

2D Gel Sample Preparation

1. Wash and extract cell pellets from your cell culture using Protocol A.
2. Dissolve cell pellets in Lysis Buffer. Sonicate for 5 seconds three time and centrifuge at 14,000 G for 30 minutes. Collect the supernatant.
3. Using Amersham 2-D Clean-Up Kit remove salts from Lysis Buffer which contain cell pellets. You will end up with protein pellets.
4. Dissolve protein pellets in Lysis Buffer and sonicate for 5 seconds.
5. Quantify the protein concentration using Bradford Method (Bio-Rad Protein Assay Kit). For using coomassie blue stain the recommended amount of protein is 300 to 500 micro grams per gel. And for Sypro Ruby stain the recommended amount is 100 to 200 micro grams.

1. Protocol A

Preparation of cell pellet:

The amount of reagents and the procedure that is described is based on collecting 100-mm dishes of cultured cells that will contain approximately 1 mg of total protein. A useful preliminary experiment is to harvest plates of cells at a variety of densities to roughly relate the yield of protein per number of cells and apparently density under the microscope.

Reagents and Materials:

- a. **TBS:** Tris buffered saline 4.4g NaCl + 1.2g tris base (FW=121) dissolved in approximately 300 mL water. Adjust pH to 7.8 with 6M HCl. Adjust total volume to 500 mL. This is the buffer that we use most often but any isotonic buffer is probably suitable for washing cells without lysis should function well. Delbeco's MEM (with or without phenol red) works well. Avoid phosphate buffered saline because of the deleterious effect of the phosphate on isoelectric focusing.
- b. **Cell Monolayer:** The cells of interest in 100-mm plates. Because of the acetone precipitation step, at least 1 mg of protein must be present in the cell pellet. It is common to combine the contents of 2 to 3 plates to get the desired amount of protein in a pellet.
- c. **15-mL Polypropylene Centrifuge Tubes:** Polypropylene is required for the acetone precipitation step.

Procedure:

The following procedure is based on combining two 100-mm plates per cell pellet. If more than two plates are combined, then the volumes used for the scraping should be adjusted so that the total volume for the dishes is less than 15 mL.

1. Pour the growth media off of the monolayer.
2. Add 5 mL of cold TBS to each dish to wash the monolayer.
3. Pour the wash off of the monolayer.
4. Add 3 mL of cold TBS to each dish.
5. Carefully scrap the cells into the buffer.
6. Collect the cells/buffer in a 15-mL centrifuge tube, combining two dishes per centrifuge tube. Keep the collected cells/buffer on ice.
7. Add a second aliquot of 3 mL of cold isotonic buffer to each dish.
8. Repeat scraping.
9. Collect the residual cells/buffer, adding it to the 15-mL centrifuge tube containing the first scrapping.
10. Pellet the cells by centrifugation at ~500 g for 5 min (longer times or higher speeds are not necessarily better and may lyse the cells).
11. Pour off the supernate and blot any excess liquid out of the tube with the kimwipe, be careful not to disturb the pellet with the kimwipe.
12. The cell pellets may be stored at this point at -20°C.

2. Lysis Buffer (Solubilization Buffer)

Lysis Buffer is good for solubilizing

- i. Human/Animal Tissue
- ii. Bacteria
- iii. Cells

7M	Urea	4.2 g
2M	Thiourea	1.6 g
4%	Chaps	400 mg
0.2%	BioLytes 3/10	50 microL (SC: 40% w/v)
0.5%	Triton X-100	50 microL
	Double Distilled water	adjust to 10 mL

NOTE: Store Lysis Buffer at -20 °C

Just before using Lysis Buffer add following additives in 10 mL of Lysis Buffer

2.5 mM	Na-pyrophosphate	100mM (Stock Conc.)	0.25 mL
1.0 mM	Na ₃ VO ₄	100mM (Stock Conc.)	0.1 mL
	Protease Inhibitor Cocktail		50 microL

Product Information

Chaps	Bio-RAD Catalog # 161-0460
BioLytes 3/10	Bio-RAD Catalog # 163-1112
Triton X-100	Bio-RAD Catalog # 161-0407
Na-pyrophosphate	Sigma Catalog # S-9515 (<i>13472-36-1</i>)
Na ₃ VO ₄	Sigma Catalog # S-6508 (<i>13721-39-6</i>)
Protease Inhibitor Cocktail	Sigma Catalog # P-8340
Amersham 2-D Clean-Up Kit	Amersham Catalog # 80-6484-51
Bio-Rad Protein Assay Kit	Catalog # 500-0002