Product Information

SP Sepharose®

S1799

Product Description

SP Sepharose® is an ion exchange chromatography resin with a sulfopropyl (SP) functional group (-CH_2-CH_2-CH_2-SO_3^-) attached to Sepharose® Fast Flow. The SP group serves as a strong cation exchanger, which is completely ionized over a broad pH range. The terms "strong" and "weak" in ion exchange chromatography refer to the extent of ionization with pH, and **not** to the binding strength of the functional group to the target species.

The parent Sepharose® Fast Flow is a cross-linked derivative of Sepharose®. The particle size range is 45-165 µm. The average bead diameter is ~90 µm. The counterion in the product is sodium (Na^+).

Recommended anionic buffers to use with SP Sepharose® include acetate, barbiturate, citrate, glycine, or phosphate. In terms of pH, it is suggested to operate within 0.5 pH unit of the buffer's pK_a. With respect to proteins, it is suggested to operate at least 1 pH unit below the pI of the protein, to facilitate binding. Oxidizing agents, and cationic detergents and buffers, should **not** be used with SP Sepharose®. Likewise, extended exposure of SP Sepharose® to conditions of pH < 4 should be avoided.

Several publications[1-4] and dissertations[5,6] cite use of product S1799 in their research.

Reagent

SP Sepharose® is offered as a suspension in 20% ethanol.

Approximate Exclusion Limit: average molecular mass of ~4 × 10^6 Daltons

Ionic Capacity: 0.18-0.25 mmol H^+/mL gel

Binding Capacity: 70 mg RNase A per mL gel

pH Stability: 4-13

Working temperature: 4-40 °C

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

General Resin Preparation Procedure

1. Allow the ion exchange medium and ~10 column volumes (CV) of buffer to equilibrate to the temperature chosen 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. Allow the buffer 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, 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 from 1-5% of the column volume. If the chromatographic run involves elution with a gradient, the applied sample mass 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 both to concentrate and to fractionate the sample.
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:
    - Either (a) washing the column with a high ionic strength salt solution, such as 1 M NaCl, or (b) changing the pH to the tolerable low and high pH extremes, is usually sufficient to remove 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 such as NaOH was used, adjust the pH of the resin to neutral before storing or using.
12. Storage: SP Sepharose® may be stored at 2-8 °C in 0.2 M sodium acetate, with 20% ethanol added as an antibacterial agent.

General Notes

Cation versus Anion Exchanger

- If sample components are most stable below their pI values, a cation exchanger should be used.
- If sample components 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. Usually, an ionic strength of at least 10 mM is required to ensure adequate buffering capacity.
- Since salts (including buffers) help to stabilize proteins in solution, their concentration should be high enough to prevent denaturation and precipitation.

References

1. Qi, J.H. *et al.*, *PLoS One*, **8(3)**, e55667 (2013).
2. Leidecker, O. *et al.*, *Nat. Chem. Biol.*, **12(12)**, 998-1000 (2016).
3. Fulcher, Y.G. *et al.*, *Structure*, **25(7)**, 1100-1110.e5 (2017).
4. Rudnizky, S. *et al.*, *Mol. Cell*, **81(16)**, 3410-3424.e4 (2021).
5. Redmond, Miranda, "The Role of N-Terminal Acidic Inserts on the Dynamics of the Tau Protein". University of Vermont, Ph.D. dissertation, p. 22 (May 2017).
6. Sessa, Gaetana, "Role of the interaction of BRCA2 and DDX5 in the DNA damage response". Université Paris-Saclay, Ph.D. dissertation, p. 151 (2020).

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