# Protein electrophoresis and troubleshooting

By

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# Troubleshooting Guide

| | Problem | | Cause | | Solution |
|---|---|---|---|---|---|
| 1. | "Smile effect" - band pattern curves upward at both sides of the gel. | a. | Center of the gel running hotter than either end. | a. | Buffer not mixed well or buffer in upper chamber too concentrated. Remake buffer, insuring thorough mixing, especially when diluting 5x or 10x stock. |
| | | b. | Power conditions excessive. | b. | Decrease power setting from 200 V to 150 V or fill lower chamber to within 1 cm of top of Short Plate. |
| 2. | Vertical streaking of protein. | a. | Sample overload. | a. | Dilute sample, selectively remove predominant protein in the sample, or reduce voltage by about 25% to minimize streaking. |
| | | b. | Sample precipitation. | b. | Centrifuge sample before addition of SDS sample buffers, or decrease % T of resolving gel.* |
| | | | | c. | The ratio of SDS to protein should be enough to coat each protein molecule with SDS, generally 1.4:1. It may require more SDS for some membrane protein samples. For example, SDS in sample can be increased to 4% and/or in running buffer increased to 0.4%. |

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| 3. | Lateral band spreading. | a. | Diffusion out of the wells prior to turning on the current | a. | Minimize the time between sample application and power start up. |
| | | b. | Ionic strength of sample lower than that of gel. | b. | Use same buffer in sample as in gel or stacking gel. |
| 4. | Skewed or distorted bands. | a. | Poor polymerization around sample wells. | a. | Degas stacking gel solution thoroughly prior to casting; increase ammonium persulfate and TEMED concentrations by 25%; for stacking gel or low%T, leave APS the same and double the TEMED concentration. |
| | | b. | Salts in sample. | b. | Remove salts by dialysis, desalting column, Micro Bio-Spin columns, etc. |
| | | c. | Uneven gel interface. | c. | Decrease the polymerization rate. Overlay gels very carefully. |
| 5. | Lanes constricted at bottom of gel. | a. | Ionic strength of sample higher than that of surrounding gel. | a. | Desalt sample and neighboring samples. |
| 6. | Run taking unusually long time. | a. | Running buffer too concentrated. | a. | Check buffer protocol, dilute if necessary. |
| | | b. | Excessive salt in sample. | b. | Desalt sample. |

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| 7. | Run too fast, poor resolution. | a. Running or reservoir buffer too dilute. | a. Check buffer protocol, concentrate if necessary. |
| | | b. Voltage too high. | b. Decrease voltage by 25–50%. |
| 8. | Doublets observed where a single protein species is expected (SDS-PAGE) | a. A portion of the protein may have been reoxidized during the run or may not have been fully reduced prior to run. | a. Prepare fresh sample buffer solutions if over 30 days old; increase 2-mercaptoethanol concentration in the sample buffer; substitute DTT for BME. |
| 9. | Observe fewer bands than expected and one heavy band at dye front. | a. Protein(s) migrating at the dye front. | a. Increase % T of resolving gel.* |
| | | b. Protein degradation. | b. Use protease inhibitors, *e.g.* PMSF, etc. |
| 10. | Upper buffer chamber leaks. | a. Upper buffer chamber over filled. | a Keep level of buffer below the top of the Spacer Plates. |
| | | b. Improper assembly. | b. Be sure u-shaped electrode core gasket is clean, free of cuts, and lubricated with buffer. Be sure Short Plate is *under* the notch on the gasket, not on top of it and press down on electrode assembly when closing cams of the frame. |

