APPLICATION NOTE

Recovery of proteins using low binding tubes and the Omni Bead Ruptor Elite bead mill homogenizer.

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Introduction

The scope of proteomics is consistently moving in a direction that requires low sample volumes and detection of minimal concentrations of protein. In order to keep up with the demand of the research industry, low-binding tubes are a necessity. Theoretically, low-binding tubes minimize the loss of protein/nucleic acid sample that binds to the sides of the tubes; this is a vital characteristic in downstream protein analysis methods, like spectrophotometry. Similarly, many intracellular proteins are located within cell membranes that are difficult to penetrate without homogenizing the cells prior to protein extraction and analysis. In this application note, standard polypropylene tubes were compared against low-binding tubes for their efficacy to bind Bovine Serum Albumin (BSA), a protein that binds readily to polypropylene.

Materials and methods

- Omni Bead Ruptor™ Elite bead mill homogenizer (Cat #19-042E)
- Omni Bead Ruptor Elite 2 mL Tube Carriage (Cat # 19-373)
- 2 mL low binding tubes (Cat #19-660-1000)
- Bulk 1.4 mm Ceramic Beads (Cat # 19-645)

Omni Bead Ruptor Elite bead mill homogenizer

For research use only. Not for use in diagnostic procedures.

Protein binding was determined by measuring the amount of protein recovered from each polypropylene tube and comparing the recovered concentration to the concentration of the spiked protein. Protein concentrations recovered from the tested tubes were quantified by bicinchoninic acid protein quantification assay (BCA) measuring BSA.

Protein binding assay

Triplicate samples were taken of standard polypropylene 2 mL tubes and low binding tubes. Each sample was spiked with 1 mL of a 100 µg/mL BSA solution. Samples were then incubated at 37 °C for 30 minutes. After incubation, 25 µL of each sample was loaded into triplicate wells on a 96 well, F-Bottom, plates (Greiner Bio-One, ref. No. 655097). Standards for the BCA assay were made according to the kit's instructions (Thermo Fisher Scientific, Cat. No. 23225), and 25 µL of each standard was also loaded into triplicate wells on the same 96 well plate. Once all samples were loaded, working reagent created according to kit instructions was loaded into all wells and the plate was incubated at 37 °C for 30 minutes. Following the incubation, the 96 well plate was placed into a Biotek ELx808 plate reader and absorbance was read at 562 nm.

Protein binding while bead milling

A second series of each tube type, standard and low binding, were each filled with 0.57 g of 1.4 mm ceramic bead media. These triplicate samples were spiked with the same 100 µg/mL BSA solution used above and were placed in an incubator set to 37 °C for 30 minutes. Following incubation all tubes were placed Omni Bead Ruptor Elite bead mill homogenizer, and the samples were processed at 6 m/s for 20 seconds. Post processing, samples were immediately analyzed spectrophotometrically for protein concentration using the protocol outlined above.

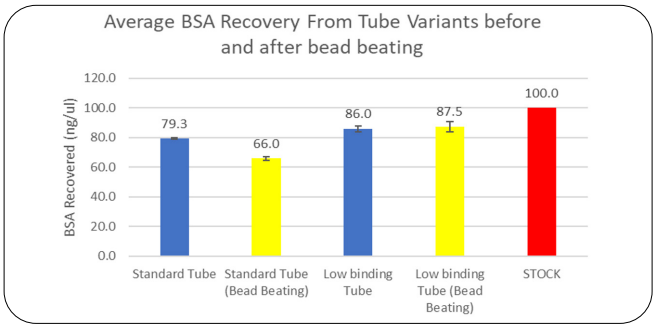

Figure 1: Average BSA recovered across triplicate samples for both the standard polypropylene tube and the low-binding tube. Bars in blue are the concentrations of BSA before bead beating and bars in yellow are concentrations after 20 seconds of bead beating. All values are in ng/µL.

Results

Standard polypropylene tubes bound various amounts of protein as seen in Figure 1. The standard 2 mL tubes bound 20.7 ng/µL of BSA on average, and the low-binding tubes bound 14 ng/µL of BSA on average. When evaluating the loss of BSA while bead beating, the low binding tubes did not lose any additional BSA during the 20 seconds of processing. In contrast, the standard 2 mL tube lost, on average, 13.3 ng/µL more BSA during the 20 seconds of bead beating.

Conclusion

The low binding tube retains less protein than standard polypropylene tubes. This tube continues to maintain its low-binding properties during bead milling at high speeds using ceramic bead media. Using the Omni Bead Ruptor Elite bead mill homogenizer, typical homogenization of soft tissue is completed in 20 seconds. During the homogenization process, intracellular proteins are released in various quantities and concentrations. Some of these proteins, such as BSA, bind quickly to plastics and can be difficult to fully extract from a lysate. These low-binding tubes can prove to be a great tool when trying to handle and extract proteins that are in small quantities because of the minimal amount of binding occurring before and after bead beating.

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