GE Healthcare

Instructions 71-7074-00 AF **Ion Exchange Media**

DEAE Sepharose CL-6B

DEAE Sepharose CL-6B is a weak anion exchanger with excellent flow properties and high capacity for proteins of all pI values. The ion exchange group is diethylaminoethyl which remains charged and maintains consistently high capacities over the entire working range, pH 3–9.

Sepharose™

Table 1. Gel characteristics

Type of ion exchanger:	Weak anion	
Total ionic capacity:	0.13–0.17 mmole/ml gel	
Available capacity*:	Thyroglobulin (MW 669 000) HSA (MW 68 000) alpha-lactalbumin (MW 14 300)	2 mg/ml 170 mg/ml 150 mg/ml
Bead structure:	6% cross-linked agarose	
Bead size range:	45–165 µm	
Mean particle size:	90 µm	
Max. flow rate:	150 cm/h	
Max. operating pressure:	0.015 MPa (0.15 bar, 2 psi)	
pH working range:	3–9	
pH stability**:	long term: short term:	3–12 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 variation due to changes in pH or ionic strength.	
Autoclavable:	In 0.1 M NaCl at 121 °C for 30 min.	

* The available capacity was determined in a 0.5x5 cm column at a linear flow rate of 300 cm/h. Starting buffer used was 0.05 M Tris, pH 8.3. Elution buffer contained 2 M NaCl.

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

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1. Preparation of the gel

DEAE Sepharose CL-6B is supplied pre-swollen in 20% ethanol. Prepare a slurry by decanting the 20% ethanol solution and replace it with starting buffer in a ratio of 75% settled gel to 25% buffer. The starting 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.

2. Packing Sepharose CL-6B gels

1. Equilibrate all material to the temperature at which the chromatography will be performed.
2. De-gas the gel 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. Do not exceed the maximum pressure given in Table 1.

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.

3. Use of an adaptor

Adaptors should be fitted as follows:

1. After the gel 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).
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, open the column outlet and start the eluent flow. Pass eluent through the column at the packing flow rate until the gel bed is stable. Re-position the adaptor on the gel surface as necessary.

4. Equilibration

Before starting a run, make sure that the ion-exchange bed has reached equilibrium. This is done by pumping starting 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.

5. Binding

- The most common procedure is to let the molecules of interest bind to the ion exchanger and allow other molecules to pass through. However, in some cases it may be more useful to bind "contaminants" and let the molecules of interest 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 above the isoelectric point (pI) of the molecule of interest.

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

Buffer	Counter ion	Concentration	pKa (25°C)
N-methylpiperazine	Cl^-	20 mM	4.8
piperazine	Cl^-	20 mM	5.7
	$HCOOL^-$		
histidine	Cl^-	20 mM	6.0
bis-Tris	Cl^-	20 mM	6.5
bis-Tris propane	Cl^-	20 mM	6.8
triethanolamine	Cl^-	20 mM	7.8
	CH_3COO^-		
Tris	Cl^-	20 mM	8.1
N-methyldiethanolamine	SO_4	50 mM	8.5
	Cl^-		
	CH_3COO		
diethanolamine	Cl^-	20 mM at pH 8.4	8.9
		50 mM at pH 8.8	
1,3-diaminopropane	Cl^-	20 mM	8.6
ethanolamine	Cl^-	20 mM	9.5
piperazine	Cl^-	20 mM	9.7
1,3-diaminopropane	Cl^-	20 mM	10.5

6. Elution

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

7. 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 decreasing pH, followed by re-equilibrating in starting 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.

8. 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 starting 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% nonionic 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 starting buffer.

9. Sanitization

Sanitization reduces microbial contamination of the gel 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 starting buffer.

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

10. Storage

It is recommended that the gel is stored for longer periods of time in 20% ethanol at 4 °C.

11. Ordering information

Product	Pack size	Code No.
DEAE Sepharose CL-6B	500 ml	17-0710-01
Ion Exchange Chromatography & Chromatofocusing Principles & Methods		11-0004-21

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