



Product Information

SEPHAROSE-BASED ION EXCHANGE MEDIA

Exact replacement for Product Code 85636

The information below is abstracted, for customer convenience, from a supplier technical manual and several supplier information sheets. Complete copies of supplier data are available upon request from Sigma Research Technical Service.

Sepharose-, Sepharose CL-, Sepharose Fast Flow and Sepharose High Performance-based ion exchange media consist of macroporous, beaded, cross-linked agarose to which charged groups are attached. The type of charged group determines the type and strength of the exchanger, while the total number and availability of the charged groups determine the capacity.

FUNCTIONAL GROUPS USED ON SEPHAROSE-BASED ION EXCHANGERS		
ANION EXCHANGER	FUNCTIONAL GROUP AND COUNTER ION	
DEAE (Diethylaminoethyl) (weak)	-OCH ₂ CH ₂ N ⁺ H(CH ₂ CH ₃) ₂	Cl ⁻
QAE (Quaternary aminoethyl) (strong)	-OCH ₂ CH ₂ N ⁺ (C ₂ H ₅) ₂ CH ₂ CH(OH)CH ₃	Cl ⁻
Q (Quaternary ammonium) (strong)	-CH ₂ N ⁺ (CH ₃) ₃	SO ₄ ²⁻
CATION EXCHANGER	FUNCTIONAL GROUP AND COUNTER ION	
CM (Carboxymethyl) (weak)	-OCH ₂ COO ⁻	Na ⁺
SP (Sulfopropyl) (strong)	-CH ₂ CH ₂ CH ₂ SO ₃ ⁻	Na ⁺
S (Methyl sulfonate) (strong)	-CH ₂ SO ₃ ⁻	Na ⁺

Sulfonic and quaternary amines form strong ion exchangers (completely ionized over a broad pH range); all others form weak ion exchangers (the degree of dissociation, and thus the exchange capacity, varies markedly with pH). "Strong" and "weak" refer to the extent of ionization with pH, and not to the strength of binding.

PRODUCT DESCRIPTION AND CHARACTERISTICS:

Sepharose: Sepharose consists of beaded agarose. Chemically unmodified gels are used for gel filtration chromatography.

Sepharose CL-6B: Sepharose CL is a cross-linked derivative of Sepharose, prepared by reacting Sepharose with 2,3-dibromopropanol under strongly alkaline conditions. After cross-linking, the gel is desulfated by alkaline hydrolysis under reducing conditions. Sepharose CL-6B stands for cross-linked 6% beaded agarose, and has an average bead diameter of ~90 μm and a particle size range of 45-145 μm. Ion exchangers based on Sepharose CL-6B can be autoclaved at 100-120°C and pH 7.

Sepharose Fast Flow: Sepharose Fast Flow is a cross-linked derivative of Sepharose. The cross-linking method is different from the one employed to make Sepharose CL, and is proprietary to the manufacturer. With a particle size range of 45-165 μm and an average bead diameter of ~90 μm, Sepharose Fast Flow gives better flow rates compared to Sepharose CL.

Sepharose High Performance: Sepharose High Performance is a crosslinked derivative of Sepharose. The cross-linking method is different from the one employed to make Sepharose CL, and is proprietary to the manufacturer. With a particle size range of 24-44 μm and an average bead diameter of $\sim 34 \mu\text{m}$, Sepharose High Performance yields better resolution compared to Sepharose CL.

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SEPHAROSE-BASED ION EXCHANGERS OFFERED BY SIGMA	
SIGMA CATALOG NUMBER	NAME - DESCRIPTION
CCL-6B-100	CM SEPHAROSE Suspension in 20% ethanol with 0.6 M NaCl Matrix: Sepharose CL-6B Approx. Exclusion Limit: Average MW 4×10^6 Exchange Capacity: 100-140 $\mu\text{eq/mL}$ gel Binding Capacity: 63 mg hemoglobin per mL gel
CCF-100	CM SEPHAROSE Suspension in 20% ethanol Matrix: Sepharose Fast Flow Approx. Exclusion Limit: Average MW 4×10^6 Exchange Capacity: 90-130 $\mu\text{eq/mL}$ gel Binding Capacity: 50 mg RNase per mL gel pH Stability: 4-13
SEPHAROSE-BASED ION EXCHANGERS OFFERED BY SIGMA	
SIGMA CATALOG NUMBER	NAME - DESCRIPTION
DCL-6B-100	DEAE SEPHAROSE Suspension in 20% ethanol with 0.5 M NaCl Matrix: Sepharose CL-6B Approx. Exclusion Limit: Average MW 4×10^6 Exchange Capacity: 130-170 $\mu\text{eq/mL}$ gel Binding Capacity: 170 mg HSA per mL gel pH Stability: 3-12
DFF-100	DEAE SEPHAROSE Suspension in 20% ethanol Matrix: Sepharose Fast Flow Approx. Exclusion Limit: Average MW 4×10^6 Exchange Capacity: 110-160 $\mu\text{eq/mL}$ gel Binding Capacity: 110 mg HSA per mL gel pH Stability: 2-13

