Sample Preparation Guidelines for 2-D Gel Electrophoresis.

A. 2D DIGE Experiments

It is required that samples submitted for 2D DIGE experiments do not contain reagents that interfere with the labeling of the protein sample with CyDye Fluor minimal dyes. These reagents include thiols used as reducing agents and polyamines used as IPG buffers (ampholytes). Samples must also be free of ionic contaminants that hamper effective isoelectric focusing of the proteins during the first dimension. Acceptable lysis buffers and sample contaminants to avoid are listed below. A typical DIGE experiment run in triplicates followed by a preparative gel experiment needs at least 600 μg of each sample. Sample solution should contain 5-10 μg/μL of protein and the accurate protein concentration should be determined. It is absolute to follow these guidelines for successful DIGE experiments.

Compatible Lysis Buffers

1. 8-9 M urea
   4% CHAPS
   30 mM Tris (pH 8.5)

2. 7 M urea
   2 M Thiourea
   4% CHAPS
   30 mM Tris (pH 8.5)

Compatible Protease Inhibitors

1. Aprotinin: compatible at manufacturer's recommended concentration.
2. (4-amidino-phenyl) methane sulphonyl fluoride (APMSF): compatible at manufacturer's recommended concentration.
3. EDTA: compatible between 0.5-10 mM.
4. Phenylmethylsulphonyl fluoride (PMSF): compatible at manufacturer's recommended concentration.
5. Pepstatin A: compatible at manufacturer's recommended concentration.
6. Roche Complete Mini EDTA-free Protease Inhibitor Tablet

Compatible Phosphatase Inhibitors

1. Phosphatase inhibitor cocktail 1 (Sigma): compatible at manufacturer's recommended concentration.
2. Phosphatase inhibitor cocktail 2 (Sigma): compatible at manufacturer's recommended concentration.

Contaminants to Avoid

1. Reducing agents (e.g., DL-dithiothreitol (DTT), Tris- (2-carboxyethyl) phosphine (TCEP), tributyl phosphine (TBP), β-mercaptoethanol)
2. IPG buffers (e.g., ampholytes, pharmalytes, resolytes)
3. Ionic Detergents (e.g., SDS)
4. Buffers other than Tris (e.g., HEPES, PPA, PBS)
5. Salts (e.g., NaCl)
6. Nucleic Acids
7. Lipids
8. Polysaccharides

Note: If samples contain components not compatible with DIGE experiment, remove these contaminants by protein precipitation. A number of 2D clean-up kits are commercially available. After protein clean-up, redissolve the protein pellet with a compatible lysis buffer. Be sure to make the final protein concentration between 5-10 μg/μL. Protein precipitation and the subsequent protein quantitation can be done in our facility for additional charge.

B. Regular 2D Gel Electrophoresis.

For regular 2D experiments, samples can contain reducing agents (e.g., DTT, TBP), IPG buffers (eg., ampholytes, pharmalytes) and detergents such as NP40 and Triton X-100. Components to be avoided include ionic detergents (e.g., SDS), salts (e.g., NaCl), nucleic acids, lipids and polysaccharides. The required protein concentration is at least 5 μg/μL and at least 400 μg of each sample is needed for a single preparative run.