

Total Sample Recovery in Eppendorf Protein LoBind Conical Tubes

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Abstract

Protein preparation and storage are critical steps in a wide range of laboratory applications including various methods in the fields of proteomics, molecular biology, forensics and bio-pharma. Nonspecific adsorption of protein/peptide molecules to polymer surfaces of lab consumables has been shown to be a substantial factor contributing to sample loss and degradation. This may adversely influence experimental results, particularly when sensitive methods/assays or small sample amounts are used. In this study, recovery rates of low concentration protein samples were compared between conical tubes from different manufacturers by using a sensitive fluorescence assay. Among the conical tubes tested, Eppendorf Protein LoBind Conical Tubes showed highest protein recovery rates (mean of 100%) and thus ensured the highest protection from sample loss.



Introduction

Preparation and storage of protein samples are crucial steps in various protocols in the fields of proteomics, molecular biology, forensics and bio-pharma. Nonspecific adsorption of protein and peptide molecules to polymer surfaces of laboratory consumables has been shown to be a substantial factor contributing to structural denaturation, diminishing activity and decrease of sample concentration [1, 2, 3]. These effects are particularly prominent when

sensitive methods/assays or small sample amounts are used in proteomic and forensic protocols.

In this Application Note, we investigated nonspecific binding of low concentration protein samples by using a sensitive fluorescence assay. Sample recovery was compared between standard polypropylene conical tubes from various manufacturers and Eppendorf Protein LoBind Conical Tubes.

Materials and Methods

Protein recovery rates were evaluated by using a fluorescent-labeled protein assay in 15 mL polypropylene tubes: Eppendorf Protein LoBind Conical Tubes and standard polypropylene conical tubes from the manufacturers Sa, Co, Gr, Bd, Nu, Br. The assay was set up as follows: 256 μL of a FITC conjugated BSA solution at 1 $\mu\text{g}/\text{mL}$ (1 x Dulbecco's PBS) were transferred into each tube and incubated for 24 h at room temperature in the dark.

After incubation, 190 μL of the solution stored in each tube were used for fluorescence measurements using the Fluoroskan Ascent™ Microplate Fluorometer. The remaining FITC-BSA concentration and the recovery rate percentage were calculated using a calibration curve established with the original solution. Two independent experiments were performed in triplicate ($n=6$).

Results and Discussion

The recovery rates of the FITC conjugated BSA samples following incubation for 24 hours in the different tubes is presented in figure 1.

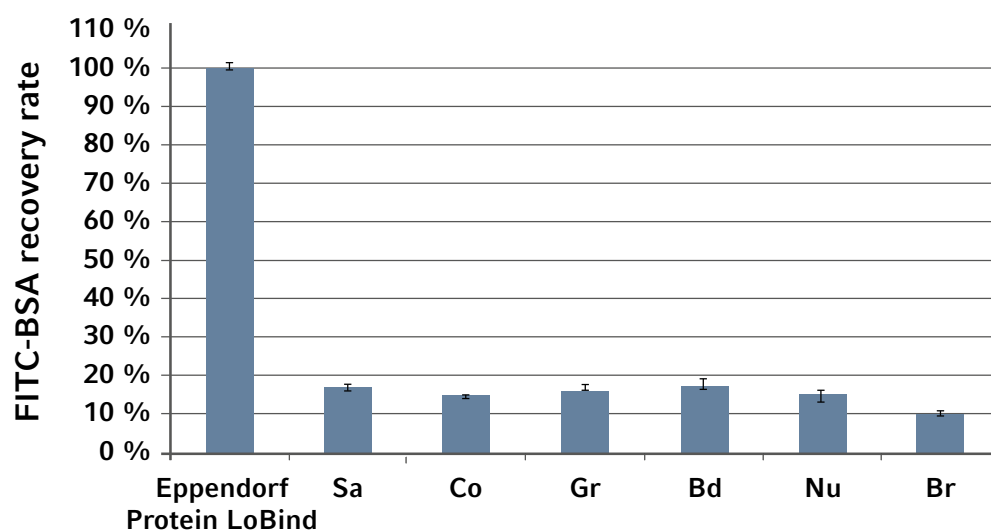


Figure 1: FITC-BSA recovery rate after 24 hours of incubation in 15 ml conical tubes from various manufacturers. Bars represent mean values of two independent experiments, each performed in triplicate ($n=6$).

