free liquid connection between the column and the pump, and column and the sample application system (LV-3 or LV-4 valves).
4. Slide the plunger slowly down the column so that the air above the net and in the capillary tubings is displaced by eluent. Valves on the inlet side of the column should be turned in all directions during this procedure to ensure that air is removed.
5. Lock the adaptor in position on the medium surface, open the column outlet and start the eluent flow. Pass eluent through the column at the packing flow rate until the medium bed is stable. Re-position the adaptor on the medium surface as necessary.

Equilibration

Before starting a run, make sure that the ion-exchange bed has reached equilibrium. This is done by pumping binding buffer through the column until the conductivity and/or pH of the effluent is the same as for that of the in-going solution.

The column is now equilibrated and ready for use.

Binding

- The most common procedure is to let the molecules of interest bind to the ion exchanger and allow the others to pass through.

  However, in some cases it may be more useful to bind "contaminants"and let the molecules of interest remain in the flow through.

- For adsorption, it is critical to choose a buffer with an appropriate pH. Please refer to Table 2. The ionic strength of the buffer should be kept low so as not to interfere with sample binding. Recommended operating pH is within 0.5 pH units of the buffer's pKa and at least one pH unit below the isoelectric point (pI) of the molecule of interest.

Table 2. Suggested buffers for use with CM Sepharose CL-6B

Buffer	Counter ion	Concentration	pKa (25 °C)
Citrate	Na^+, Li^+	20 mM	3.1
Acetate	Na^+, Li^+	50 mM	4.8
Malonate	Na^+, Li^+	50 mM	5.7
Phosphate	Na^+	50 mM	7.2
BICINE	Na^+	50 mM	8.4

Note: CM Sepharose CL-6B will function as a pure ion exchanger in the pH range 6 to 12. The media is, however, stable down to pH 3. In the pH range 3 to 6 there will be other interactions and other selectivities.

Elution

Desorption may be done using either an increasing salt gradient (linear or step) or an increasing pH gradient (linear or step).

Regeneration

Depending on the nature of the sample, regeneration is normally performed by washing with a high ionic strength buffer (*e.g.* 1–2 M NaCl) and/or increasing pH, followed by re-equilibrating in binding buffer.

In some applications, substances such as denaturated proteins or lipids do not elute in the regeneration procedure. These can be removed by cleaning-in-place procedures.

Cleaning-in-place (CIP)

Remove ionically bound proteins by washing the column with 0.5 bed volumes of a 2 M NaCl solution, contact time 10–15 minutes, reversed flow direction.

Remove precipitated proteins, hydrophobically bound proteins and lipoproteins by washing the column with 1 M NaOH solution at a linear flow rate of approximately 40 cm/h, contact time 1–2 hours, reversed flow direction.

Wash with at least 3 bed volumes of binding buffer.

Remove strongly hydrophobically bound proteins, lipoproteins and lipids by washing the column with 4 bed volumes of up to 70% ethanol or 30% isopropanol, reversed flow direction. Apply increasing gradients to avoid air bubble formation when using high concentrations of organic solvents.

Alternatively, wash the column with 2 bed volumes of detergent in a basic or acidic solution. Use, for example, 0.1–0.5% non-ionic detergent in 0.1 M acetic acid. Wash at a linear flow rate of approximately 40 cm/h, contact time 1–2 hours, reversed flow direction. After treatment with

detergent always remove residual detergent by washing with 5 bed volumes of 70% ethanol.

In both cases wash with at least 3 bed volumes of binding buffer.

Sanitization

Sanitization reduces microbial contamination of the medium bed to a minimum.

Wash the column with 0.5–1 M NaOH at a flow rate of approximately 40 cm/h, contact time 30–60 minutes, reversed flow direction.

Re-equilibrate the column with 3–5 bed volumes of sterile binding buffer.

Column performance is normally not significantly changed by the cleaning-in-place or sanitization procedures described above.

Storage

For longer period of storage, keep the medium at 4–8 °C in a suitable bacteriostat, *e.g.* 20% ethanol. The medium must not be frozen.

Ordering Information

Designation	Pack size	Code No.
CM Sepharose CL-6B	500 ml	17-0720-01

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