

Rapid Desalting of Protein Solutions

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ProteoSpin CBED (Concentration, Buffer Exchange and Desalting) Micro Kit

Purpose

The purpose of this application note is to evaluate the ability of the ProteoSpin™ CBED Micro Kit to effectively and rapidly desalt protein solutions.

Overview

Building on an innovative and recently patented silicon carbide (SiC) technology, the ProteoSpin CBED Kit provides a fast and easy desalting solution. Protein desalting is an important preparation step for many biological samples. The purified samples can then be used for a variety of downstream proteomics applications including western blotting, SDS-PAGE gel electrophoresis, NMR spectroscopy, X-ray crystallography, and mass spectrometry.

SiC has many superior features compared to the commonly used matrices such as agarose, acrylamide, and even silica, which have limited usefulness. SiC is a very hard man-made material, which is capable of withstanding high-speed centrifugation.

The discovery that SiC can reversibly bind biomolecules with high capacity led to its adaption as a chromatographic matrix. SiC binds proteins due to its electrostatically charged surface, therefore the basic principle for binding biomolecules to SiC is similar to that of a cation exchanger. SiC is chemically very inert, and is suitable for applications that require harsh conditions, such as extreme pH values and high temperature.

Desalting Methods and Materials

Samples containing 4 µg of gold-tagged rabbit IgG (Nanoprobes) and 50 µg of carrier BSA were prepared in the presence or absence of 100 ppm LiCl (lithium chloride) or CsCl (cesium chloride), in 50mM sodium acetate buffer, pH 4.5. The solutions were loaded onto spin columns containing 25 mg of pre-activated SiC matrix, followed by centrifugation. The bound proteins were then eluted by using the elution buffer, and all flowthrough samples following the binding, washing, and elution steps were collected. Quantitative analysis was then carried out by ICP/MS (Inductively Coupled Plasma Mass Spectrometry).

The experimental measurements were made on a commercial DRC^{plus} 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 to 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 yield of 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.

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

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 (ppm)	
	100	< 0.005

Conclusion

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 prove 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.