Rapid Desalting of Protein Solutions Using the ProteoSpin™ CBED Kit

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INTRODUCTION

The ProteoSpin™ CBED (Concentration, Buffer Exchange and Desalting) Kit provides a fast and easy method for desalting protein solutions. The kit is based on spincolumn chromatography using Norgen's proprietary protein resin as an ion exchanger. Protein desalting is an important sample preparation step for many downstream proteomic applications including western blotting, SDSPAGE gel electrophoresis, NMR spectroscopy, X-ray crystallography, and mass spectrometry.

In this application note, we evaluate the ability of the ProteoSpin™ CBED Micro Kit (P/N 10100) to effectively and rapidly desalt a BSA solution that is spiked with goldtagged rabbit IgG.

METHODS AND MATERIALS:

Samples containing 4 µ g of gold-tagged rabbit IgG (Nanoprobes NANOGOLD®-anti human, Catalog #2052) and 50 µg of carrier BSA were prepared in the presence or absence of 100 ppm LiCl (lithium chloride) or CsCl (cesium chloride), in 50 mM sodium acetate buffer, pH 4.5. The protein solutions were then applied to activated spin columns by centrifugation, and washed with the provided Column Activation and Wash Solution. The bound proteins were then eluted using the supplied elution buffer. Quantitative analysis was then carried out by ICP/MS (Inductively Coupled Plasma Mass Spectrometry).

The experimental measurements were made on a commercial DRCplus ICP/MS (PerkinElmer SCIEX), a quadrupole-based mass spectrometer designed for elemental analysis. The ICP/MS allows elemental measurements in the mass range of 5-250 amu. The operating plasma conditions are sufficient to disintegrate, atomize, and ionize the sample's constituent elements. Therefore, ICP/MS technology is a convenient way to directly quantify the atomic composition of a tag conjugated to a biologically active material.

RESULTS AND DISCUSSION:

The desalting capability of ProteoSpin™ columns was demonstrated with CsCl and LiCl, as spike materials at 100 ppm, in solutions of IgG-gold conjugates. Due to low environmental levels, Cs+ and Li+ provide a convenient way to track the flow of salt through a system. ICP/MS results show that Cs+ and Li+ ion concentrations were reduced to less than 0.5% of their original levels.

To determine the column efficiency to capture IgG-gold conjugates in solution, the eluted samples were subjected to further ICP/MS analysis for gold particles. The results show that the recovery of the conjugates was 94 ± 11%. Since the original solution contained 4 µg of conjugate and 50 µg of BSA, the total protein mass recovered is estimated at 50.8 µg. The results are summarized in Table 1.

Samples	Before	After
4 µg Au-IgG and 50 µg carrier BSA	Protein	
	54 µg	50.8 µg
4 µg Au-IgG and 50 µg carrier BSA and LiCl or CsCl	LiCl or CsCl	
	100 ppm	< 0.005 ppm

Table 1: Yields of Protein and LiCl or CsCl Salts Before and After ProteoSpin™ CBED Processing

RESULTS AND DISCUSSION:

The performance of the ProteoSpin™ CBED Kit is best measured by its efficiency to simultaneously remove salts and to effectively recover the protein. ICP/MS analysis provides a direct approach to measure salt and protein levels in all steps of the desalting process. The excellent recovery of protein at 94% efficiency, and the reduction of Cs and Li salt levels to lower than 0.5% of their original levels proves the exceptional capability of ProteoSpin™ columns. As a preparative spin column tool, the ProteoSpin™ CBED Kit significantly enhances the efficiency and effectiveness of proteome-level analysis.

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