MCLAB
Molecular Cloning Laboratories

User Manual

Version 2.0

Product name: SP Sepharose Big Beads

Cat #: SPS-100

Description:

MCLAB's SP Sepharose Big Beads are cross-linked agarose beads that are modified with sulphopropyl (SP) strong cation exchange groups. The product is designed to obtain resolution and capacity through intermediate purification and polishing applications. It is often used in ion exchange chromatography, protein chromatography and cation exchange media.

Protocol:

1. Allow the resin and 10 resin bed volume (BV) of buffer to equilibrate to room temperature for a chromatographic run.
2. Mix the resin suspension with the starting buffer at a ratio of 75% settled gel and 25% liquid.
3. Degas the resin/buffer mixture under vacuum at room temperature for a chromatographic run to prevent bubble formation in the column.
4. Mount the chromatographic column vertically and pour some buffer to get rid of air pockets.
5. Gently pour the prepared resin/buffer mixture to the chromatographic column to carefully avoid bubble formation.
6. Connect an operable pump to the column for reproducible separations.
7. Fill the column with buffer to the rim and allow 5 BV to flow through the column.
8. Once the column is prepared with MCLAB's SP Sepharose Big Beads, apply or inject the specific sample dissolved in the starting buffer to the column. Sample volume should range from 1-5% of BV for isocratic separations. Low ionic strength medium and sample mass applied is more important than sample volume when a gradient elution is used. Ion exchange chromatography can help concentrate or fractionate samples.
9. Elution: The starting buffer can be used for elution if a clear separation of the target samples is achieved. More often, gradient elution is applied for successful separation and elution. This is done by changing the pH and/or ionic strength of Elution Buffer to selectively decrease the affinity of molecules for the charged groups on the resin.
10. Regeneration: Wash the resin with high ionic strength salt solution or with low and high pH to remove reversibly bound materials. Lipids and precipitated proteins can be removed with sequential washings of 1 BV of 1 M NaCl and then 1 BV of 0.1 M NaOH in 0.5 M NaCl. Equilibrate the resin containing column with the starting buffer again and be sure to adjust the pH of the resin to neutral.

Recommended Anionic Buffers:

Acetate
Barbiturate
Citrate
Glycine
Phosphate

*pH of buffer ions should operate within 0.5 pH unit of buffer's pK_a value