

Comparison of Protein Features Detected On 2D Gels Using Two Solubilization/Rehydration Cocktails

Proteomic Application Note

INTRODUCTION

To demonstrate why one should consider alternative solubilization/rehydration solutions for the preparation of protein samples prior to 2D gel electrophoresis.

Particular considerations:

1. Zwittergent® (Calbiochem) 3-10 (a component of Genomic Solutions' product 70-4019) can be insoluble in the presence of high concentrations of urea at room temperature. This obviously could reduce the efficiency of protein solubilization.
2. ASB-14 is a zwitterionic amidosulfobetaine detergent (Calbiochem) that is readily soluble in the presence of high concentrations of urea. It has also been shown to enable the identification of previously undetected membrane proteins separated by 2D gels (Molloy, MP et. al. (1999) *Electrophoresis* **20**: 701).
3. Triton X-100 is a non-ionic detergent that can improve the solubility of some membrane proteins (Observation made in lab; Phadke, et.al. (2001) *Proteomics* **1**:705-720).
4. The reducing reagent tris (2-Carboxyethyl) phosphine, HCl (TCEP) is water-soluble, odorless and more effective than DTT for the reduction of disulfide bonds in proteins. It reduces the most stable, water soluble alkyl disulfides within 5 minutes. It is active in the pH range of 1.5-8.5 and is more resistant to air-oxidation than DTT. It can be stored and is stable for months at 4°C in solution (unlike DTT). (Han, J. et. al. (1999) *Previews* **2**(4):16-21.)
5. The use of different solubilization conditions can increase the total number of features detected. The researcher must investigate the alternatives to find the method(s) and reagents that meets their requirements (Phadke et. al. *Proteomics* (2001)**1**:705-720).

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PROTOCOLS

The following shows an example of an *E. coli* sample treated with various solubilization/rehydration solutions. The individual researchers must determine the optimal conditions for their study.

1. Duplicate cell pellets containing 10^9 *E. coli* were resuspended in 400 μ l of either Zwittergent 3-10 or ASB-14 solubilization/rehydration solution or with 200 μ l of each solution (total 400 μ l).
2. The samples were sonicated with 10- 1 second bursts.
3. The samples were incubated for 1 hour at 30°C.
4. The samples were then centrifuged at 20,000g for 20 minutes at 20°C.
5. The resulting supernatants were used to rehydrate 18 cm 4-7 IPG strips.
6. The IPG strips were rehydrated overnight.
7. Isoelectric focusing was then carried out at 5,000 volts, 70 μ amps/gel, for a total of 100,000 volt-hours.
8. During the first 8 hours of focusing, the electrode wicks were changed at 2 hour intervals.
9. Upon completion of focusing, the gels were equilibrated twice for 10 minutes with equilibration solution I and once for 10 minutes with equilibration solution II.
10. The equilibrated gels were applied to 10% Tris-Tricine SDS-PAGE and electrophoresed at 500 volts and 14,000 mW per gel until the dye front was approximately 1 cm from the bottom of the gel.
11. The gels were stained with SYPRO[®] ruby and imaged on the ProXpress[™] (Perkin-Elmer, Inc.) imaging system with the following parameters: 3 second exposure time; top illumination; 100 μ m resolution; 480/30 nm excitation; 620/30 nm emission.

BUFFERS

Zwittergent solubilization-rehydration solution	ASB-14 solubilization-rehydration solution
7M Urea	7M Urea
2M Thiourea	2M Thiourea
2% CHAPS	5mM TCEP
65mM DTT	1% w/v ASB-14
1% Zwittergent 3-10	1% v/v Triton X-100
0.01% bromophenol blue	0.5% w/v CHAPS
0.8% 3-10 ampholytes	0.5% v/v 3-10 Ampholytes

Equilibration Buffer I (cat.# 80-0233)	Equilibration Buffer II (cat# 80-0232)
6M Urea	6M Urea
2% DTT	2.5% Iodoacetamide
30% glycerol	30% glycerol
45mM tris base	45mM tris base
1.6% SDS	1.6% SDS
acetic acid (ph to 7)	acetic acid (ph to 7)
0.002% bromophenol blue	0.002% bromophenol blue

