GE Healthcare

Instructions 71–7100–00 AC **Ion Exchange**

DEAE Sephacel

DEAE Sephacel™ is a weak anion exchanger based on beaded cellulose. The ion exchange group is diethylaminoethyl, which remains charged and maintains consistently high capacity over the entire working range, pH 2–9.

DEAE Sephacel is macroporous and has an exclusion limit of approximately 1 000 000 daltons for globular proteins.

Sephacel™

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Table 1. Medium characteristics

Type of ion exchanger:	Weak anion
Total ionic capacity:	0.11–0.14 mmol/ml medium
Available capacity*:	10 mg Thyroglobulin (MW 669 000)/ml drained medium 160 mg HSA (MW 68 000)/ml drained medium
Bead structure:	Beaded cellulose
Bead size range:	40–160 µm
Mean particle size:	100 µm
Max linear flow rate**:	50 cm/h
pH working range:	2–9
pH stability***	
long term:	2–12
short term:	2–12
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 estimated in 0.5 M Tris, pH 8.3. The 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 estimates 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 subsequent chromatographic performance.
pH stability, short term refers to the pH interval for regeneration and cleaning.

1. Preparing the medium

DEAE Sephacel is supplied pre-swollen in 24 % ethanol. Prepare a slurry by decanting the 24 % ethanol solution and replace it with starting buffer in a ratio of 75 % settled medium 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 DEAE Sephacel

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 linear flow rate, do not exceed 75 % of this in subsequent chromatographic procedures.
7. Maintain the packing flow rate for 3 bed volumes after a constant bed height is reached.

3. Using 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.
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.

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 bed is stable. Re-position the adaptor on the medium surface as necessary.

4. Equilibrating

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 that of the in-going solution.

The column is now equilibrated and ready for use.

Table 2. Suggested buffers for use with DEAE Sephacel.

Buffer	Counter ion	Concentration	pKa (25 °C)
N-methylpiperazine	Cl^-	20 mM	4.8
piperazine	Cl^-	20 mM	5.7
	$HCOO^-$		
L-histidine	Cl^-	20 mM	6.2
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	8.2
N-methyldiethanolamine	SO_4^{2-}	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-diaminopropionate	Cl^-	20 mM	10.5

5. 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. The 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.

6. Elution

Desorption is achieved using either an increasing salt gradient (continuous or step-wise) or an decreasing pH gradient (continuous or step-wise).

7. Regeneration

Depending of 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-equilibration 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 procedures described below.

8. Cleaning

Remove ionically bound proteins by washing the column with 0.5–1 bed volume of a 2 M NaCl solution.

Remove precipitated proteins, hydrophobically bound proteins and lipoproteins by washing the medium with 1 bed volume of a 0.1 M NaOH solution followed by binding buffer until free from alkali.

Strongly hydrophobically bound proteins, lipoproteins and lipids can be removed by washing the medium with up to 70 % ethanol or 30 % isopropanol.

Alternatively, wash the medium with 2 bed volumes of detergent in a basic or acidic solution. Use for example, 0.1–0.5 % non-ionic detergent (e.g. Triton X-100) in 0.1 M acetic acid. After treatment with detergent always remove residual detergents by washing with 5 bed volumes of 70 % ethanol.

After cleaning the medium, re-equilibrate the ion-exchanger according to the recommendations above.

9. Storage

Store the medium at 4–8˚C in 20 % ethanol.

10. Ordering information

Product	Pack size	Code No.
DEAE Sephacel	500 ml	17-0500-01

www.gehealthcare.com/protein-purification

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