APPLICATION NOTE No. 382 I October 2016

Comparative Analysis of Protein Recovery Rates in Eppendorf LoBind® and Other "Low Binding" Tubes

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Abstract

Protein preparation and storage poses a critical step in a wide range of laboratory applications. Unspecific adsorption of protein molecules or peptides to polymer surface of lab consumables has been shown to be a substantial factor contributing to sample loss during storage/handling and to influence experimental results. Binding of protein samples was investigated here by using a sensitive fluorescence assay, and recovery rates were compared between tubes of different manufacturers referred to as "low binding". The majority of tubes of different manufacturers tested showed very poor recovery rates (4 % - 12 %) after 24 h storage time and do not protect sufficiently against unspecific loss of protein samples. Eppendorf LoBind Tubes provided highest recovery rates of proteins (95 %) and thus ensure utmost protection of protein samples.

Introduction

Protein preparation and storage pose critical steps in a wide range of laboratory applications including various methods in proteomics, molecular biology, forensics, and bio-pharma. Protein sample purity and yield in these methods have a strong effect on experimental results. They are a function of biological material quality and availability, of preparation and handling methods, but also of conditions and consumables used during preparation and storage [1].
Unspecific adsorption of protein molecules and peptides to polymer surface has been shown to be a substantial factor contributing to sample loss during storage and handling in lab consumables [2, 3, 4]. This process is largely conveyed via unspecific binding of hydrophobic domains in peptides and proteins to hydrophobic polymer surface, leading both to structural denaturation and decrease in concentration over relatively short time: up to 90 % of protein sample may be adsorbed within 24 h. [5] (fig. 1A). Unspecific sample and activity loss may be a critical factor influencing experimental results particularly when sensitive methods/assays or small sample amounts are used in proteomic, forensic, and other protocols.

Several manufacturers of lab consumables approached this problem by various material modifications. The most common method is coating (siliconization), which is designed to create a barrier between protein and polymer surface and diminish binding. Depending on experimental conditions, the coating may however be not sufficiently stable and migrate into the sample (leaching), which can have adverse effects on sample purity and experimental results [6, 7]. Another approach uses low retention modification of surface, which decreases retaining of sample during liquid handling steps but per se doesn't lead to decreased adsorption at molecular level.

Third approach is direct material optimization, where its hydrophobicity is reduced (Eppendorf LoBind), leading to diminished adsorption of protein molecules to the surface of consumables (fig. 1B).
These three approaches rely on very different molecular mechanisms and allow various degrees of adsorption reduction of protein samples. In this Application Note we investigated unspecific binding of protein samples by using a sensitive fluorescence assay. Recovery rates were compared and indicate large differences between various manufacturers of low binding tubes.

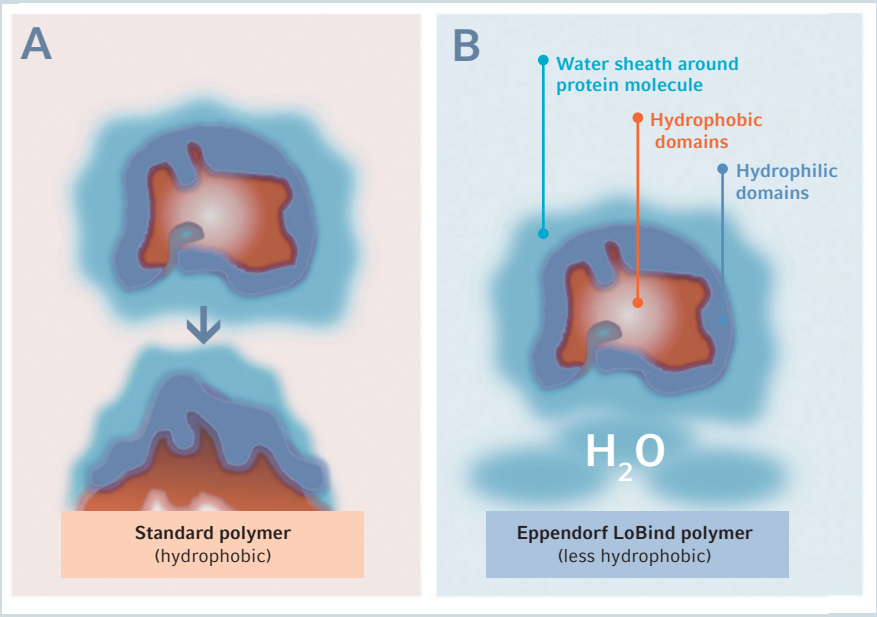

Figure 1:
A) Protein molecules bind via hydrophobic domains to standard polymer surface. This results in structural denaturation and activity/concentration loss.
B) Water molecules migrate between polymer and protein molecules and prevent binding to the surface and denaturation.

