

CONTAMINANTS THAT AFFECT 2-D RESULTS

Contaminant	Reason for Removal	Removal Techniques
Salts, residual buffers, and Other charged small molecules that carry over from sample preparation	Salts disturb the electrophoresis process and must be removed. Salts in the IPG strip result in high strip conductivity. Focusing of the proteins will not occur until the ions have moved to the ends of the strip, prolonging the time required for IEF. Water movement can also result, causing one end of the strip to dry out and the other to swell. Salt in the IPG strip can result in large regions at either end of the IPG strip where proteins do not focus (seen as horizontal streaking in the final result) The salt concentration in the rehydration solution <u>must</u> be lower than 10mM	Desalting can be performed by: <ul style="list-style-type: none"> • Dialysis • Spin dialysis • Gel filtration • Precipitation/resuspension. <p>Dialysis is very effective method for salt removal, resulting in minimal sample loss; however, the process is time consuming and requires large volumes of solution.</p> <p>Spin Dialysis is quicker, but protein adsorption onto the dialysis membrane may be a problem. Spin dialysis should be applied to samples prior to addition of urea and detergent.</p> <p>Gel filtration can be acceptable but often results in protein losses.</p> <p>Precipitation/resuspension is an effective means of removing salts and other contaminants, but can also result in losses.</p>
Endogenous small ionic molecules (Nucleotides, metabolites, phospholipids, etc.)	Endogenous small ionic molecules are present in any cell lysate. These substances are often negatively charged and can result in poor focusing toward the anode.	TCA/acetone precipitation is particularly effective at removing this sort of contaminant. Other desalting techniques may be applied (see above).
Ionic detergent	Ionic detergent (Usually SDS) is often used during protein extraction and solubilization, but strongly interferes with IEF . SDS forms complexes with proteins, and the resulting negatively charged complex will not properly focus unless the SDS is removed.	SDS containing samples must be diluted to a final concentration of 0.25% SDS or less. This may result in application of less protein per IEF run. Acetone precipitation of the protein will only partially remove SDS. Protein precipitation is more complete at -20 degrees.

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Nucleic Acids (DNA, RNA)	<p>Nucleic acids increase sample viscosity and cause background smears.</p> <p>High-molecular-weight nucleic acids can clog gel pores.</p> <p>Nucleic acids can bind to proteins through electrostatic interaction, preventing focusing.</p> <p>If the separated sample proteins are visualized by silver staining, nucleic acids present in the gel will also stain, resulting in a background smear on the 2-D gel.</p>	<p>Treat samples rich in nucleic acids with a protease-free Dnase/Rnase mixture to reduce the nucleic acids to mono- and oligonucleotides. This is often done by adding 0.1 X volume of a solution containing 1 mg/ml Dnase I, 0.25mg/ml Rnase A, and 50mM MgCl₂ followed by incubation on ice.</p> <p><i>Note:</i> The Dnase and Rnase proteins may appear on the 2-D map.</p> <p>Ultracentrifugation can be used to remove large nucleic acids; however, this technique may also remove high-molecular-weight proteins from the sample.</p> <p>When using low-ionic strength extraction conditions, negatively charged nucleic acids may form complexes with positively charged proteins. High-ionic strength extraction and/or high-pH extraction may minimize these interactions. (Note that salts added during extraction must be subsequently removed; see above.)</p>
Polysaccharides	<p>Polysaccharides can clog gel pores, causing either precipitation or extended focusing times and resulting in horizontal streaking.</p> <p>Some polysaccharides contain negative charges and can complex with proteins by electrostatic interactions.</p>	<p>Precipitate the sample in TCA, ammonium sulphate, or phenol/ammonium acetate, then centrifuge.</p> <p>Ultracentrifugation will remove high-molecular-weight polysaccharides.</p> <p>Employing the same methods used for preventing protein-nucleic acid interactions may also be helpful (solubilize sample in SDS or at high pH).</p>

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Lipids	<p>Many proteins, particularly membrane proteins, are complexes with lipids. This reduces their solubility and can affect both the pI and the molecular weight.</p> <p>Lipids form complexes with detergents, reducing the effectiveness of the detergent as a protein solubilizing agent.</p> <p>When extracts of lipid-rich tissues are centrifuged, there is often a lipid layer that can be difficult to remove.</p>	<p>Strongly denaturing conditions and detergents minimize protein-lipid interactions. Excess detergent may be necessary.</p> <p>Precipitation with acetone removes some lipid.</p>
Phenolic compounds	<p>Phenolic compounds are present in many plant tissues and can modify proteins through an enzyme-catalyzed oxidative reaction</p>	<p>Prevent phenolic oxidation by employing reductants during tissue extraction (e.g., DTT BME)</p> <p>Rapidly separate proteins from phenolic compounds by precipitation techniques.</p> <p>Inactivate polyphenol oxidase with inhibitors such as Diethyldithiocarbamic acid or thiourea.</p> <p>Remove phenolic compounds by adsorption to polyvinylpyrrolidone (PVP) or polyvinylpolypyrrolidone (PVPP).</p>
Insoluble material	<p>Insoluble material in the sample can clog gel pores and result in poor focusing.</p> <p>Insoluble material is particularly problematic when the sample is applied using sample cups: It can prevent protein entry into the IPG strip.</p>	<p>Samples should always be clarified by centrifugation prior to submission.</p>