

Rapid Concentration of Dilute Protein Solutions

E. Bibby, M.Sc., J. Ramwani, Ph.D., and D. Bautista, Ph.D.
Norgen Biotek Corporation, St. Catharines, Ontario, Canada

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

Purpose

This application note evaluates the ability of the ProteoSpin™ CBED Micro 25 Kit to effectively and rapidly concentrate proteins in eluted BSA solutions.

Overview

Building on an innovative and recently patented silicon carbide (SiC) technology, the ProteoSpin CBED kit provides a fast and easy solution to concentrate protein samples. Protein concentration is a critical 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 distinct features from 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 adaptation 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.

Concentration Methods and Materials

ProteoSpin columns containing 25 mg of SiC were used to concentrate 1 mL samples containing 5, 10, 15, 25, 40, and 50 µg BSA (Sigma). BSA samples were prepared in 50 mM sodium acetate buffer, pH 4.5. The protein samples were applied into six different activated spin columns, followed by centrifugation. Once bound, the proteins were washed twice then eluted using the ProteoSpin elution buffer. The protein elute samples were quantified using the Bio-Rad Protein Assay, and with BSA as the protein standard. The results are shown in Table 1.

Table 1: Elution (E) of BSA from Columns at Various Input Amounts

Input (µg)	E1 + E2 (µg)	% Recovered
5	2.7	54.9%
10	9.4	93.6%
15	12.9	86.2%
25	24.2	96.9%
40	36.5	91.4%
50	41.9	83.8%

Results and Discussion

Using the ProteoSpin CBED Kit (P/N 10400), a 10-fold concentration factor for BSA was achieved, with a final volume of 100 µL. In other experiments, larger volumes of protein sample (up to 5 mL) were loaded onto the columns and concentration factors from 9- to 50-fold were achieved. This method provides an exceptional yield of protein recovery from all the BSA samples (see Table 1). The data, displayed in Figure 1, reveals a good fit ($r^2 = 0.9902$) to a hyperbolic function with parameters $a = 85.69$, and $b = 63.86$. The plot shows a distinct linear range at lower protein amounts (up to 40 µg) and appears to saturate at higher amounts. The linear range was further analyzed by fitting the first six data points to a linear function and the regression equation $y = 0.9588x - 1.0566$ ($r^2 = 0.9942$) was obtained. The 95% confidence interval for the slope was 0.958 ± 0.096 . Since the slope of this line represents the fraction of the amount of input protein that was eluted, the percent recovery of BSA from the columns is $95.8 \pm 9.6\%$ at the indicated confidence interval.

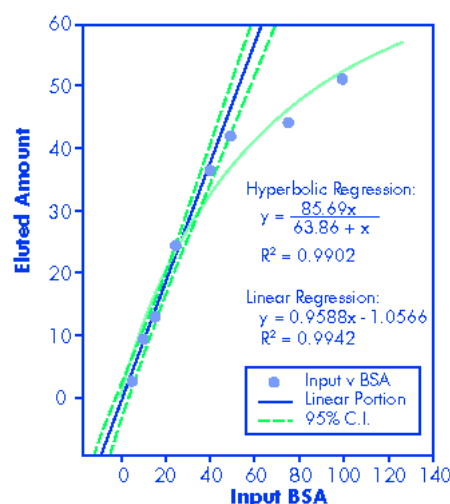


Figure 1: Relationship between Input Amount versus Eluted Amount for BSA

Conclusion

By using the ProteoSpin columns, a 10-fold concentration of BSA solutions is easily achieved. Further analysis showed that the ProteoSpin columns are able to achieve 96% efficiency in the linear range for the binding and elution of BSA. The entire procedure can be easily carried out in less than 20 minutes.

SiC proves to be a very robust material to enhance the efficiency and effectiveness of spin column technology, particularly for those applications that require high throughput protein processing.