

---

## Preparation of Eukaryotic Cell Proteins for Analysis by 2DE

The following protocol has been developed for preparing soluble cellular proteins from cell lines grown *in vitro* using typical culture conditions. The procedure described was originally developed for the analysis of cells grown as monolayers in cell culture dishes.

### **Stock solutions**

In addition, to the growth media that are specific for the cell line under investigation the following lysis buffer is required:

### **2D Lysis Buffer**

0.01M	Tris-HCl, pH7.4
1mM	EDTA
8M	Urea
0.05M	DTT
10% (v/v)	glycerol
5%(v/v)	NP40
6% (w/v)	ampholytes

The buffer is made up using high purity MilliQ water

### **NOTES:**

1. Use Aristar grade Urea from Merck-BDH - or a similar high purity urea.
2. The ampholyte solution currently used is "Resolyte" pH 3.5-10 from Merck-BDH. The buffer requires an alkaline ampholyte solution which reduces protease activity as well as precipitating DNA in the sample. Commercially produced ampholyte stock solutions are at a concentration of 40% (w/v).
3. A protease inhibitor can be added to the lysis buffer. PMSF at 1-2 mM can be used, but the compound is not stable for long term storage.
4. Nucleases (e.g. DNase and RNase A) can be added at ca. 0.2 mg/ml to help remove nucleic acid contaminants.
5. Once prepared, the 2D Lysis Buffer is divided into smaller volumes (e.g. 1-1.5 ml) and stored frozen in sterile microfuge tubes at -70°C until required. A tube of lysis buffer is thawed as required and any remaining lysis buffer is discarded - avoid re-freezing the 2D Lysis Buffer.

### **PBS-A**

171mM	NaCl
3.3mM	KCl
10mM	Na <sub>2</sub> PO <sub>4</sub>
2mM	NaH <sub>2</sub> PO <sub>4</sub>

### **Method**

The following procedure gives the buffer volumes for cells grown as a monolayer on a 30mm culture dish. Typically for Hep-2 cells (a “classic” epithelial cell line) there are approximately 10<sup>6</sup> cells in a confluent monolayer. The volumes used for the lysis buffer can be adjusted for different cell concentrations and culture vessels but it is generally better to keep the protein sample as concentrated as reasonably possible.

The protocol provides the preparation of a sample that is enriched for cytoplasmic proteins but may not be fully representative of membrane and nuclear proteins.

The procedure as described permits the detection of the abundant cellular proteins using the colloidal Coomassie blue G250 stain but for the detection of minor proteins silver stain may be required. The cellular proteins can be radiolabelled using times and amounts of radiolabel specific for the individual experiment.

### **Basic Lysis Procedure**

1. Gently pour off the culture media and wash the surface of the cell monolayer twice with 0.8 ml ice cold PBS-A. This buffer is added using a P1000 Gilson pipette. The buffer should be added gently by directing the buffer from the Gilson to the side of the culture vessel - this ensures that the cells are not dislodged from the surface of the plastic dish.

### **NOTES.**

- *The purpose of the washes is to remove culture media and serum proteins - increase the number of washes if these are likely to be a problem.*
  - *After the first wash the buffer can be discarded simply by pouring from the dish into a waste bottle. For the second wash pour off the majority of the buffer and then use a Gilson to remove the remaining buffer from the dish.*
  - *During the washes it is essential that the cell monolayer is not allowed to dry.*
2. Place the dish on ice and add 0.2 ml of 2D lysis buffer. Leave for 5 minutes. Some cell lines will lyse in less time than this but this can be checked using an inverted microscope.
  3. Using a P100 or P200 Gilson gently run the buffer over the cell monolayer and scrape the surface of the dish with the end of the pipette tip.
  4. Remove all of the cell suspension and transfer to a 1.5 ml microfuge tube. Centrifuge at 11,000 x g for 5 minutes - this is equivalent to 13000 rpm on a MSE MicroCentaur.
  5. Collect the supernatant - avoid sucking up the pellet which contains mainly nuclei and large insoluble cell debris. The supernatant is stored at -70°C until analysed. Discard the pellet.

### *Suggested Modifications*

The following are suggested modifications to the basic procedure that may be necessary for different cell lines.

1. The wash buffer suggested is PBS-A this can be changed if necessary. The important point being that any buffer used should be iso-tonic and not lead to cell lysis and should also have a low salt concentration. The latter reduces artefacts in 2DE when carrier ampholytes are used for the first dimension separation.
2. Cells grown in suspension can be harvested and washed simply by centrifugation. The speed depends on the specific cell line but should not lead to cell disruption. It is recommended that after the final wash the cells are collected using a microfuge running at 6500 rpm - providing this will not destroy the cells. Ensure that all of the final buffer is removed from the cell pellet.
3. For some T cells (e.g. MOLT-4 and Jurkat) as well as monocytes (e.g. U937 cells) it may not be necessary to remove the nuclei which primarily removes DNA contamination from the sample which may interfere with the 2DE separation.
4. Loading of the 2D gels is determined empirically by analysing the protein samples by 1D-PAGE. If it is necessary to determine the actual protein concentration this must be done before lysing the cells with the 2D Lysis Buffer, since the latter can interfere with many standard protein assays.
5. 2D gels are either loaded with equivalent amounts of sample or balanced for radioactive counts - the specific method used will depend on the purpose of the analyses