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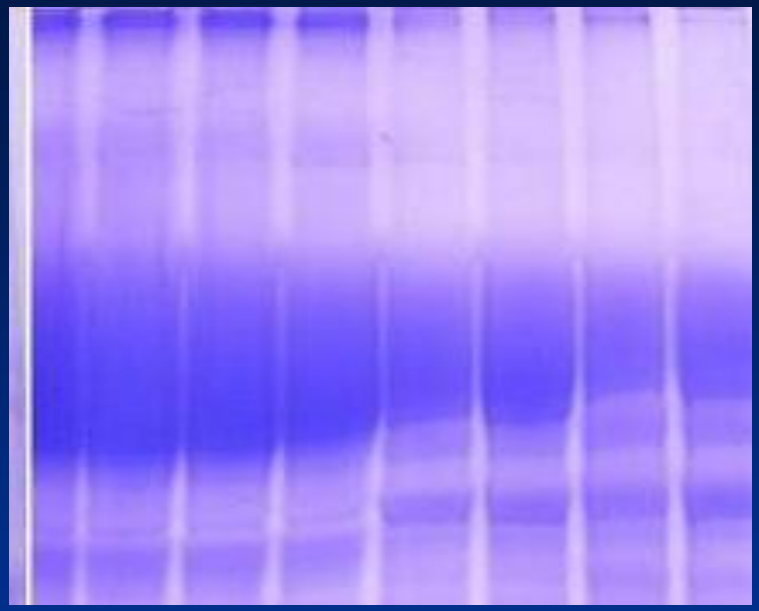
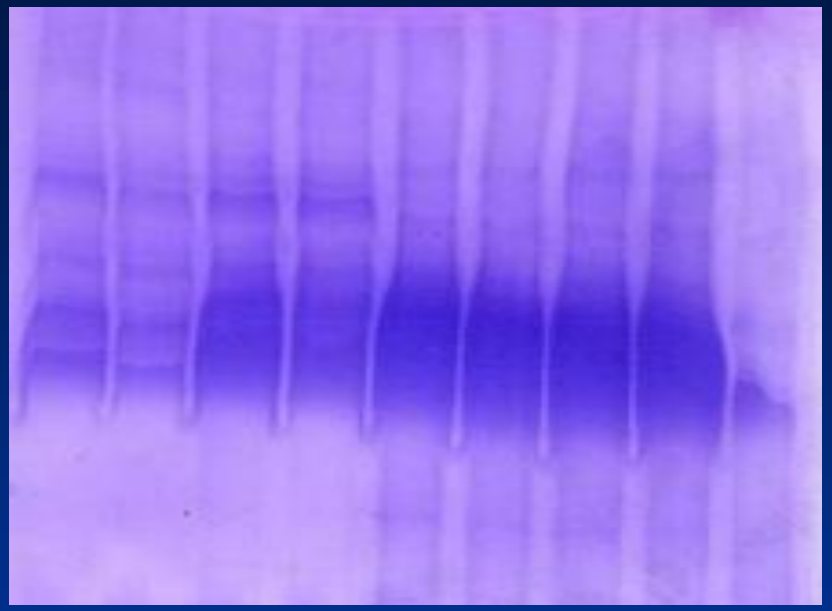
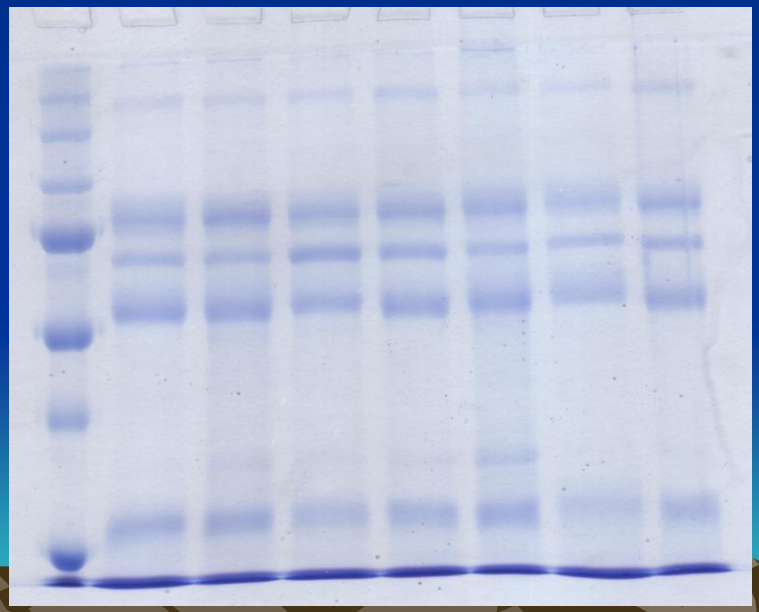
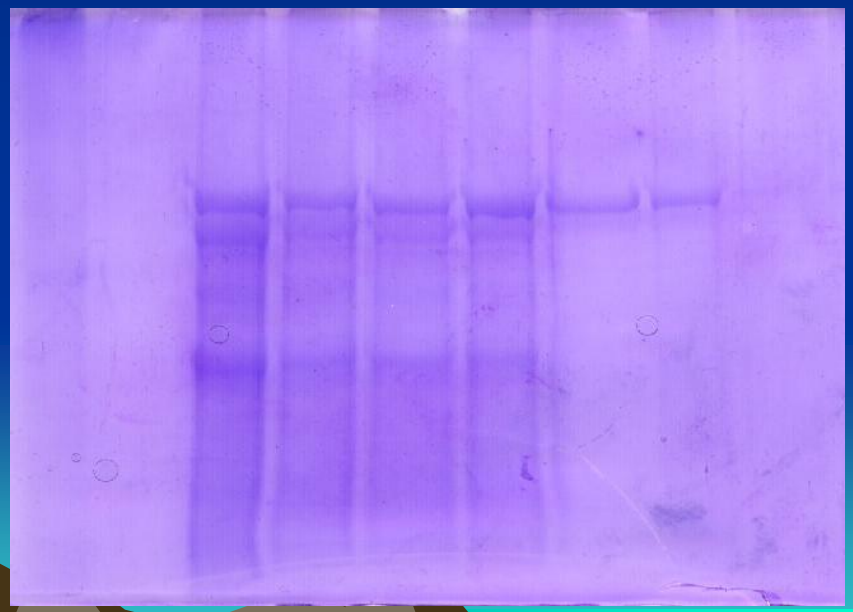
| 11. | Leaking during gel casting. | a. | Chipped glass plates. | a. | Insure glass plates are free of flaws. |
| | | b. | Spacer Plate and Short Plate not level. | b. | Insure cassette is aligned correctly. |
| | | c. | Casting Stand gasket is flawed or worn out. | c. | Replace casting stand gaskets. |
| 12. | Poor end well formation. | a. | Incorrect catalyst concentration. | a. | Prepare fresh catalyst solution, or increase catalyst concentration of stacking gel to 0.06% APS and 0.12% TEMED. |
| | | b. | Monomer solution not degassed. Oxygen inhibits polymerization. | b. | Degas monomer solution immediately prior to casting the stacking gel. |
| 13. | Webbing/excess acrylamide behind the comb. | a. | Incorrect catalyst concentration. | a. | Prepare fresh catalyst solution, or increase catalyst concentration of stacking gel to 0.06% APS and 0.12% TEMED. |
| 14. | The pressure cams on the casting frame are difficult to close or make a noise when closed. | a. | A build up of a powder residue at the pivot point of the pressure cams. | a. | Rinse or wipe off the powder residue before each use. |










Questions