Efficient Removal of Both Free and Tightly-Bound SDS from Protein Solutions using the ProteoSpin™ Detergent Clean-up Kit

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INTRODUCTION

Biological detergents are commonly used in research laboratories to disrupt the bipolar lipid membrane of cells. This process frees membrane-bound proteins and subsequently solubilizes them. Some detergents can also be used for stabilization, crystallization, or denaturation of proteins.

Detergents are surface-active agents (surfactants) that contain a hydrophobic portion, which is soluble in oil-like solutions, and a hydrophilic portion, which is soluble in water. This characteristic results in the formation of stable micelles with hydrophobic cores in an aqueous solution.

Depending on the charge of the hydrophilic portion, detergents are divided into four groups: anionic (negatively charged), cationic (positively charged), zwitterionic (containing both positive and negative charges, with a net zero charge) or non-ionic (containing no charge) detergents. Anionic and cationic detergents can be used for processes ranging from solubilizing membrane proteins under non-denaturing conditions, to the denaturing conditions of SDS-PAGE electrophoresis. Common ionic detergents include sodium deoxycholate and SDS. Zwitterionic detergents, such as CHAPS 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate, protect the native state of the protein while preventing protein aggregation during purification procedures. Non-ionic detergents are non-denaturing, making them suitable for solubilizing membrane proteins without altering biological activity. NP-40, Triton X-100, and Tween 20 are all examples of commonly used non-ionic detergents.

In principle, all proteins retain their structures and activities in the presence of non-ionic detergents, although their stability varies. In contrast, water-soluble proteins are denatured by SDS and form special complexes with this ionic detergent; i.e., a protein-decorated micelle structure, in which the polypeptide is wound around SDS micelles, interacting to a large extent with the micelle surface.

Detergents must be removed from protein solutions prior to many downstream applications. Detergents can interfere with purification procedures such as chromatography and electrophoresis, and also interfere with structural characterization techniques such as mass spectrometry and sequence analysis. Conventional methods for detergent removal include hydrophobic adsorption, gel chromatography, dialysis, ion-exchange chromatography, and precipitation techniques. However, these methods are time-consuming, tedious, and not applicable to small volumes of protein solutions.

This application note describes the use of the ProteoSpin™ Detergent Clean-up Micro Kit (P/N 10200) for the effective removal of the anionic detergent SDS from protein solutions. The ProteoSpin™ Detergent Clean-up Micro Kit provides a fast and simple procedure for the effective removal of SDS, Triton X-100, and other detergents from small protein samples. The kit employs spin-column chromatography using Norgen's proprietary protein resin as an ion exchanger.

METHODS AND MATERIALS

The performance of the ProteoSpin™ columns was examined under various conditions to identify the effects of biological detergents, specifically that of SDS, on column performance. BSA was used as a test protein for all experiments and comparisons. The procedure for acidic proteins as described in the Application Manual was followed. Solutions containing 50 µg of BSA and specified amounts of a detergent were prepared in 50 mM sodium acetate buffer pH 4.5. Each solution was processed through ProteoSpin™ spin columns containing proprietary protein resin, washed with the standard binding buffer, and the bound protein was then eluted. The Sigma Protein Assay determined the amount of eluted protein.

RESULTS AND DISCUSSION

The tests investigated column performance for the binding and eluting of proteins when various detergents (SDS, Triton X-100, CHAPS, Tween 20, and NP-40) were present in the protein sample. To determine the precise effect of the

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detergent on protein recovery using the ProteoSpin™ columns, detergents were added individually to solutions of BSA. The results show that SDS exhibited the greatest interference with column performance (see Figure 1). The percent recovery of BSA in the presence of SDS was 1.5% ± 1.1%, which is extremely low when compared to the recovery of BSA in the absence of detergent, which was found to be 96.9% ± 2.6%. The effects of other detergents, such as NP-40, Triton X-100, CHAPS, and Tween 20, were not as profound as that of SDS, however their presence in protein solutions caused significant reduction of column performance (t-test, P = 0.0040).

SDS, being an anionic detergent, was expected to affect protein binding not by blocking binding sites on the resin but by its ability to bind to proteins. The complex formed when SDS associates with proteins is expected to contain a net negative charge. This charge prevents binding of the protein to the resin, which has a negatively charged binding surface. Other detergents used were non-ionic (NP-40, Triton X-100, and Tween 20) and zwitterionic (CHAPS), and their relatively small effect on column performance may be attributed to their inability to interfere with resin binding sites or affect charges on the protein in the solution.

