

Eppendorf LoBind®: Evaluation of Protein Recovery in Eppendorf Protein LoBind® Tubes and Plates

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Abstract

Eppendorf LoBind® consumables were created specifically to minimize sample loss caused by adsorption to the reaction tube wall. The experiments described herein provide proof that Protein LoBind products yield 80 % higher sample recovery than vessels made from standard material. In addition, the recovery rate of Eppendorf LoBind Tubes was compared to low binding tubes made by other manufacturers. Even after 96 h of incubation, Eppendorf LoBind Tubes enabled a recovery rate of 90 %, as demon-

strated by the assay described herein. Furthermore it is shown that subsequent analyses, such as MALDI-TOF, yield superior results. Thus, the Eppendorf Protein LoBind Tubes and Deepwell Plates are ideally suited for applications involving proteins, peptides and viruses; especially when working with limited sample quantities.

Introduction

The preparation and storage of samples (i.e., cells and tissues, as well as DNA, RNA and proteins) are often the basis for successful experiments. Apart from the purity of the sample, recovery after preparation is most important. In cases where biological sample preparation is difficult, labor-intensive or expensive, most often the amount of sample concentration available is quite small. In these cases especially, losses are critical, leading to faulty or ambivalent analytical results, or none at all. Since cost must not be underestimated when it comes to the use of expensive reagents, products whose surfaces have been optimized to guarantee low affinity binding of biological samples will provide an obvious advantage, both from a financial and scientific perspective.

In this context, working with proteins presents a special challenge. Proteins consist of hydrophilic as well as hydrophobic domains, the latter being located on the inside of the globular protein structure in an aqueous environment. When the protein comes into contact with a solid surface, the three-dimensional protein structure can become modified

such that the hydrophobic regions move to the surface of the molecule and seek contact with the hydrophobic surface of the container [1, 2] (Fig. 1).

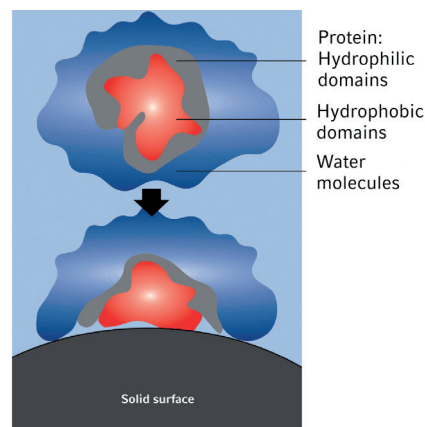


Fig. 1: Schematic drawing of a globular protein. Hydrophobic chains bind to a solid surface, leading to a change in protein conformation.

As a result, proteins in contact with the vessel may denature, leading to the loss of valuable sample, or, in the case of enzyme solutions, to a reduction in enzyme activity. The effect on the sample increases with decreasing sample concentration.

Apart from specialized applications in the medical/pharmaceutical field where protein binding to surfaces (such as polypropylene) plays a role [3, 4, 5], the following methods are employed in the laboratory to minimize adsorption of protein sample material:

- 1) the use of coated (i.e., siliconized) reaction vessels. This may result in leaching of the coating and interference with the sample, possibly influencing downstream applications.
- 2) A further possibility is the addition of BSA that will bind primarily to the vessel surface and thus protect the protein sample. However, the high BSA concentrations necessary will have an adverse effect on the precision of pipetting

and may also influence further analyses.

For these reasons, Eppendorf is focusing on manufacturing consumables featuring protein-repelling surface characteristics without a potentially contaminating coating. The tubes and plates in Eppendorf Protein LoBind quality consist of high quality polypropylene, manufactured by a special process. Hence, protein binding to the wall of the tube/plate will be minimized, leaving a high proportion of the sample available for analysis.

