Product Information

Q Sepharose® High Performance

Q1754

Product Description

Q Sepharose® High Performance is an ion exchange chromatography resin with a quaternary amine (Q) functional group [-CH_2-$N^+(CH_3)_3$] attached to Sepharose® High Performance. The Q group serves as a strong anion 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® High Performance matrix is a crosslinked Sepharose® derivative that is distinct from the separate matrix Sepharose® CL. The particle size range is 24-44 µm, with a mean bead size of 34 µm. The counterion in the product is sulfate (SO_4^{-2}).

Recommended cation buffers to use with Q Sepharose® High Performance include alkylamines, ammonium, ethylenediamine, imidazole, pyridine, or Tris. In terms of pH, it is suggested to operate within 0.5 pH unit of the buffer's pK_a. With proteins, it is suggested to operate at least 1 pH unit above the pI of the protein, to facilitate binding. Oxidizing agents and anionic detergents should **not** be used with Q Sepharose® High Performance. Several references cite use of product Q1754 in their research.[1,2]

Reagent

Q Sepharose® High Performance is offered as a suspension in 20% ethanol.

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

Ionic Capacity: 0.14-0.20 mmol Cl^-/mL gel

Binding Capacity: ~70 mg bovine serum albumin (BSA) or human serum albumin (HSA) per mL gel

pH Stability: 2-12

Working temperature: 4-30 °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 needed, 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: Q Sepharose® may be stored at 2-8 °C in water 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.
- When 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.
- As salts (such as buffers) help to stabilize proteins in solution, their concentration should be high enough to prevent denaturation and precipitation.

References

1. Banerjee, R. *et al*., *Methods Enzymol.*, **596**, 239-290 (2017).
2. Banerjee, Rahul, "Kinetic and Spectroscopic Characterization of Intermediates in the Soluble Methane Monooxygenase Catalytic Cycle: The Old and The New". University of Minnesota, Ph.D. dissertation, p. 62 (February 2013).

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