Materials and Methods

Protein recovery rates were evaluated by using fluorescently labeled protein assay: 234 µL of an FITC conjugated BSA solution (1 µg/mL in 1 x Dulbecco's PBS) were transferred in triplicates into low binding tubes of each manufacturer and incubated for 24 h at room temperature in the dark. After incubation, 190 µL of the solution stored in the tubes was used for fluorescence measurements using the Fluoroskan Ascent™ Microplate Fluorometer (Thermo Fisher Scientific®).

The recovery rates of FITC-BSA were calculated using a calibration curve performed with the standard solution. The labeled BSA solution used for the calibration curve was measured before the transferring to the tubes. Two independent experiments in triplicates were performed (n=6). Standard polypropylene tubes were used as negative control.

Results and Discussion

The recovery rates of the FITC conjugated BSA samples after incubation in the different tubes are presented in figure 2. The majority of the investigated tubes referred to as "low binding" (F, Sa, A, So, W, B) showed very poor protein recovery rates ranging between 4 % - 12 %, and were comparable to standard polypropylene material (Ctl. – recovery rate 5%). This indicates that under applied experimental conditions the majority of the tubes specified as "low binding" effectively offer no advantage over standard material. Low binding tubes from two manufacturers (C and Su) provided sub-optimal recovery rates of 48 % and 73 % respectively.

Highest protein recovery (95 %) was obtained with the Eppendorf Protein LoBind Tubes after 24-hours incubation. The Eppendorf LoBind Tube showed in two independent experiments very similar results providing consistent and reproducible data.

These substantial differences of recovery rates observed might be due to very different technologies applied by manufacturers to achieve low binding properties of their consumables. Our results indicate that direct material optimization with reduced hydrophobicity offers, under experimental conditions we tested, the most effective approach to minimize unspecific protein adsorption and therefore sample loss. Additionally, since this technology uses no surface modification (i.e. silicon coating), it offers higher experimental safety and data reliability.

