GE Healthcare

Instructions 71-7104-00 AD **Ion Exchange**

DEAE Sephadex A-25
DEAE Sephadex A-50
QAE Sephadex A-25
QAE Sephadex A-50

CM Sephadex C-25
CM Sephadex C-50
SP Sephadex C-25
SP Sephadex C-50

DEAE Sephadex™ A-25 and DEAE Sephadex A-50 are weak anion exchangers. The ion exchange group is diethylaminoethyl which remains charged and maintains consistently high capacity over the entire working range, pH 2–9.

QAE Sephadex A-25, and QAE Sephadex A-50 are strong anion exchangers. The ion exchange group is diethyl-(2-hydroxy-propy)aminoethyl which remains charged and maintains consistently high capacity over the entire working range, pH 2–12.

CM Sephadex C-25 and CM Sephadex C-50 are weak cation exchangers. The ion exchange group is a carboxy methyl group which remains charged and maintains consistently high capacity over the working range of pH 6–10.

SP Sephadex C-25 and SP Sephadex C-50 are strong cation exchangers. The ion exchange group is a sulphopropyl group which remains charged and maintains consistently high capacity over the entire working range of pH 4–13.

Sephadex

Table 1. Medium characteristics

	DEAE Sephadex A-25
Type of ion exchanger	Weak anion
Total ionic capacity (mmol/g dry powder)	3–4
Available capacity* (mg/ml drained medium)	
thyroglobulin (M_r 669 000)	1
HSA (M_r 68 000)	30
α-lactalbumin (M_r 14 300)	140
Matrix	Cross-linked dextran
Dry particle size range (µm)	40–120
Max operating linear flow rate)**	475 cm/h at 25 °C, HR 16/10 column, 5 cm bed height
pH working range***	2–9
pH stability***	
Long term	2–13
Short term	2–13
Chemical stability	Stable to all commonly used aqueous buffers additives such as 8 M urea and 6 M guanidine hydrochloride.
Physical stability	Negligible volume variation due to changes in pH or ionic strrength.
Autoclavable	In 0.1 M sodium chloride at 121 °C for 30 min.

* The available binding capacity was estimated in 0.05 M Tris-HCl, pH 8.3.

** 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 to the best of our knowledge and experience. Please note the following:

i) *pH working range* means the pH interval where the medium functions normally.

ii) *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 procedures.

DEAE Sephadex A-50	QAE Sephadex A-25	QAE Sephadex A-50
Weak anion	Strong anion	Strong anion
3–4	2.6–3.4	2.6–3.4
2 110 50	1.5 10 110	1.2 80 30
Cross-linked dextran	Cross-linked dextran	Cross-linked dextran
40–120	40–120	40–120
45 cm/h at 25 °C, HR 16/10 column, 5 cm bed height	475 cm/h at 25 °C, HR 16/10 column, 5 cm bed height	45 cm/h at 25 °C, HR 16/10 column, 5 cm bed height
2–9	2–12	2–12
2–12 2–12	2–13 2–13	2–12 2–12
	Stable to all commonly used aqueous buffers and and additives such as 8 M urea and 6 M guanidine hydrochloride.	
Volume changes due to changes in pH or ionic strength.	Negligible volume variation due to changes in pH or ionic strength.	Volume changes due to changes in pH or ionic strength.
	In 0.1 M sodium chloride at 121 °C for 30 min.	

Table 2. Medium characteristics

	CM Sephadex C-25 CM
Type of ion exchanger	Weak cation
Total ionic capacity (mmol /g dry powder)	4–5
Available capacity* (mg/ml drained medium)	
IgG (M_r 160 000)	1.6
Bovine COHb (M_r 69 000)	70
Ribonuclease (M_r 13 700)	190
Matrix	Cross-linked dextran
Dry particle size range (µm)	40–120
Max operating linear flow rate** HR 16/10 column, 5 cm bed height	475 cm/h at 25 °C, HR 16/10 column 5 cm bed height
pH working range***	6–10
pH stability***	
Long term 2–13	2–12
Short term 2–13	2–12
Chemical stability	Stable to all commonly used aqueous buffers and additives such as 8 M urea and 6 M guanidine hydrochloride.
Physical stability	Negligible volume variation due to changes in pH or ionic strength.
Autoclavable	In 0.1 M sodium chloride at 121 °C for 30 min.

* The available binding capacity was estimated in 0.1 M Acetate buffer pH 5.0.

** 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 to the best of our knowledge and experience. Please note the following:

i) *pH working range* means the pH interval where the medium functions normally

ii) *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 procedurs.