The analysis revealed that most of the protein sample was adsorbed to the wall of standard PP tubes and that the overall recovery rate after 24 hours of storage was only approximately 15%.

All investigated tubes, Sa, Co, Gr, Bd, Nu and Br, with the exception of Eppendorf Protein LoBind, showed very poor protein recovery rates, ranging between 10% and 17%. This indicates that under applied experimental conditions the standard polypropylene material used in these tubes poses a high risk of losing significant amounts of protein sample.

In contrast, Eppendorf Protein LoBind Conical Tubes showed very high recovery rates of the tested protein samples: virtually no loss was observed after 24 hours of incubation, with a mean recovery rate of 100%. This guarantees utmost sample protection and assures reliable results for respective downstream applications.

Conclusions

In this study, we employed a sensitive fluorescence assay to measure sample recovery rates in various standard conical tubes, as well as Eppendorf Protein LoBind Conical Tubes, following incubation at room temperature in the dark over a period of 24 hours. We showed that the recovery rates of samples incubated in standard conical tubes from various manufacturers was low (10% to 17%) and that these tubes may therefore not sufficiently protect against the non-specific loss of protein sample.

In contrast, the Eppendorf Protein LoBind Conical Tubes allowed nearly complete sample recovery (mean recovery rate of 100%) and thus ensured the highest possible protection of samples, assuring the integrity of the sample and thus safeguarding various downstream applications.

Literature

- [1] Kristensen K, Henriksen JR, Andresen TL. Adsorption of cationic peptides to solid surfaces of glass and plastic. PLoS One 2015; 10(5).
- [2] Hoofnagle AN1, Whiteaker JR2, Carr SA3, Kuhn E3, Liu T4, Massoni SA5, Thomas SN6, Townsend RR7 et al. Recommendations for the Generation, Quantification, Storage, and Handling of Peptides Used for Mass Spectrometry-Based Assays. Clin Chem 2016; 62(1):48-69.
- [3] Kraut A, Marcellin M, Adrait A, Kuhn L, Louwagie M, Kieffer-Jaquinod S, Lebert D, Masselon CD, Dupuis A, Bruley C, Jaquinod M, Garin J, Gallagher-Gambarelli M. Peptide storage: are you getting the best return on your investment? Defining optimal storage conditions for proteomics samples. J Proteome Res 2009; 7:3778-85.

Ordering information

Description	Order no. International	Order no. North America
Eppendorf Conical Tubes 15 mL sterile, pyrogen-, DNase, RNase and DNA-free, 500 tubes (10 bags x 50 tubes)	0030 122.151	0030122151
Eppendorf Conical Tubes 50 mL sterile, pyrogen-, DNase, RNase and DNA-free, 500 tubes (20 bags x 25 tubes)	0030 122.178	0030122178
Eppendorf Conical Tubes 15 mL, Protein LoBind PCR clean, clear, 200 tubes (4 bags x 50 tubes)	0030 122.216	0030122216
Eppendorf Conical Tubes 50 mL, Protein LoBind PCR clean, clear, 200 tubes (4 bags x 50 tubes)	0030 122.240	0030122240
Eppendorf Conical Tubes 15 mL, DNA LoBind PCR clean, clear, 200 tubes (4 bags x 50 tubes)	0030 122.208	0030122208
Eppendorf Conical Tubes 50 mL, DNA LoBind PCR clean, clear, 200 tubes (4 bags x 50 tubes)	0030 122.232	0030122232

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