PRINCETON SEPARATIONS

Technical Tip

T-1

Maximizing sample recovery when using Centri-Spin[™] columns for desalting Proteins

Introduction:

Reduced recovery of protein samples may occur when using Centri-Spin spin columns due to specific or non-specific interaction with the column matrix. Below is a list of common modes of interaction and suggestions for minimizing their effect on sample recovery.

Interaction Possible Solution(s) Hydrophobic Interaction Hydrophobic proteins in high molarity lyotrophic Decrease ionic strength of hydration buffer. salts solutions may adsorb to the Centri-Spin Increase pH of hydration buffer. matrix. Subsequent elution of the protein with Add organic solvent to hydration buffer water is indicative of a hydrophobic interaction. (ie. 5% isopropanol or ethanol) Add 10 % ethylene glycol to hydration buffer Add suitable non-ionic detergent to 0.1 % v/v (ie. Tween, Triton, Nonidet P40, Brij, Span, Lubrol) **D**nic Interactions The Centri-Spin matrix has a slight net Increase ionic strength of hydration buffer negative charge at neutral pH. Positively (>50mM) charged molecules may be slightly retained. Sample Precipitates on Column Precipitation may be caused by salt removal, Increase ionic strength of hydration buffer sample dilution, or a change in pH. **Protease Degradation** Add suitable inhibitor Lectin Binding Carbohydrate binding proteins may adsorb to Add suitable competing binding sugar to the the Centri-Spin matrix. hydration buffer

Using the above guide, modify the Centri-Spin hydration buffer to reduce the suspected interaction. In addition, check that the molecular weight of the sample exceeds the exclusion limit of the Centri-Spin column used.



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