The following experiments will compare the Eppendorf Deepwell Plate in Protein LoBind quality with deepwell plates produced by other manufacturers, thereby focusing on sample recovery. Furthermore, the recovery of proteins from Eppendorf LoBind Tubes and low binding tubes made by other manufacturers is investigated. Another experiment involving mass spectrometry analysis will illustrate the effects of different sample recovery rates on downstream applications.

Material and Methods

Determination of sample recovery via fluorescence measurement

Protein binding to polypropylene was tested using fluorescein-labeled BSA. The tests involving plates employed the Eppendorf Deepwell Plates 96/1000 μL and 384/200 μL , alongside competitors' products of the equivalent format. Four wells of each plate were filled with a protein solution (fluorescein-BSA, 1 $\mu\text{g}/\text{mL}$) and incubated in the dark at room temperature for 24 h.

For the tests involving tubes, three 1.5 mL Eppendorf LoBind Tubes each were used, as well as 4 or 5 competing products of the 1.5 mL or 1.7 mL format, respectively. Incubation was performed once for 24 h and, in a second experiment, for 96 h. At different times during the incubation, samples were removed from two wells of a plate, or from all tubes, and transferred to a black 96 well flat bottom plate.

The samples originated from the plates were measured in the Synergy HT Multi Detection Microplate Reader (BioTek), and the samples originated from the tubes were measured in the Safire2 (Tecan).

MALDI-TOF analysis of peptides

1.3 $\mu\text{g}/\text{mL}$ (10 pmol) and 130 ng/mL (1 pmol) of the peptide angiotensin I were dissolved in 10 μL H_2O each and refrigerated for one week in either Protein LoBind tubes or in standard tubes. Following this incubation 1 μL of each sample was subjected to MALDI-TOF spectral analysis.

Results and Discussion

The first two figures illustrate the results of the protein-incubation experiments performed in deepwell plates. It is obvious that significantly less protein adsorbed to the wells of the Eppendorf Deepwell Plate Protein LoBind (Figs. 2 and 3) than to the wells of other manufacturers' plates made from standard materials. For instance, loss of sample after 24 h incubation in Eppendorf Deepwell Plate 96/1000 μ L is less than 5 %, whereas sample loss after incubation in the competitors' plates can be as high as 85 %.

Following the first incubation experiment for 24 h in tubes, protein recovery from Eppendorf Protein LoBind Tubes was determined at close to 90 %, whereas between 13 % and 52 % BSA could be detected in the tubes from other manufacturers (Fig. 4). A second test using an incubation period of 96 h was designed in order to assess whether the amount of available protein decreased further after 24 h.

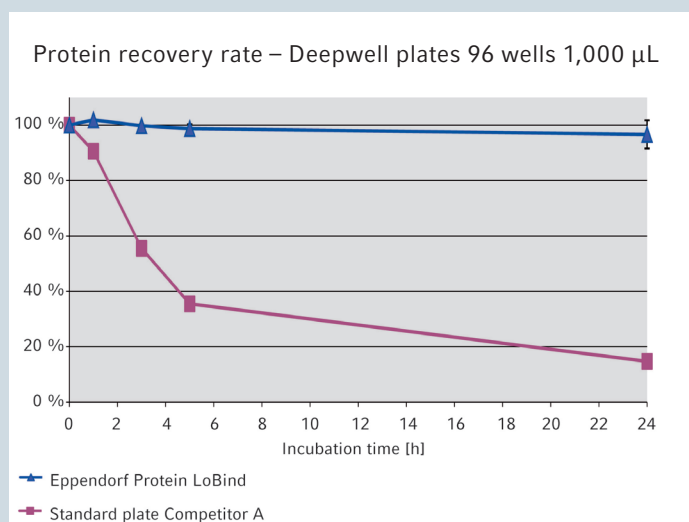


Fig. 2: Recovery rate of proteins after incubation in 96-well deepwell plates with a total volume of 1,000 μ L.

