Rapid Concentration of Dilute Protein Solutions Using the ProteoSpin™ CBED Kit

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APPLICATION NOTE #1

INTRODUCTION

The (P/N 10100) provides a fast and easy solution to concentrate dilute protein samples. The kit is based on spin-column chromatography using Norgen's proprietary protein resin as an ion exchanger. Protein concentration is a critical preparation step for many downstream proteomic applications including western blotting, SDS-PAGE gel electrophoresis, NMR spectroscopy, X-ray crystallography, and mass spectrometry.

Here we investigate the ability of the ProteoSpin™ CBED Micro Kit to concentrate 1 mL samples containing increasing amounts of the test protein BSA. The efficiency of protein recovery from the ProteoSpin™ columns is also investigated.

METHODS AND MATERIALS

ProteoSpin™ micro columns were used to concentrate 1 mL samples containing 5, 10, 15, 25, 40, and 50 µg of BSA (Sigma A-4503). The BSA samples were prepared in 50 mM sodium acetate buffer, pH 4.5. The protein samples were applied to six different activated spin columns by centrifugation. Once bound, the proteins were washed twice and then eluted using the ProteoSpin™ elution buffer. The eluted protein samples were then quantified using the Bio-Rad Protein Assay (#500-0006) with BSA as the protein standard.

RESULTS AND DISCUSSION

Using the ProteoSpin™ CBED Kit, a 10-fold concentration factor for BSA was achieved. In other experiments (results not shown), larger volumes of protein sample (up to 5 mL) were loaded onto the columns and concentration factors from 9- to 50-fold were achieved. The ProteoSpin™ CBED Kit also provided exceptional protein recovery from all the BSA samples (see Table 1).

Figure 1 shows a graphical representation of the relationship between the input amount and the eluted amount of the BSA. The data displayed in Figure 1reveals 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% ± 9.6% at the indicated confidence interval.

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

Input (µg)	Elution (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

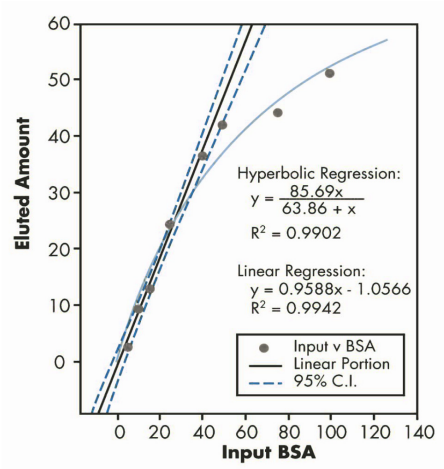

Figure 1: Relationship between input amount and eluted amount of BSA using ProteoSpin™ columns (amounts shown in µg)

CONCLUSION

By using the ProteoSpin™ columns, a 10-fold concentration of BSA solutions is easily achieved. BSA solutions containing from 5 to 50 µg of protein were concentrated into 100 µL from 1 mL. Further analysis showed that the ProteoSpin™ columns are able to achieve 96% efficiency in the linear range for the binding and elution of BSA.

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