Detergent Optimization for Total Protein Extraction from Chinese Hamster Ovary (CHO) Cells

Jodi M. Zobrist, Jennifer R. Cresswell, and Rafael Valdes-Camin
Sigma-Aldrich Corporation, St. Louis, Missouri, USA

Introduction

Proteomic sample preparation for 2-dimensional gel electrophoresis (2-DE) presents challenges, as no universal method has been developed for the wide range of sample types. Within the range of sample types, protein type adds to the challenge of solubilizing as much of the protein content as possible. Thierry Rabilloud and colleagues have made significant contributions in the area of proteomic sample preparation by developing new detergents to help overcome issues of solubility for 2-DE work [1]. In collaboration with Dr. Rabilloud, Sigma-Aldrich has developed a detergent sample pack that contains a variety of nonionic and zwitterionic detergents to aid in determining optimal detergent combinations for solubilizing specific protein sample types.

The ProteoPrep™ Detergent Sample Pack was used to determine the optimal detergent combination for total protein extraction from Chinese Hamster Ovary (CHO-K1) cells. The goal of the experiments was to extract as much of the total protein content for the purpose of discovering potential protein indicators via 2-DE for the optimal production of IgG in culture. Zwitterionic detergents were chosen for the total protein extraction based on published research describing successful solubilization of membrane proteins [2]. While not every detergent supplied in the ProteoPrep™ Detergent Sample Pack was used, the utility of the kit for setting up matrix style experiments is demonstrated, and is applicable to other sample types.

Methods

Cell culture

CHO-K1 cells were grown as a suspension culture in animal component-free CHO medium supplemented with 4 mM L-Glutamine. Duplicate spinner flasks (Techne Inc., Princeton, NJ) were innoculated at 2 x 10^5 cells/ml in 200 ml media and incubated at 37 °C with humidified air and 5% CO_2 at 80 rpm on a magnetic stirrer platform (Thermolyne, Dubuque, IA). After four days, cultures were 96% viable with a cell density of 2.5 x 10^6 viable cells/ml as determined by Vi-Cell™ XR Cell Viability Analyzer (Beckman Coulter, Inc., Fullerton, CA). Cells were harvested by centrifugation at 200 xg for 5 minutes then washed twice with chilled PBS.

Sample preparation

Total protein from 5 x 10^7 cells was extracted in 1 ml of extraction solution. The extraction solution contained various detergents, prepared in a solution of 7 M urea, 2 M thiourea, and 40 mM Tris. Cells suspended in extraction solution were sonicated using an ultrasonic probe (4 x 15 sec). Samples were then diluted 1:4 in water containing 500 U/ml benzonase and incubated on ice for 30 minutes. Samples were reduced with tributylphosphine and alkylated with iodoacetamide using the ProteoPrep™ Reduction and Alkylation Kit, followed by precipitation with trichloroacetic acid using the ProteoPrep Protein Precipitation Kit. Pellets were re-suspended in the extraction solution.

Separation by 2-DE

IPG strips (11 cm, pH 3-10 or 4-7) were rehydrated with the samples and focused at 8,000 Volts for 85,000 Volt hours. The IPG strips were equilibrated for 20 minutes with IPG equilibration buffer, and SDS-PAGE was performed using 4-20% or 8-16% precast gels. The gels were electrophoresed for 10 minutes at 80 Volts, followed by 70 minutes at 170 Volts. Protein bands were visualized in the gels using EZBlue™ Gel Staining Reagent. The gels were imaged using a Flour-S™ MultiImager (BioRad), and analysis of the gels was performed using Phoretix 2-D Expression Software (Nonlinear Dynamics, Durham, NC).

Results

In order to evaluate an optimal method for total protein extraction from CHO cells, three detergents (CHAPS, C7BzO, and ASB-14) of the ProteoPrep™ Detergent Sample Pack, which contains a 2-DE compatible selection of 10 detergents, were tested. The detergents and combinations of these were used for sample preparation and 2-D electrophoretic separations comprising the pH ranges 3 to 10 and 4 to 7 were carried out (Figure 1). The effectiveness of the detergents was determined based on the number of well-resolved protein spots and clarity of the gel. Extraction with 2% C7BzO demonstrated the greatest number of well-resolved spots under the various conditions, as determined by the 2-DE analysis software (Nonlinear Dynamics). Normalizing the total spot number for the pH 3-10 IPG strips of 2% C7BzO to 100% extraction, 1% ASB-14/2% C7BzO resulted in 85% of the total spots, 1% ASB-14 /1% C7BzO resulted in 83% of the total spots, 2% ASB-14 /1% C7BzO resulted in 78% of the total spots, and 4% CHAPS resulted in 83% of the total spots. Normalizing the total spot number for the pH 4-7 IPG strips of 2% C7BzO to 100% extraction, 1% ASB-14/2% C7BzO resulted in 85% of the total spots, 1% ASB-14 /1% C7BzO resulted in 90% of the total spots, 2% ASB-14 /1% C7BzO resulted in 76% of the total spots, and 4% CHAPS resulted in 86% of the total spots. Overall, using either pH 4-7 or pH 3-10 IPG strips, the 2% C7BzO gave the highest number of well-resolved spots using the 2-D expression software, without manipulation of the chosen

spots. Because the goal was total protein extraction at this point, the 2% C7BzO was determined to be the best extraction method, as it resulted in the most protein spots.

