

proteomics

C7BzO: A Powerful New Detergent for 2-D Gel Electrophoresis Sample Preparation

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Application Notes

- Extracts more proteins
- Allows higher protein loads in 2-D electrophoresis
- Reduces streaking in 2-D electrophoresis

Introduction

Two-dimensional gel electrophoresis (2DE) is a powerful tool used to separate proteins in complex samples. Improvements made in various formulation components have helped with protein solubility but none have been more important than the detergents. Detergents are the key reagents making proteins soluble and keeping them soluble during separation by 2DE. This is important since the first step is separation of proteins by their isoelectric point, which is typically the pH in which the protein is the least soluble. Over the last six years, various groups have investigated new detergents to use in two-dimensional gels. Among them, ASB-14 and C7BzO (Figure 1), have been shown to be very powerful solubilizing detergents. In addition, the C7BzO has been shown to be very useful with many different types of samples such as bacterial, mammalian, and plant.

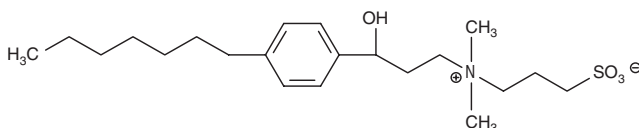


Figure 1. Structure of C7BzO.

Superior extraction power

In order to illustrate the differences in the extraction power of the traditional CHAPS-containing reagent versus the C7BzO-containing reagent, side-by-side comparison of a lyophilized *E. coli* sample (EC-1) was performed. The traditional reagent, Protein Extraction Reagent Type 1 (Product Code [C 0481](#)), contained 8 M urea, 4% CHAPS, and 40 mM Trizma® base. The C7BzO-containing reagent, Protein Extraction Reagent Type 4 (Product Code [C 0356](#)), consisted of 7 M urea, 2 M thiourea, 1% C7BzO, and 40 mM Trizma base. Ten milligrams of the *E. coli* was extracted with 2 ml of each of the extraction reagents. The cells were sonicated for 2 minutes on ice and the material was then allowed to mix for 10 minutes. Insoluble material was removed by centrifugation for 20 minutes at 20,000 x g at 15 °C. The supernatant was then removed and placed into a clean tube. Aliquots of the extracts were removed and the protein content was measured by the Bradford assay (Product Code [B 6916](#)). As seen in Figure 2 the protein extraction reagent with the C7BzO detergent, extracted about 23% more protein than the protein extraction reagent utilizing the CHAPS detergent. The superior performance of C7BzO was verified by how well the proteins were resolved by 2DE. The protein samples were reduced using tributylphosphine for 30 minutes at 25 °C and then alkylated using iodoacetamide for 1 hour at 25 °C. Protein samples were then applied to 11-cm, pH 4-7 strips (Product Code [I 3531](#)) and were allowed to rehydrate for 6 hours. The strips were focused for 80,000 volt-hours. The strips were incubated in IPG Equilibration Buffer (Product Code [I 7281](#)) for 30 minutes at 25 °C and then the focused proteins were separated on a 4-20% Tris-Glycine gel. After electrophoresis, the gels were stained with EZBlue™ Gel Staining Reagent (Product Code [G 1041](#)) and thoroughly destained with water.

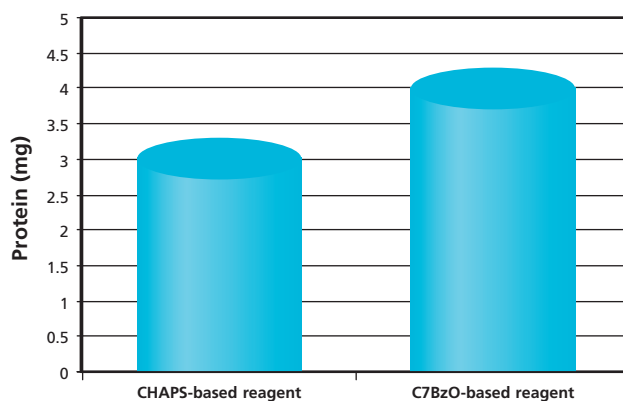
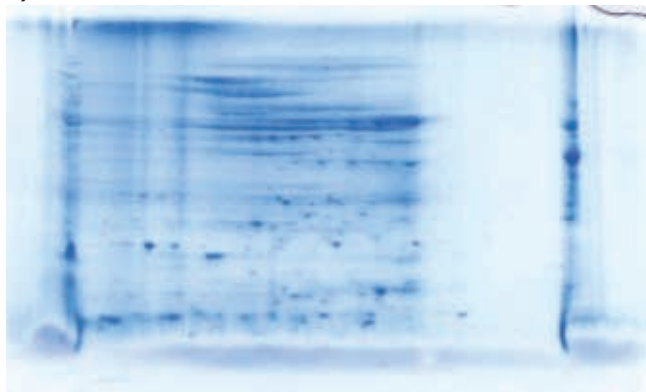


