cytiva

DEAE **Sephacel**

Ion exchange

Instructions for Use

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 to 9.

DEAE Sephacel is macroporous and has an exclusion limit of $M_p \sim 1 \times 10^5$ for dextrans.

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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 BioProcess™ resins

DEAE Sephacel™ is part of the Cytiva range of resins. BioProcess™ chromatography resins are developed and supported for production scale chromatography. BioProcess resins are produced with validated methods and are tested to meet manufacturing requirements. Secure ordering and delivery routines give a reliable supply of resins for production scale. Regulatory Support Files (RSF) are available to assist process validation and submissions to regulatory authorities. BioProcess resins cover all purification steps from capture to polishing.

2 Characteristics

Table 1. Resin characteristics

Matrix	Beaded cellulose
Particle size, d_{50V} [1]	~ 100 µm
Type of ion exchanger	Weak anion
Ionic capacity	0.10 to 0.14 mmol Cl^-/mL resin
Available capacity [2]	~ 10 mg Thyroglobulin (MW 669 000)/mL resin ~ 160 mg HSA (MW 68 000)/mL resin
Recommended operating flow velocity	≥ 40 cm/h [3]
pH stability, operational [4]	2 to 12
pH stability, CIP [5]	2 to 12
pH ligand fully charged [6]	Below 9
Chemical stability	Stable to commonly used aqueous buffers
Physical stability	Negligible volume variation due to changes in pH or ionic strength
Autocalvability	20 min at 121 °C in 0.1 M NaCl, 1 cycle

[1] Median particle size of the cumulative volume distribution.

[2] The available capacity was estimated in 0.5 M Tris, pH 8.3. The elution buffer contained 2 M NaCl.

[3] 5 cm diameter, 10 cm bed height, at room temperature using 0.02 M sodium chloride.

[4] pH range where resin can be operated without significant change in function.

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

[6] pH range where ligand is fully charged; although the ligand is fully charged at the range stated, only use the resin within the stated stability ranges.

3 Preparing the resin

DEAE Sephacel 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.

4 Packing DEAE Sephacel

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.

Step	Action
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.
6	Open the bottom outlet of the column and set the pump to run at the desired flow rate. This must be at least 133% of the flow rate to be used during subsequent chromatographic procedures. However, the maximum flow rate is typically employed during packing ***Note:*** *If you have packed at the maximum 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.

5 Using 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.
2	Insert the adapter 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 must be turned in all directions during this procedure to make sure that air is removed.
5	Lock the adapter in position on the resin 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 adapter on the resin surface as necessary.

6 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^- $HCOO^-$	20 mM	5.7
L-histidine	Cl^-	20 mM	6.2
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	8.2
N-methyldiethanolamine	SO_4^{2-} 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
ethanolamine	Cl^-	20 mM	9.5
piperazine	Cl^-	20 mM	9.7
1,3-diaminopropane	Cl^-	20 mM	10.5

7 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 can 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. 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.

8 Elution

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

9 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-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.

10 Cleaning

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

Remove precipitated proteins, hydrophobically bound proteins and lipoproteins by washing the resin with 1 bed volume of a 0.01 M NaOH solution followed by binding buffer until free from alkali.

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

11 Storage

Store the resin at 4°C to 30°C in 20% ethanol.

12 Ordering information

Product	pack size	Product code
DEAE Sephacel	500 mL	17050001
	10 L	17050005

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cytiva.com/protein-purification

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

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