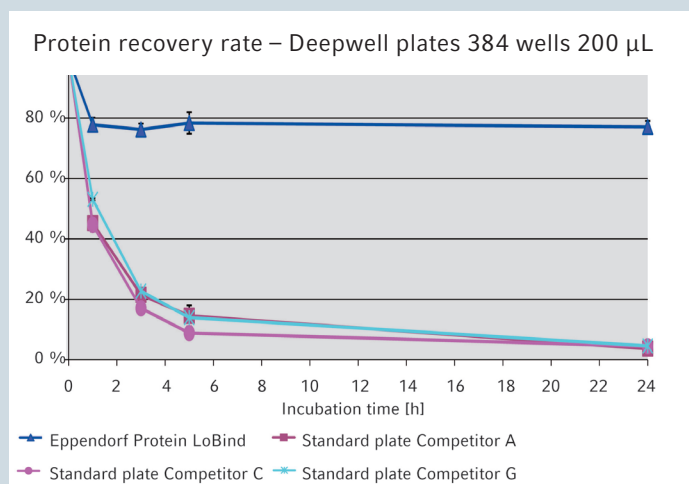


Fig. 3: Recovery rate of proteins after incubation in 384-well deepwell plates with a total volume of 200 μ L.

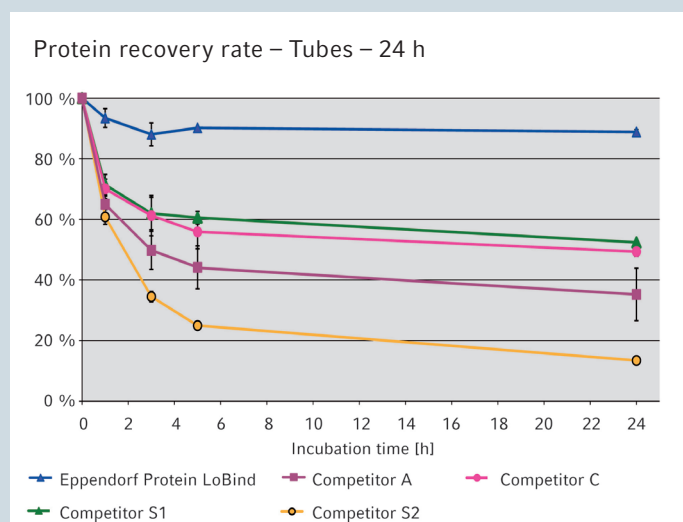


Fig. 4: Recovery rate of proteins after incubation for 24 h in low binding tubes.

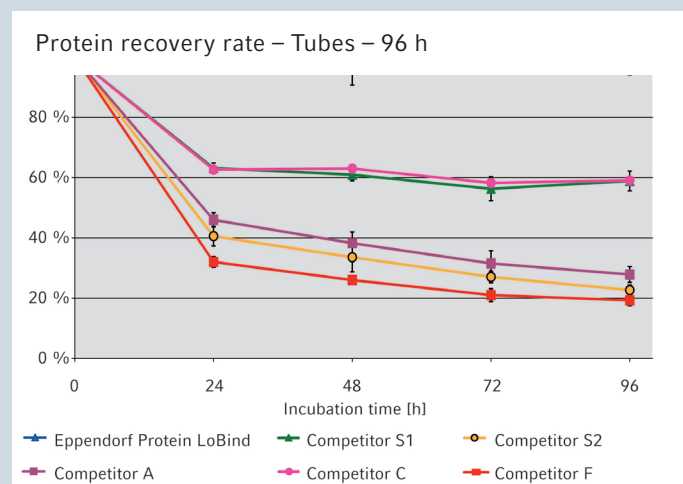


Fig. 5: Recovery rate of proteins after incubation for 96 h in low binding tubes.

