上海伊卡生物技术有限公司

Sepharose® Ion Exchange Media

Catalog Numbers
DCL6B100 – DEAE–Sepharose CL6B
DFF100 – DEAE–Sepharose Fast Flow
Q1126 – Q Sepharose Fast Flow
Q1754 – Q Sepharose High Performance
CCF100 – CM Sepharose Fast Flow
S1799 – SP Sepharose Fast Flow
Storage Temperature 2–8 °C

Synonyms: Sepharose® CL, Sepharose Fast Flow, and Sepharose High Performance ion exchange media

Product Description
Sepharose CL, Sepharose Fast Flow, and Sepharose High Performance 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.

Ion Exchange Functional Groups

Anion Exchanger		**Functional Group and Counter Ion**	
DEAE (Diethylaminoethyl)	weak	$-OCH_2CH_2N^+H(CH_2CH_3)_2$	Cl^-
Q (Quaternary ammonium)	strong	$-CH_2N^+(CH_3)_3$	SO_4^{2-}
Cation Exchanger		**Functional Group and Counter Ion**	
CM (Carboxymethyl)	weak	$-OCH_2COO^-$	Na^+
SP (Sulfopropyl)	strong	$-CH_2CH_2CH_2SO_3^-$	Na^+

Sulfonic and quaternary amines form strong ion exchangers, which are completely ionized over a broad pH range. All others form weak ion exchangers, where 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.

Matrices
Sepharose CL6B: Sepharose CL is a crosslinked derivative of Sepharose, prepared by reacting Sepharose with 2,3-dibromopropanol under strongly alkaline conditions. After crosslinking, the gel is desulfated by alkaline hydrolysis under reducing conditions. Sepharose CL6B stands for cross-linked 6% beaded agarose, and has an average bead diameter of ~90 μm and a particle size range of 45–165 μm. Ion exchangers based on Sepharose CL6B can be autoclaved at 100–120 °C and pH 7.

Sepharose Fast Flow: Sepharose Fast Flow is a crosslinked derivative of Sepharose. The crosslinking 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 crosslinking 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 ~34 μm, Sepharose High Performance yields better resolution compared to Sepharose CL.

Sepharose Anion Exchangers

Catalog Number	Type and Properties
DCL6B100	**DEAE–Sepharose** Suspension in 20% ethanol with 0.5 M NaCl **Matrix:** Sepharose CL6B **Approx. Exclusion Limit:** Average MW 4×10^6 **Exchange Capacity:** 130–170 μeq/mL gel **Binding Capacity:** 170 mg HSA per mL gel **pH Stability:** 3–12
DFF100	**DEAE–Sepharose** Suspension in 20% ethanol **Matrix:** Sepharose Fast Flow **Approx. Exclusion Limit:** Average MW 4×10^6 **Exchange Capacity:** 110–160 μeq/mL gel **Binding Capacity:** 110 mg HSA per mL gel **pH Stability:** 2–13
Q1126	**Q SEPHAROSE** Suspension in 20% ethanol **Matrix:** Sepharose Fast Flow **Approx. Exclusion Limit:** Average MW 4×10^6 **Exchange Capacity:** 180–250 μeq/mL gel **Binding Capacity:** 120 mg HSA per mL gel **pH Stability:** 2–12
Q1754	**Q SEPHAROSE** Suspension in 20% ethanol **Matrix:** Sepharose High Performance **Approx. Exclusion Limit:** Average MW 4×10^6 **Exchange Capacity:** 150–200 μeq/mL gel **Binding Capacity:** 120 mg HAS per mL gel **pH Stability:** 2–12

Sepharose Cation Exchangers

Catalog Number	Type and Properties
CCF100	**CM SEPHAROSE** Suspension in 20% ethanol **Matrix:** Sepharose Fast Flow **Approx. Exclusion Limit:** Average MW 4×10^6 **Exchange Capacity:** 90–130 μeq/mL gel **Binding Capacity:** 50 mg RNase per mL gel **pH Stability:** 4–13
S1799	**SP SEPHAROSE** Suspension in 20% ethanol **Matrix:** Sepharose Fast Flow **Approx. Exclusion Limit:** Average MW 4×10^6 **Exchange Capacity:** 180–250 μeq/mL **Binding Capacity:** 120 mg BSA per mL gel **pH Stability:** 4–13

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store Sepharose ion exchanger media at 2–8 °C in water or a dilute buffer of choice with 20% ethanol added as an antibacterial agent.

Procedure

Cationic versus 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.

Strong versus 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.

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	**Cationic**	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.
DEAE Sepharose CL6B	(e.g., alkylamines, alcohol, ammonium, ethylenediamine, imidazole, Tris, pyridine, etc...)	
DEAE Sepharose Fast Flow		
Q Sepharose Fast Flow		
Q Sepharose High Performance		
Cation Exchanger	**Anionic**	Operate within 0.5 pH unit of the buffer's pK_a and, to facilitate binding, at least 1 pH unit below the pI of the protein.
CM Sepharose Fast Flow	(e.g., acetate, barbiturate, citrate, glycine, phosphate, etc...)	
SP Sepharose Fast Flow		

1. Allow the ion exchange medium and ~10 column volumes (CV) of buffer to equilibrate to the temperature selected for the chromatographic run.

2. Mix the pre-swollen suspension with starting buffer to form a moderately thick slurry, which consists of ~75% settled gel and 25% liquid.

3. Degas the gel under vacuum at the temperature of column operation.

4. Mount the column vertically on a suitable stand, out of the way of direct sunlight or drafts, which may cause temperature fluctuations.

5. Pour a small amount of buffer into the empty column and allow it to flow through spaces to eliminate air pockets.

6. Pour the suspension of ion exchange medium prepared in step 3 into the column by allowing it to flow gently down the side of the tube in order to avoid bubble formation.

7. For consistent flow rates and reproducible separations, connect a pump to the column.

8. Fill the remainder of the column to the top with buffer. Allow ~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.

9. 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 1–5% of the column volume. If the chromatographic run involves elution 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.

10. 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 are achieved by selectively decreasing the affinity of the molecules for the charged groups on the resin by changing the pH and/or ionic strength of the eluent, this procedure is termed gradient elution.

11. 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.

References

The information was 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.

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