1. Sample preparation guideline for 2D/DIGE gel analysis

Sample preparation is a key factor in successful 2DE, with complete solubilization and denaturation of sample proteins being the ultimate aim.

Protein solubilization varies widely with samples. In general, improved solubilization of proteins is obtained by the use of chaotropic agents, detergents, reducing agents, buffers and ampholytes. It is absolutely necessary to remove cell debris, nucleic acids, lipids, polysaccharides and other particulates from the protein samples before performing any 2D gel analysis. Prefractionation of whole extracts is generally recommended in order to reduce the sample complexity and to improve gel separation resolution for given amount loaded samples. Which type of prefractionation method used is dependent on the sample sources and the researcher's goals. In general, the total salt concentration should not exceed 20 mM, to ensure that the proteins migrate properly to the voltage and current during focusing. It is extremely important that the sample does not contain any SDS or ionic detergent, which will have significantly negative impact on IEF running.

The first dimensional analysis (IEF) is performed in a loading/rehydration buffer made of Urea, CHAPS, and DTT. We strongly recommend that researchers submit their sample in the following buffer with estimated concentration around 1mg/mL:

- 8 M Urea
- 4% CHAPS
- 50 mM DTT

If your sample is not soluble in this buffer, an alternative could consist of:

- 7 M Urea
- 2 M Thiourea
- 2 mM tributyl phosphine (TBP)
- 4% CHAPS
50mM DTT can be substituted for TBP. Reagents used should be of the highest quality to ensure good results. **Note that DTT should be added to these solutions just prior to use. Never heat the sample after adding urea to avoid protein carbamylation.**

If you prefer to submit pellet protein sample, make sure the pellet was completely washed with pre-cold acetone and/or methanol to **remove residual phenol or TCA** etc. It is important to keep washed protein pellet from dryness. The wet pellet will help facilitate solublize proteins in reconstitution process prior to IEF analysis.