Figure 5 shows that protein recovery from Protein LoBind Tubes remains relatively stable between 24 h and 96 h, at a level above 96 %. It appears that possible binding sites may already be saturated by this time. The recovery rates of the competitors' tubes S1 and C are around 60 % during this period of time. For the other three low binding tube types the proportion of detectable protein has dropped to 23 % for manufacturer S2, to 28 % for manufacturer A and to 19 % for manufacturer F after 96 h. In these cases, a downward trend is evident, which may suggest that the recovery rates could drop further past the 96 h time point.

The large proportion of protein lost due to binding to the vessel surface is thus not available for the actual experiments. In contrast, the optimized surface of the Protein LoBind plates achieves very high sample recovery, which is subsequently available for all downstream applications.

The effects of sample loss on the analysis of proteins and peptides are demonstrated using different mass spectrometric detection methods; during sample preparation and storage either Eppendorf standard tubes or Eppendorf Protein LoBind tubes were used for comparison [6]. Please refer to Fig. 6 for a representative experiment. The use of Eppendorf Protein LoBind tubes will yield a significantly higher signal during MALDI-TOF analysis of the peptide angiotensin I than the use of tubes made from standard materials. When the amount of incubated peptide is reduced to 1 pmol, analysis becomes impossible if standard tubes are used.

In addition, Kersten and Halder investigated the influence of the tube surface on peptide binding. MALDI-TOF analysis of a tryptic digest of various proteins yielded a higher coverage of sequences along with a better signal-to-noise ratio of the samples that had been prepared in Eppendorf LoBind tubes compared to preparations in conventional tubes [7].

The data show that it is advisable to employ surface-optimized vessels in order to prevent critical sample loss. The Eppendorf Protein LoBind products are exceptionally well suited for this purpose. The advantageous protein-repelling features of this product line become evident during other applications, such as the purification of viruses whose surface is protein-rich. A recent publication describes a test in which viruses were incubated in nine different types of tubes for up to 120 h. During the course of this experiment, eight of the tubes led to considerable sample loss due to adsorption, whereas almost complete recovery of viruses was achieved only with Eppendorf Protein LoBind tubes [8].

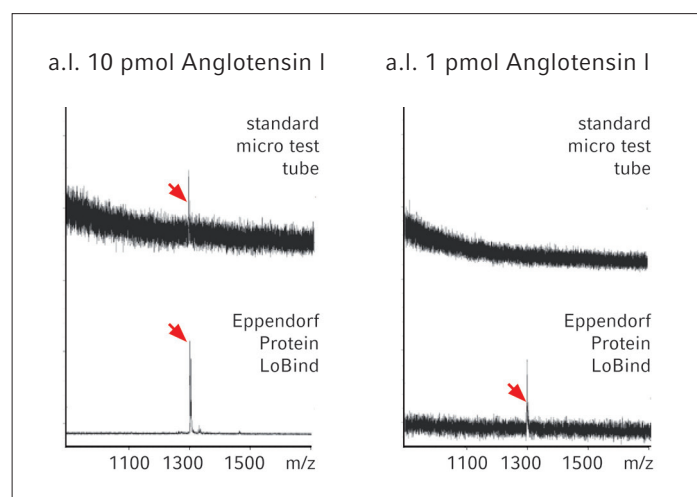


Fig. 6: MALDI-TOF Mass spectrometry following storage of two different concentrations of peptide at 4 °C. (Source: Dr. S. Seeber and Dr. A. Humeny, Institute of Biochemistry, University of Erlangen-Nürnberg, Erlangen). The arrows identify the signals in each experiment.

Conclusion

The analyses presented here show unequivocally that the material of the vessel surface has a tremendous influence on sample recovery. The use of Eppendorf Protein LoBind plates and tubes leads to drastically improved sample recovery rates over standard vessels, especially under conditions of

limited sample amounts, thus allowing these experiments to be performed with confidence for the first time. Furthermore, efficient use of limited sample material will allow the user to save precious time and money in the laboratory.

Literature

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