

2D Sample Preparation

2D sample preparation is dependent on what is desired in the final result. Is the goal to view as many proteins as possible or is the interest in only a subset of proteins? The optimal procedure must be determined empirically for each sample type. In general, the preparation should be as simple as possible to increase the reproducibility. Any protein modifications during sample preparation must be minimized.

The three fundamental steps in sample preparation are

1. Cell disruption
2. Inactivation or removal of interfering substances
3. Solubilization of proteins

Inactivation or Removal of Interfering Compounds

Proteases:	
Cause protein degradation.	Inactivate proteases to prevent protein degradation
Salt ions:	
Interfere with electrophoretic separation. Greater than 100mM must be removed.	Remove by dialysis. Remove by protein precipitation by TCA or organic solvents. Use 2D cleanup kits (Amersham Biosciences) Dilution of the sample so the salt concentration is below 100 mM During rehydration, apply 50V to "desalt" the sample
Lipids:	
May interact with membrane proteins and "consume" detergents.	Use high-speed centrifugation followed by removal of the lipid layer or extract the proteins with organic solvents (may lead to protein loss)
Nucleic Acids and Polysaccharides:	
Interact with the carrier ampholytes and proteins, causing horizontal streaking in the gel.	Use TCA/acetone (20% TCA in pure acetone) precipitation to remove (may lead to protein loss)
Phenols (present in plant material)	
May interact with proteins and give rise to horizontal streaking in the gel.	Removed by binding to insoluble polyvinylpolypyrrolidone (PVPP) (not PVP) or precipitation of proteins with TCA and extracting the phenols with ice-cold acetone.

Solubilization of Proteins prior to IEF

Samples containing Urea must not be heated to avoid carbamylation of the proteins that will lead to charge heterogeneities.

- Urea lysis buffer (preferred buffer):
8M Urea, 2% CHAPS, 0.5% (v/v) carrier ampholytes
- Thiourea/urea lysis buffer:
2M Thiourea, 7M Urea, 2% CHAPS, 0.5% (v/v) carrier ampholytes
- Boiling with SDS sample buffer, followed by dilution with urea or thiourea/urea lysis buffer. Horizontal streaking in the gel may be observed if sample with 0.5-1% SDS are not diluted at least 8X. The final SDS concentration must be <0.25%. This dilution will displace the SDS from the protein and replace it with a nonionic or zwitterionic detergent. Avoiding the use of SDS is ideal

The rehydration buffer used to dilute the sample prior to application of the sample into the IEF boat: 8M Urea, 2% CHAPS, 0.5% IPG Buffer, trace of bromophenol blue, add 2.8 mg/mL DTT prior to use

Sample Amount to load

7 cm IPG strip: 50 µg in <50 µL, total volume loaded including the rehydration buffer is 125 µL

18 cm IPG strip: 500 µg in <150 µL, total volume loaded including the rehydration buffer is 350 µL

Typical IEF run conditions:

2 hr rehydration

50V for 10 hours

500V for 1 hour

1,000V for 1 hour

8,000V for 4 hours

Ideal voltage to obtain is 8,000V with >30,000 Vhrs

There is a detailed class procedure for 542E: Introduction to Proteomics that goes through (in detail) 2D procedures. It is available online at <http://www.protein.iastate.edu>.

References:

Simpson, R.J., Purifying Proteins for Proteomics: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2004.