cytiva

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

Ion exchange resins

Instructions for Use

cytiva.com

71710400 AG

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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 Introduction

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 below pH 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 pH range.

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 above pH 6.

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 pH range.

2 BioProcess 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.

3 Resin Characteristics

Table 1. Anion exchangers resin characteristics

	DEAE Sephadex A-25	DEAE Sephadex A-50	QAE Sephadex A-25	QAE Sephadex A-50
Matrix	Cross-linked dextran, spherical			
Type of ion exchanger	Weak anion	Weak anion	Strong anion	Strong anion
Ionic capacity (mmol/g dry resin)	3.0-4.0	3.0-4.0	2.6-3.4	2.6-3.4
Available capacity[1]				
Thyroglobulin (M_r 669 000)	1	2	1.5	1.2
HAS (M_r 68 000)	30	110	10	80
α-lactalbumin (M_r 14 300)	140	50	110	30
Particle size distribution, dry (µm)[2]	40-100	40-100	40-100	40-100
Recommended operating flow velocity[3]	≥ 120 cm/h	≥ 60 cm/h	≥ 100 cm/h	≥ 60 cm/h
pH stability, operational[4]	2-13	2-12	2-13	2-12
pH stability, CIP[5]	2-13	2-12	2-13	2-12
pH ligand fully charged[6]	Below 9	Below 9	Entire pH range	Entire pH range
Chemical stability	Stable to commonly used aqueous buffers			
Physical stability	Negligible volume variation due to changed in pH or ionic strength.	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
Autoclavability	30 min at 121°C in 0.1 M sodium chloride			

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

2 ≥ 80% volume share within given range.

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 throughout the range stated, only use the resin within the stated stability ranges.

Table 2. Cation exchangers resin characteristics

	CM Sephadex C-25	CM Sephadex C-50	SP Sephadex C-25	SP Sephadex C-50
Matrix	Cross-linked dextran, spherical			
Type of ion exchanger	Weak cation	Weak caton	Strong cation	Strong cation
Ionic capacity (mmol/g dry resin)	4.0-5.0	4.0-5.0	2.0-2.6	2.0-2.6
Available capacity[1]				
IgG (M_r 160 000)	1.6	7	1.1	8
Bovine COHb (M_r 69 000)	70	140	70	110
Ribonuclease (M_r 13 700)	190	120	230	100
Particle size distribution, dry (µm)[2]	40-100	40-100	40-100	40-100
Recommended operating flow velocity[3]	≥ 120 cm/h	≥ 100 cm/h	≥ 100 cm/h	≥ 100 cm/h
pH stability, operational[4]	2-13	2-12	2-13	2-12
pH stability, CIP[5]	2-13	2-12	2-13	2-12
pH ligand fully charged[6]	Above 6	Above 6	Entire pH range	Entire pH range
Chemical stability	Stable to commonly used aqueous buffers			
Physical stability	Negligible volume variation due to changed in pH or ionic strength.	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

	CM Sephadex C-25	CM Sephadex C-50	SP Sephadex C-25	SP Sephadex C-50
Autoclavability	30 min at 121°C in 0.1 M sodium chloride			

[1] The available binding capacity was estimated in 0.1 M Acetate buffer, pH 5.0.

[2] ≥ 80% volume share within given range.

[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 throughout the range stated, only use the resin within the stated stability ranges.

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 resin.

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 can have a higher available capacity for molecules with molecular weights over 100 000 since such molecules only bind on the surface of the resin bead. Here the higher charge density of the A-25 resins can be advantageous.

4 Operation

Preparing the resin

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. For a general guideline, see the table below.