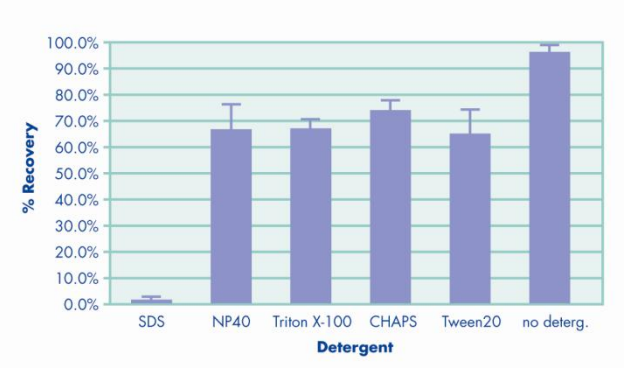

Figure 1: Effect of various detergents on BSA recovery

To test the ability of isopropanol to reverse the negative effects of SDS on column performance, BSA solutions were prepared for binding that contained different strengths of isopropanol (the Application Manual specifies to add an equivalent volume of isopropanol to protein solutions, which is indicated here as 100% strength). The results (see Figure 2) show that the amount of BSA recovered from solutions containing SDS increased with the concentration of isopropanol added to the protein in the binding solution. At 30% of the recommended isopropanol strength, only 17.9% (± 2.3%) of the input 50 µg BSA was recovered; at 50% strength, the recovery of the protein was 66.8% (±7.2%); and at 100% strength, the protein recovery was 97.3% (± 2.2%), indicating that full column performance was restored despite the presence of SDS in the protein solution.

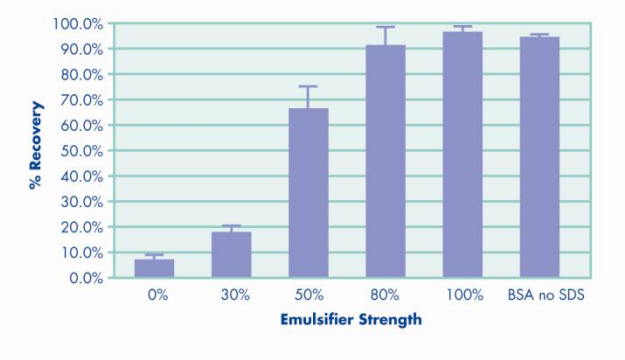

Figure 2: Restoration of column performance for purification of proteins with SDS.

In order to determine the capacity of the columns for removing SDS, increasing concentrations were tested for their ability to be neutralized using isopropanol. Again, 50 µg samples of BSA solutions were prepared in solutions that were spiked with SDS (0%–1.0%), and isopropanol was added at full strength into the binding mixture. The protein samples were bound to and then eluted from the columns. The percentage of recovery of BSA was determined. The results shown in Figure 3 indicate that the inhibitory effect of SDS from levels up to 1.0% can be removed with isopropanol.

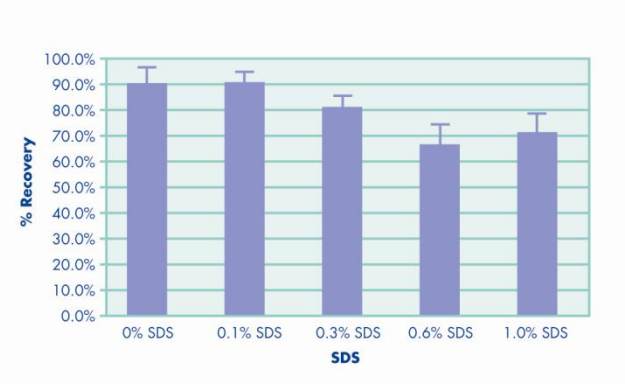

Figure 3: Protein recovery in the presence of high concentrations of SDS

The final test determined the ability of the Detergent Clean-up Micro Kit to remove free and tightly bound SDS from the protein solution. To evaluate this, a commercially available assay was used to measure the SDS content of BSA which was purified using the ProteoSpin™ kit. The input consisted of 50 µg BSA, 0.1% SDS, and the isopropanol solution at full strength. The eluted protein was then assayed for SDS using the Detergent-OUT kit by Geno Technologies, Inc. This test was performed in duplicate as shown in Table 1. The results in Table 1 clearly

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show that up to 99.98% of the total SDS added to the protein solution, a certain portion of which is bound to BSA, was removed. Since the assay can detect SDS that is both protein-bound and free in the solution, the protein recovered after the spin column purification is virtually free of all forms of SDS. Thus the Detergent Clean-up Micro Kit is able to remove both free and tightly bound SDS from protein solutions

Table 1: Removal of SDS from Proteins in Solution

Sample	A_{600}	% SDS Remaining	%SDS Removed	mg SDS Removed
Control (water)	0.0	0.0	0.0	0.0
0.1% SDS	0.142	-	-	-
ProteoSpin™ 1	0.024	0.017	99.98	2.457
ProteoSpin™ 2	0.03	0.02	99.98	2.45

CONCLUSION

The ProteoSpin™ Detergent Clean-up Micro Kit is a powerful tool for the removal of SDS from protein solutions. It was found that greater than 99% of SDS can be removed using this kit, including both the free and the tightly bound SDS. The inhibitory effects of SDS on the performance of the columns can be overcome by adding the isopropanol solution to the protein samples before applying to the column for binding the protein. The protein recovery is found to be greater than 95% when the isopropanol is used at full strength, coupled with the removal of virtually all SDS. The ProteoSpin™ Detergent Clean-up Micro Kit provides a method for obtaining SDS-free protein samples with high recovery.

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