Figure 2. Total protein extracted from 10 mg of lyophilized *E. coli* using a CHAPS-based reagent (Product Code [C 0481](#)) and a C7BzO-based reagent (Product Code [C 0356](#)). The protein was measured using the Bradford assay.

Higher protein-loads with C7BzO

The 2DE gels shown in Figure 3 illustrate that C7BzO allows higher protein-loads on gels of equivalent dimension due to superior solubilizing properties. The protein concentration in the CHAPS-based detergent was approximately 400 μg of total protein, while the C7BzO-based extraction reagent allowed for a load of 500 μg of total protein. This corresponds to a 20% increase in the amount of protein loaded onto the gel with no loss of resolution and thereby potentially allowing more low-abundance proteins to be observed. In addition, streaking in the crude samples was significantly reduced in the C7BzO gel and many more proteins were visualized than in the CHAPS-based extraction. The results support that C7BzO can extract and keep more protein solubilized for subsequent resolution than with traditional extraction buffers using CHAPS.

A)



B)

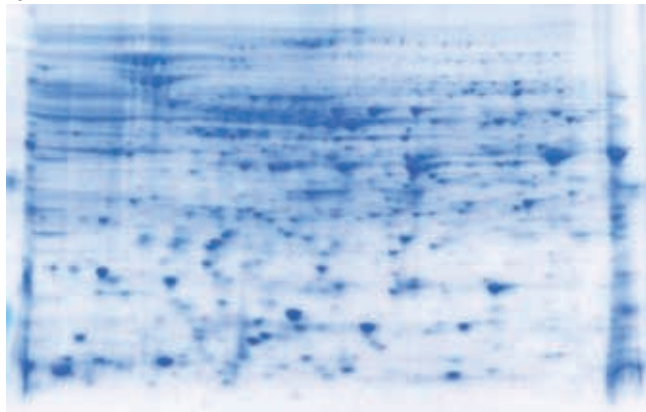


Figure 3. Two-dimensional gels of the extracted *E. coli* proteins. The first dimension was an 11-cm, pH 4-7 strip focused for 80,000 volt-hours and then separated by a 4-20% SDS-PAGE gel. Gels were stained for protein using EZBlue Gel Staining Reagent. Gel A was loaded with 400 μg of protein extracted with CHAPS-based reagent (Product Code [C_0481](#)) and Gel B was loaded with 500 μg of protein extracted with C7BzO-based reagent (Product Code [C_0356](#)).

Summary

Extraction of biological samples with reagents containing a new detergent, C7BzO, yields more extracted protein than traditional protein extraction reagents. In addition, C7BzO-containing extraction reagents allow for higher protein loads with reduced sample streaking, leading to higher resolution detection of more proteins in 2DE protein analysis. This new detergent should be a useful and powerful protein extraction tool in a variety of proteomic applications.

References

- Chevallet, M., et al., New zwitterionic detergents improve the analysis of membrane proteins by two-dimensional electrophoresis. *Electrophoresis*, **19**, 1901-1909 (1998).
- Tastet, C., et al., Structure-efficiency relationships of zwitterionic detergents as protein solubilizers in two-dimensional electrophoresis. *Proteomics*, **3**, 111-121 (2003).
- Luche, S., et al., Evaluation of nonionic and zwitterionic detergents as membrane protein solubilizers in two-dimensional electrophoresis. *Proteomics*, **3**, 249-253 (2003).

Ordering Information

Product	Description	Unit
C_0856	C7BzO	1 g