Table 3. Swelling volumes for Sephadex resins

Resin	Approximate volume per 1 g dry powder in saline buffer
DEAE Sephadex A-25	~ 7 mL
QAE Sephadex A-25	~ 7 mL
DEAE Sephadex A-50	~ 20 mL
QAE Sephadex A-50	~ 25 mL
CM Sephadex C-25	~ 7 mL
SP Sephadex C-25	~ 7 mL
CM Sephadex C-50	~ 25 mL
SP Sephadex C-25	~ 25 mL

Sephadex ion exchangers must be swollen at the pH to be used in the experiment. Note that in ultra pure water, the bands will swell too much, too quickly, which can cause breakage. Complete swelling takes 1 to 2 days at room temperature. Swelling at high temperature also serves to degas the resin. Vigorous stirring, for example with a magnetic stirrer, must 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 resin to 25% buffer. The binding 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.

Packing the Sephadex resins

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, mount 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 4* and *Table 2, on page 5*, 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	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 4* and *Table 2, on page 5*, is typically employed during packing.
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 must 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 adapter will be fitted.

Using an adapter

An adapter 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.

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.
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 resin bed is stable. Re-position the adapter on the resin surface as necessary.

Equilibration

Before starting a run, make sure that the resin 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.

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. See the tables below. The ionic strength of the buffer must 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 4. Buffers for cation exchange chromatography

pH interval	Substance	Conc. (mM)	Counter-ion	pK_a (25°C)[1]
1.4-2.4	Malic 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

pH interval	Substance	Conc. (mM)	Counter-ion	pK_a (25°C)[1]
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.

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	Ethanoliamine	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.

Elution

For DEAE Sephadex and QAE Sephadex resins, 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 resins, elution is achieved using either an increasing salt gradient (continuous or step wise) or an increasing pH gradient (continuous or step wise).

5 Maintenance

Regeneration

Depending of 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/increasing 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).

Cleaning-In-Place (CIP)

Remove ionically bound proteins by washing the column with 0.5 to1 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 0.1 M or 0.001 M NaOH (see pH stability, CIP in *Table 1, on page 4* and *Table 2, on page 5*) solution followed by binding buffer until free from alkali.

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

Alternatively, wash the resin with 2 bed volumes of detergent in a basic or acidic solution. Use for example, 0.1% to 0.5% nonionic detergent 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 resins in organic solvents, we recommend washing with organic solvents on a Büchner funnel, since the resin needs to be repacked after such treatment.*

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

CAUTION
70% ethanol can require the use of explosion-proof areas and equipment.

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 must be stored at 4°C to 30°C.

Store swollen resin in the presence of a suitable bacteriostat, for example 20% ethanol or 0.01 M NaOH[1] at 4°C to 30°C. Sodium azide or thiomersal must not be used as bacteriostat.

1 In most cases, no long term stability data has been generated by Cytiva in 0.01 M NaOH. In some cases, accelerated studies at elevated temperature indicate that storage in 0.01 M NaOH can be a viable option but no guarantees can be made regarding retained function of the product.

6 Ordering information

Product	Pack size	Product code
DEAE Sephadex A-25	100 g	17017001
DEAE Sephadex A-25	500 g	17017002
DEAE Sephadex A-25	5 kg	17017003
DEAE Sephadex A-50	100 g	17018001
DEAE Sephadex A-50	500 g	17018002
DEAE Sephadex A-50	5 kg	17018003
DEAE Sephadex A-50	40 kg[1]	17018007
QAE Sephadex A-25	500 g	17019002
QAE Sephadex A-25	5 kg	17019003
QAE Sephadex A-50	100 g	17020001
QAE Sephadex A-50	5 kg	17020003
CM Sephadex C-25	100 g	17021001
CM Sephadex C-25	500 g	17021002
CM Sephadex C-25	5 kg	17021003
CM Sephadex C-50	100 g	17022001
CM Sephadex C-50	500 g	17022002
CM Sephadex C-50	5 kg	17022003
SP Sephadex C-25	100 g	17023001
SP Sephadex C-25	500 g	17023002
SP Sephadex C-25	5 kg	17023003
SP Sephadex C-50	100 g	17024001
SP Sephadex C-50	5 kg	17024003

[1] Pack sizes available upon request

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

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