Sephadex C-50	SP Sephadex C-25	SP Sephadex C-50
Weak cation	Strong cation	Strong cation
4–5	2–2.6	2–2.6
7 140 120	1.1 70 230	8 110 100
Cross-linked dextran	Cross-linked dextran	Cross-linked dextran
40–120	40–120	40–120
45 cm/h at 25 °C, HR 16/10 column 5 cm bed height	475 cm/h at 25 °C, HR 16/10 column 5 cm bed height	45 cm/h at 25 °C, HR 16/10 column 5 cm bed height
6–10	4–13	4–13
2–13 2–13	2–12 2–12	
	Stable to all commonly used aqueous buffers and additives such as 8 M urea and 6 M guanidine. hydrochloride	
Volume changes due to changes in pH or ionic strength.	Negligible volume variation due to changes in pH or ionic strength.	Volume changes due to changes in pH or ionic strength.
	In 0.1 M sodium chloride at 121 °C for 30 min.	

Sephadex is particular suitable as a basis for an ion exchange matrix, since it is hydrophilic and shows very low non-specific adsorption. Proteins, nucleic acids and other labile biological molecules are not adsorbed to or denatured by the medium.

DEAE Sephadex A-50, QAE Sephadex A-50 , CM Sephadex C-50 and SP Sephadex C-50 are prepared from Sephadex G-50 and have a greater porosity and higher available capacity for larger molecules (M_r > 30 000) than DEAE Sephadex A-25, QAE Sephadex A-25, CM Sephadex C-25 and SP Sephadex C-25, which are prepared from Sephadex G-25.

DEAE Sephadex A-25, QAE Sephadex A-25, CM Sephadex C-25 and SP Sephadex C-25 may have a higher available capacity for molecules with molecular weights over 100 000 since such molecules only bind on the surface of the medium bead. Here the higher charge density of the A-25 media may be advantageous.

1. Preparing the medium

DEAE Sephadex A-25, DEAE Sephadex A-50, QAE Sephadex A-25, QAE Sephadex A-50, CM Sephadex C-25, CM Sephadex C-50, SP Sephadex C-25 and SP Sephadex C-50 are supplied as dry powders.

Weigh out the required amount of dry powder and suspend it in the binding buffer. Note that the swelling factor is dependent on the buffer used. As a general guideline; 1 g dry powder DEAE Sephadex A-25 or QAE Sephadex A-50 gives about 25 ml final volume of medium, 1 g dry powder CM Sephadex C-25 or SP Sephadex C-25 gives about 7 ml final volume of medium and 1 g dry powder CM Sephadex C-50 or SP Sephadex C-50 gives about 30 ml final volume of medium, when swollen in saline buffer. Sephadex ion exchangers should be swollen at the pH to be used in the experiment. Complete swelling takes 1-2 days at room temperature or 2 hours, at 100 °C, over boiling water. Swelling at high temperature also serves to deaerate the medium. Vigorous stirring, e.g. with a magnetic stirrer, should be avoided in order not to damage the particles. Stir the required amount of ion exchanger into an excess of binding buffer. The binding buffer must contain the same ion as that originally present in the ion exchanger.

After the initial swelling, remove the supernatant and wash the ion exchanger extensively on a Büchner funnel with binding buffer.

Prepare a slurry 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.

2. Packing Sephadex media

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 procedure.
7. Maintain the packing flow rate for 3 bed volumes after a constant bed height is reached.
8. After packing columns with DEAE Sephadex A-50, QAE Sephadex A-50, CM Sephadex C-50 or SP Sephadex C-50 we recommend layering about 0.5 cm Sephadex G-25 Coarse, swollen in the same buffer as the ion exchanger, onto the top of the bed to act as a bed surface protectant.

This should not be done for a column packed with DEAE Sephadex A-25, QAE Sephadex A-25, CM Sephadex C-25 or SP Sephadex C-25 where an adaptor will be fitted.

3. Using an adaptor

An adaptor is less suitable for columns packed with DEAE Sephadex A-50, QAE Sephadex A-50, CM Sephadex C-50 or SP Sephadex C-50 because of bed volume variations, due to changes in pH or ionic strength, during elution.

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-freee liquid connection between the column and the pump.
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.

4. Equilibration

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

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 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 appropiate 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.

