cytiva

DEAE **Sepharose** CL-6B

Ion exchange resin

Instructions for Use

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 to 9.

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Read these instructions carefully before using the products.

Safety

For use and handling of the products in a safe way, refer to the Safety Data Sheets.

1 Characteristics

Table 1. Characteristics of DEAE Sepharose CL-6B

Matrix	Cross-linked agarose, 6%, spherical
Particle size, d_{50V} [1]	~ 90 µm
Type of ion exchanger	Weak anion
Ionic capacity	0.14 to 0.18 mmol Cl^-/mL resin
Available capacity[2]	Thyroglobulin (MW 669 000) 2 mg/mL HSA (MW 68 000) 170 mg/mL alpha-lactalbumin (MW 14 300) 150 mg/mL
Recommended maximum operating flow velocity	50 to 150 cm/h[3]
Recommended maximum operating pressure	0.045 MPa (0.45 bar, 6.53 psi)[4]
pH stability, operational[5]	3 to 12
pH stabilty, CIP[6]	3 to 14
pH stability[7]	Below 9
Chemical stability	Stable to commonly used aqueous buffers
Physical stability	Negligible volume variation due to changes in pH or ionic strength

1 Median particle size of the cumulative volume distribution.

2 The available capacity was determined in a 0.5 x 5 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.

3 The recommended maximum operating flow velocity depends on the column dimensions and bed height used.

4 The recommended maximum operating pressure depends on the column dimensions and bed height used.

5 pH range where resin can be operated without significant change in function.

6 pH range where resin can be subjected to cleaning-or-sanitization-in-place without significant change in function.

7 pH range where ligand is fully charged; although the ligand is fully charged throughout the range stated, only use the resin within the stated stability ranges.

2 Preparing the resin

DEAE Sepharose CL-6B is supplied preswollen in 20% ethanol. Prepare a slurry by decanting the 20% ethanol solution and replace it with starting buffer in a ratio of 75% settled resin to 25% buffer. The starting buffer must not contain agents which significantly increase the viscosity. The column can be equilibrated with viscous buffers at reduced flow rates after packing is completed.

3 Packing the Sepharose CL-6B

Step	Action
1	Equilibrate all material to the temperature at which the chromatography will be performed.
2	Degas the resin 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, attach the column top piece onto the column and connect the column to a pump.

Step	Action
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, on page 3*, is typically employed during packing. Do not exceed the maximum pressure given in *Table 1, on page 3*. ***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.

4 Use of an adapter

Adapters must be fitted as follows:

Step	Action
1	After the resin 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 adapter at an angle into the column, ensuring that no air is trapped under the net.

Step	Action
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 make sure that air is removed.
5	Lock the adapter in position, open the column outlet and start the eluent flow. Pass eluent through the column at the packing flow rate until the resin bed is stable. Re-position the adapter on the resin surface as necessary.

5 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.

6 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 might 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. Refer to the table below. The ionic strength of the buffer must 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.

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

Buffer	Counter ion	Concentration	pKa (25°C)
ethanolamine	Cl^-	20 mM	9.5
piperazine	Cl^-	20 mM	9.7
1,3-diaminopropane	Cl^-	20 mM	10.5

7 Elution

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

8 Regeneration

Depending on the nature of the sample, regeneration is normally performed by washing with a high ionic strength buffer (e.g., 1 to 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.

9 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 to 15 minutes, reversed flow direction.

Remove precipitated proteins, hydrophobically bound proteins and lipoproteins by washing the column with 1.0 M NaOH solution at a low velocity of approximately 40 cm/h, contact time 1 to 2 hours, reversed flow direction.

Wash with at least 3 bed volumes of starting buffer.

10 Sanitization

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

Wash the column with 0.5 to 1.0 M NaOH at a flow rate of approximately 40 cm/h, contact time 30 to 60 minutes, reversed flow direction.

Re-equilibrate the column with 3 to 5 bed volumes of sterile starting buffer.

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

11 Storage

It is recommended that the resin is stored for longer periods of time in 20% ethanol at 4°C to 30°C.

12 Ordering information

Product	Pack size	Product code
DEAE Sepharose CL-6B	500 mL	17071001
	10 L	17071005
Ion Exchange Chromatography Principles & Methods		11000421

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cytiva.com/protein-purification

For local office contact information, visit cytiva.com/contact

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