Q-1126	Q SEPHAROSE Suspension in 20% ethanol Matrix: Sepharose Fast Flow Approx. Exclusion Limit: Average MW 4×10^6 Exchange Capacity: 180-250 $\mu\text{eq/mL}$ gel Binding Capacity: 120 mg HSA per mL gel pH Stability: 2-12
Q-1754	Q SEPHAROSE Suspension in 20% ethanol Matrix: Sepharose High Performance Approx. Exclusion Limit: Average MW 4×10^6 Exchange Capacity: 150-200 $\mu\text{eq/mL}$ gel Binding Capacity: 120 mg HSA per mL gel pH Stability: 2-12
S-1799	SP SEPHAROSE Suspension in 20% ethanol Matrix: Sepharose Fast Flow Approx. Exclusion Limit: Average MW 4×10^6 Exchange Capacity: 180-250 $\mu\text{eq/mL}$ Binding Capacity: 120 mg BSA per mL gel pH Stability: 4-13

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OPERATION, REGENERATION AND STORAGE:

1. **CHOICE OF CATIONIC VS. ANIONIC EXCHANGER:** If the sample components are most stable below their pI values, a cation exchanger should be used; if they are most stable above their pI values, an anion exchanger should be used; if stability is good over a wide pH range on both sides of the pI, either or both types of ion exchanger may be used.
2. **CHOICE OF STRONG VS. WEAK ION EXCHANGER:** Most proteins have pI values within the range 5.5-7.5, and can thus be separated on both strong and weak ion exchangers. In cases where maximum resolution occurs at an extreme pH and the molecules of interest are stable at that pH, a strong ion exchanger should be used.
3. **CHOICE OF BUFFER, pH AND IONIC STRENGTH:** The highest ionic strength which permits binding should normally be used. The required buffer concentration varies from substance to substance, but usually an ionic strength of at least 10 mM is required to ensure adequate buffering capacity. Since salts (including buffers) help stabilize proteins in solution, their concentration should be high enough to prevent denaturation and precipitation.

ION EXCHANGER	RECOMMENDED BUFFER IONS	pH
ANION EXCHANGER DEAE Sepharose Cl-4B DEAE Sepharose Fast Flow Q Sepharose Fast Flow Q Sepharose High Performance	CATIONIC (e.g., alkylamines, alcohol, ammonium, ethylene-diamine, imidazole, Tris, pyridine, etc...)	Operate within 0.5 pH unit of the buffer's pK^a and, to facilitate binding, at least 1 pH unit above the pI of the protein.
CATION EXCHANGER CM Sepharose CL-6B CM Sepharose Fast Flow SP Sepharose Fast Flow S Sepharose Fast Flow	ANIONIC (e.g., acetate, barbiturate, citrate, glycine, phosphate, etc...)	Operate within 0.5 pH unit of the buffer's pK^a and, to facilitate binding, at least 1 pH unit above the pI of the protein.

4. Allow the ion exchanger and about 10 column volumes (CV) of buffer to equilibrate to the temperature selected for the chromatographic run.
5. Mix the pre-swollen suspension with starting buffer to form a moderately thick slurry which consists of about 75% settled gel and 25% liquid.
6. Degas the gel under vacuum at the temperature of column operation.
7. Mount the column vertically on a suitable stand out of the way of direct sunlight or draughts which may cause temperature fluctuations.

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OPERATION, REGENERATION AND STORAGE: (continued)

8. Pour a small amount of buffer into the empty column and allow it to flow through spaces to eliminate air pockets.
9. Pour the ion exchanger suspension prepared in step 6 into the column by allowing it to flow gently down the side of the tube in order to avoid bubble formation.
10. For consistent flow rates and reproducible separations, connect a pump to the column.
11. Fill the remainder of the column to the top with buffer and allow about 5 CV of buffer to drain through the bed at a flow rate at least 133% of the flow rate to be used in the procedure. The bed height should have settled to a constant height.

2. Using a syringe or similar instrument, apply the sample dissolved in starting buffer to the column. For isocratic separations, the sample volume should range between 1% and 5% of the column volume. If the ion exchanger is to be developed with a gradient, the sample mass applied is of much greater importance than the sample volume, and the sample should be applied in a low ionic strength medium. Ion exchange is used to concentrate the sample as well as fractionate it.
13. ELUTION: If only unwanted substances in the sample are adsorbed, or if sample components are differentially retarded under isocratic conditions, the starting buffer can also be used as the eluent. Normally, however, separation and elution is achieved by selectively decreasing the affinity of the molecules for the charged groups on the resin by changing the eluent's pH, its ionic strength, or both; this procedure is termed gradient elution.
14. REGENERATION: Washing the column with a high ionic strength salt solution (e.g., 1 M NaCl), or changing the pH to the low and high extremes tolerable, is usually sufficient to remove all reversibly bound material. When necessary, lipids and precipitated proteins can be removed by washing with 1 CV of 1-2 M NaCl, followed by 1 CV of 0.1 M NaOH in 0.5 M NaCl. Rinse with several CV of water, then re-equilibrate the resin with starting buffer. If base was used, adjust the pH of the resin to neutral before storing or using.
15. STORAGE: Store Sepharose-based ion exchangers at 2-8°C in water or a dilute buffer of choice with 20% ethanol added as an antibacterial agent.

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