Table 3. Buffers for cation exchange chromatography

pH interval	Substance	Conc. (mM)	Counter-ion	pK_a (25˚C)[1]
1.4–2.4	Maleic acid	20	Na+	1.92
2.6–3.6	Methyl malonic acid	20	Na+ or Li+	3.07
2.6–3.6	Citric acid	20	Na+	3.13
3.3–4.3	Lactic acid	50	Na+	3.86
3.3–4.3	Formic acid	50	Na+ or Li+	3.75
3.7–4.7	Succinic acid	50	Na+	4.21
5.1–6.1	Succinic acid	50	Na+	5.64
4.3–5.3	Acetic acid	50	Na+ or Li+	4.75
5.2–6.2	Methyl malonic acid	50	Na+ or Li+	5.76
5.6–6.6	MES	50	Na+ or Li+	6.27
6.7–7.7	Phosphate	50	Na+	7.20
7.0–8.0	HEPES	50	Na+ or Li+	7.56
7.8–8.8	BICINE	50	Na+	8.33

[1] Handbook of chemistry and physics, 83rd edition, CRC, 2002–2003.

Table 4. Buffers for anion exchange chromatography.

pH interval	Substance	Conc. (mM)	Counter-ion	pK_a (25°C)[1]
4.3–5.3	N-Methylpiperazine	20	Cl^-	4.75
4.8–5.8	Piperazine	20	Cl^- or $HCOO^-$	5.33
5.5–6.5	L-Histidine	20	Cl^-	6.04
6.0–7.0	Bis-Tris	20	Cl^-	6.48
6.2–7.2	Bis-Tris propane	20	Cl^-	6.65
8.6–9.6	Bis-Tris propane	20	Cl^-	9.10
7.3–8.3	Triethanolamine	20	Cl^- or CH_3COO^-	7.76
7.6–8.6	Tris	20	Cl^-	8.07
8.0–9.0	N-Methyldiethanolamine	20	SO_4^{2-}	8.52
8.0–9.0	N-Methyldiethanolamine	50	Cl^- or CH_3COO^-	8.52
8.4–9.4	Diethanolamine	20 at pH 8.4 50 at pH 8.8	Cl^-	8.88
8.4–9.4	Propane 1,3-diamino	20	Cl^-	8.88
9.0–10.0	Ethanolamine	20	Cl^-	9.50
9.2–10.2	Piperazine	20	Cl^-	9.73
10.0–11.0	Propane 1,3-diamino	20	Cl^-	10.55
10.6–11.6	Piperidine	20	Cl^-	11.12

[1] Handbook of chemistry and physics, 83rd edition, CRC, 2002–2003.

6. Elution

For DEAE Sephadex and and QAE Sephadex media, elution is achieved using either an increasing salt gradient (continuous or step wise) or a decreasing pH gradient (continuous or step wise). For CM Sephadex and SP Sephadex media, elution is achieved using either an increasing salt gradient (continuous or step wise) or an increasing 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/incresing 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, CIP (Cleaning-in-place).

8. CIP, Cleaning-in-place

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.

Note: Due to the relatively large volume changes of Sephadex based media in organic solvents, we recommend washing with organic solvents on a Büchner funnel, since the medium needs to be repacked after such treatment.

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

9. Storage

Dry powders of DEAE Sephadex A-25, DEAE Sephadex A-50, QAE Sephadex A-25, QAE Sephadex A-50, CM Sephadex C-25, CM Sephadex C-50, SP Sephadex C-25 and SP Sephadex C-50. should be stored between 4–25 °C.

Store swollen medium in the presence of a suitable bacteriostat, e.g. 20% ethanol or 0.01 M NaOH at 4–8 °C. Sodium azide or thiomersal should not be used as bacteriostat.

10. Ordering information

Product	Pack size	Code No.
DEAE Sephadex A-25	100 g	17-0170-01
DEAE Sephadex A-25	500 g	17-0170-02
DEAE Sephadex A-50	100 g	17-0180-01
DEAE Sephadex A-50	500 g	17-0180-02
QAE Sephadex A-25	100 g	17-0190-01
QAE Sephadex A-25	500 g	17-0190-02
QAE Sephadex A-50	100 g	17-0200-01
QAE Sephadex A-50	500 g	17-0200-02
CM Sephadex C-25	100 g	17-0210-01
CM Sephadex C-25	500 g	17-0210-02
CM Sephadex C-50	100 g	17-0220-01
CM Sephadex C-50	500 g	17-0220-02
SP Sephadex C-25	100 g	17-0230-01
SP Sephadex C-25	500 g	17-0230-02
SP Sephadex C-50	100 g	17-0240-01
SP Sephadex C-50	500 g	17-0240-02

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