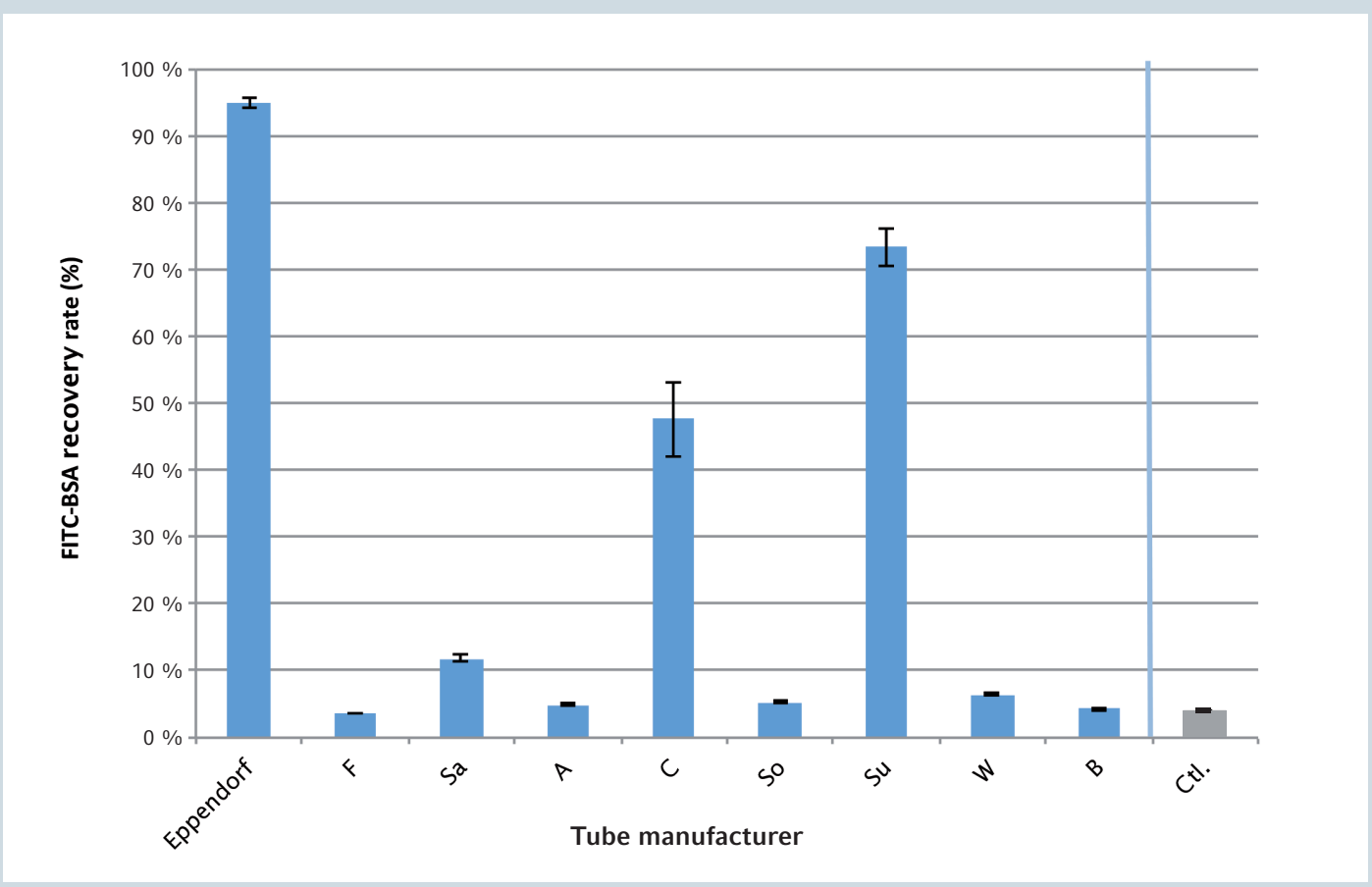

Figure 2: FITC-BSA recovery rate after 24-hours incubation in 1.5 mL low binding tubes from different manufacturers. Negative control (Ctl.): standard polypropylene tubes. Two independent experiments in triplicates are shown (n=6).

Conclusion

In this study, we have demonstrated that the sample recovery rates of various tubes referred to as "low binding" vary greatly. Out of 9 manufacturers tested, 6 showed very poor recovery rates (4 % - 12 %) and do not protect sufficiently against unspecific loss of protein samples.

Eppendorf LoBind Tubes show the highest recovery rates of protein samples (95 %) and therefore ensure safe protection of protein samples. This leads to more stable protein concentrations and in turn more reproducible and reliable experimental results.

Literature

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[2] Kristensen K, Henriksen JR, Andresen TL. Adsorption of cationic peptides to solid surfaces of glass and plastic. PLoS One 2015; 10(5).
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Ordering information

Description		Order no. international	Order no. North America
Eppendorf Protein LoBind Tubes, 1.5 mL, PCR clean, colorless		0030 108.116	022431081
Eppendorf Protein LoBind Tubes, 0.5 mL, PCR clean, colorless		0030 108.094	022431064
Eppendorf Protein LoBind Tubes, 2.0 mL, PCR clean, colorless		0030 108.132	022431102
Eppendorf Safe-Lock Tubes, 1.5 mL, Eppendorf Quality™, colorless*		0030 120.086	022363204
Eppendorf Protein LoBind Tubes 5.0 mL, PCR clean, colorless, 100 tubes (2 bags of 50 ea.)		0030 108.094	022431064
	OptiTrack® frame color		
Microplate 384/V-PP, Protein LoBind			
PCR clean, 80 plates (5 × 16 plates)	☐ white	0030 624.300	951040589
PCR clean, 240 plates (10 × 24 plates)	☐ white	0030 628.306	951040601
Deepwell Plate 96/2000 µL, Protein LoBind			
PCR clean, 20 plates (5 bags × 4 plates)	☐ white	0030 504.305	0030504305
Deepwell Plate 96/1000 µL, Protein LoBind			
PCR clean, 20 plates (5 bags × 4 plates)	☐ white	0030 504.208	951032905
PCR clean, 20 plates (5 bags × 4 plates)	■ yellow	0030 504.216	–
PCR clean, 80 plates (10 bags × 8 plates)	☐ white	0030 508.203	951033308
Deepwell Plate 96/500 µL, Protein LoBind			
PCR clean, 40 plates (5 bags × 8 plates)	☐ white	0030 504.100	951032107
PCR clean, 40 plates (5 bags × 8 plates)	■ yellow	0030 504.119	–
PCR clean, 120 plates (10 bags × 12 plates)	☐ white	0030 508.106	951032506
Deepwell Plate 384/200 µL, Protein LoBind			
PCR clean, 40 plates (5 bags × 8 plates)	☐ white	0030 524.101	951031305
PCR clean, 120 plates (10 bags × 12 plates)	☐ white	0030 528.107	951031704

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