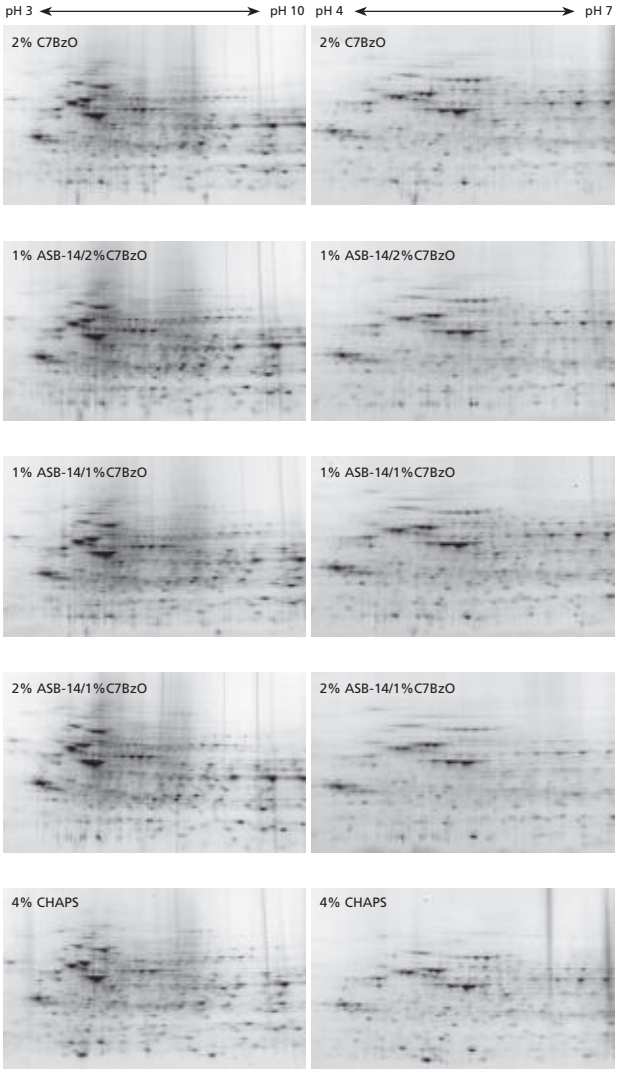

Figure 1. *Detergent extraction optimization of CHO cells. Three detergents from the kit were tested in different concentration blends. Gels resulting from five of the detergent combinations tested are illustrated above. In every case, the cell extracts (250 µg) were focused on either pH 3-10 or pH 4-7 IPG strips. SDS-PAGE was performed on 4-20% gels.*

Table 1.
Total spot number from represented 2-DE gels from Figure 1

Detergent Combination	pH 4-7 IPG strips	pH 3-10 IPG strips
2% C7BzO	317	477
1% ASB-14/2% C7BzO	270	404
1% ASB-14/1% C7BzO	287	397
2% ASB-14/1% C7BzO	242	372
4% CHAPS	274	397

Discussion

Challenges for proteomic sample preparation optimization exist, as there are no universal methods for extracting various sample types. A matrix approach was employed to determine the most effective detergent combination for total protein extraction from CHO cells. Using various detergents from the ProteoPrep Sample Pack, the most efficient extraction method was identified as 2% C7BzO. The variety of detergents provided in the kit enables researchers to determine the best extraction method for their samples of interest.

References

1. Rabilloud, T., *et al.,* Structure-efficiency relationships of zwitterionic detergents as protein solubilizers in two-dimensional electrophoresis, *Proteomics,* **3,** 111-121 (2003).
2. Rabilloud, T., *et al.,* Evaluation of nonionic and zwitterionic detergents as membrane protein solubilizers in two-dimensional electrophoresis, *Proteomics,* **3,** 249-253 (2003).
3. Lunardi, J., *et al.,* Improvement of the solubilization of proteins in two-dimensional electrophoresis with immobilized pH gradients, *Electrophoresis,* **18,** 307-316 (1997).

Ordering Information

Cat. No.	Description	Unit
PROTDT	ProteoPrep™ Detergent Sample Kit	1 kit
PROTRA	ProteoPrep® Reduction and Alkylation Kit	1 kit
PROTPR	ProteoPrep® Protein Precipitation Kit	1 kit
C5467	EX-CELL™ ACF CHO Medium	500 ml 1 L 6 x 1 L
G7513	L-Glutamine solution	20 ml 100 ml
B6916	Bradford Reagent	500 ml
I3531	ProteoGel™ IPG Strips, ph 4-7	1 Pak 12 ea
I3406	ProteoGel™ IPG Strips, ph 3-10	1 Pak 12 ea
I7281	ProteoGel™ IPG Equilibration Buffer	1 btl
G1041	EZBlue™ Gel Staining Reagent	500 ml 3.8 L