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2-D Analysis

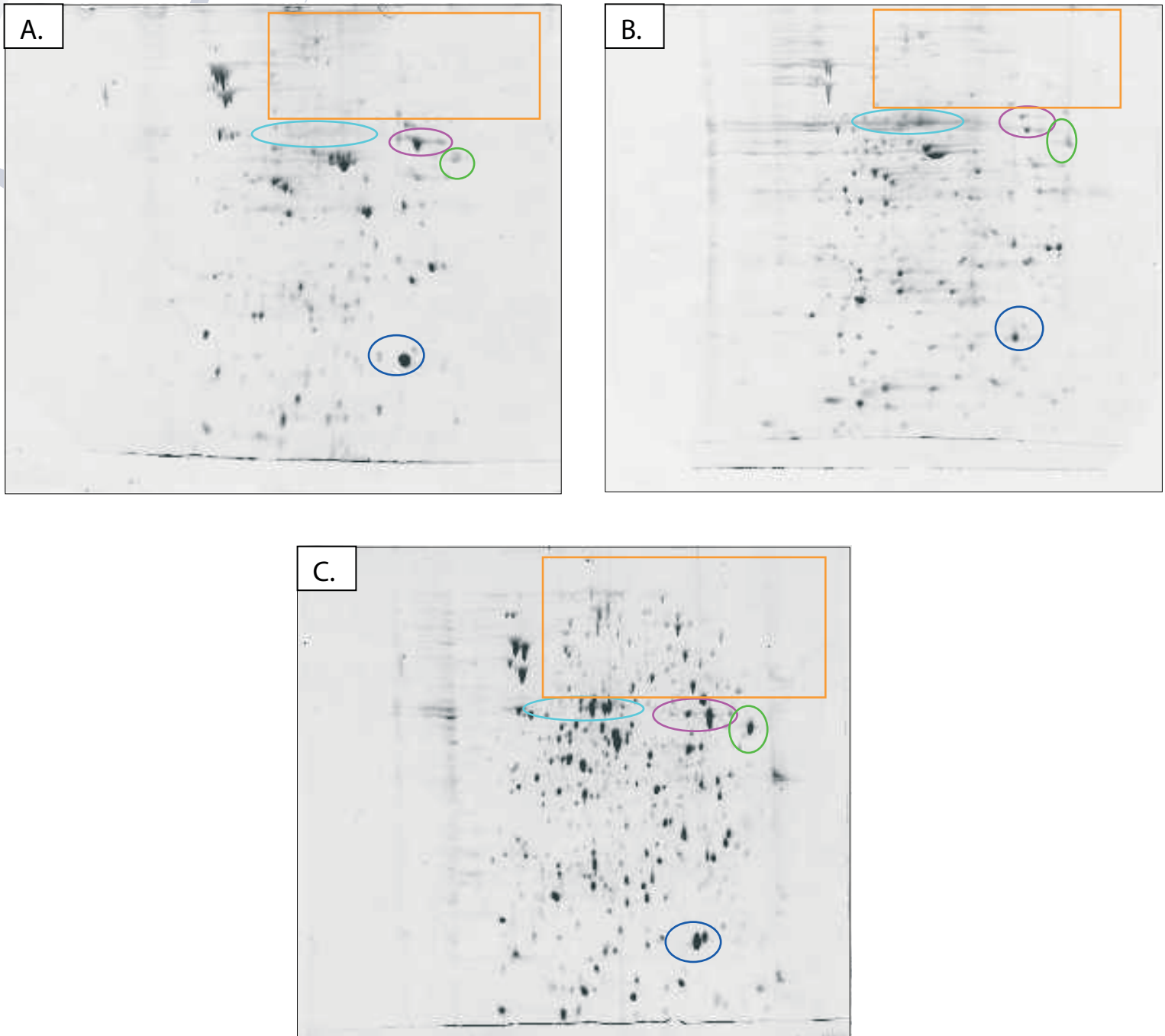


Figure 1: Duplicate cell pellets containing 10^9 E. coli were resuspended in 400 μ l of either A) ASB-14 rehydration solution, B) zwittergent rehydration solution, C) 1:1 ASB-14:zwittergent rehydration solution. 18 cm 4-7 IPG strips were rehydrated with the resulting supernatants. Isoelectric focusing was then carried out for a total of 100,000 volt-hours. The equilibrated gels were applied to 10% Tris-Tricine SDS-PAGE. The gels were stained with SYPRO[®] Ruby. The outlined areas indicate some of the observed differences.

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RESULTS

The use of different solubilization conditions can increase the total number of features detected. The example of *E. coli* treated with various solubilization/rehydration solutions was shown to demonstrate that a combination of the two solubilization/rehydration solutions yields more protein features than is seen when using either alone. Some of the observed differences are outlined on the gel images in Figure 1. The area of the gels enclosed in the orange box shows that there are many higher molecular weight, more basic proteins resolved when using the combination of detergents. There are several components one must consider for the preparation of protein samples prior to 2D gel electrophoresis. The extraction/solubilization of proteins from the sample is one of the initial steps. The researcher must investigate the alternatives to find the method(s) and reagents that meets their requirements and determine the optimal conditions for their study.

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