PROTEIN GEL ELECTROPHORESIS TIPS AND TROUBLESHOOTING GUIDE

TIPS FOR SUCCESSFUL GEL ELECTROPHORESIS

1. Use high quality acrylamide and bis.

2. Typical Laemmli sodium dodecyl sulphate (SDS-PAGE) systems include SDS in both the gel and running buffer (1). However, it was found that molecular weight determinations could be accurately performed with gels lacking SDS if both the running buffer and sample buffer contain SDS. Since excess SDS forms micelles which can interfere with accurate Rf (relative mobility) determinations it may be advantageous to limit the amount of SDS in the gel. Also, single % gels lacking SDS could be stored for longer periods than normal gels. If the gel is enclosed in a sealed bag containing tissue paper soaked in 0.02% sodium azide it could last for up to 1 month.

When working with large sample volumes (30 microliter) the SDS concentration should be limited to prevent excess micelle formation which results in diminish resolution. SDS concentration should not exceed 200 microgram/30 microliter sample. Also it has been found that high concentrations of SDS may interfere with Coomassie blue staining times. SDS grade is of utmost importance. We have found that a protein stained background along individual gel tracts with indistinct or slightly distinct protein bands are indicative of old or poor quality SDS. When fresh high quality SDS was used with the same sample sharp protein bands were observed.

3. The stacking gel length should be 1 cm from the well bottom to the top of the separating gel for proper stacking of the protein sample.

4. Band resolution could be improved by doubling the salt concentration in stacking and separating gels, but the gel must be run at lower voltages.

5. To avoid edge effects, add 1x sample buffer to unused wells.

6. If electrophoresis is carried out at low temperatures use lithium dodecyl sulfate (LiDS) instead of SDS. LiDS does not precipitate at low temperatures.

7. During protein sample treatment the sample should be mixed by vortexing before and after the heating step for best resolution.

8. Add 0.1mM thioglycolic acid to upper gel buffer if proteins will be subjected to sequence analysis.

9. When doing protein renaturation or sequencing applications, leave gel for at least 5 hours post-polymerization to allow the ammonium persulfate and the TEMED to react with the gel components which reduces their chance of reacting with the amino-terminal end of the peptide.

10. If the sample is too dilute add trichloroacetic acid (TCA) to 10% (w/v) and incubate for 5 minutes at 4°C. Centrifuge and wash pellet with cold acetone. Resuspend pellet in volume desired.

11. If salt concentration of sample is high concentrate protein as in number 10 and resuspend in appropriate buffer. High salt concentrations causes gel artifacts.

12. Although a constant power condition is still used by some scientists for electrophoresis, Hames (2) found that only constant voltage gives constant protein mobility during electrophoresis.

13. Centrifuge all samples in a microfuge tube at 12,000 g for 2-5 mins prior to loading (to remove any aggregates).

14. Never overfill wells. This could lead to artifacts.

15. Always check the protein concentration before loading. Mini-gel wells should not contain more than 150 microgram of protein even for complex mixtures of proteins.

16. Prepared samples (i.e. those which were boiled in sample buffer) could be aliquoted and stored at -20°C for 3-4 weeks or at 4°C for at least a week. Repeated freeze-thawing results in protein degradation. Before using these samples they should be warmed at 37°C for a few minutes to redissolve SDS which precipitates out of solution.

17. For the best resolution of proteins, two-dimensional gel electrophoresis (3) should be utilized.

18. A proper record should be kept for every gel and possibly a gel electrophoresis record made up similar to the one shown in the Appendix. Assign each gel a gel number.

19. Mild cleaning solution**. A** 1% SDS and 1% oxalic acid (left for an overnight soak) cleans scale, rust, fingerprints and traces of grease from gel plates. Wash plates thoroughly before soaking. SDS gradually hydrolyzes; the solution becomes turbid in about six months.

20. **Important- Make sure Tris base is used and not Tris-HCl for electrode and gel buffers.**

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PRECAUTIONS NEEDED

1. Wear gloves at all times when using acrylamide. Acrylamide is a neurotoxin. Even after polymerization some unpolymerized acrylamide may be present. If the skin should come into contact with acrylamide solution or powder, wash immediately with soap and copious water.

2. The high electrical power used in gel electrophoresis is very dangerous as such one should never disconnect the electrodes before first turning off the power supply.

TROUBLESHOOTING GUIDE

1. Poor resolution

a) Sample volume too large. Concentrate samples.

b) Excess micelle formation. Do not exceed 200 microgram SDS/30 microliter sample.

c) Gel is old. The buffer in the gel degrades over time and depending on the type of gel could last from as little as 1 week to more than 1 year. Use newly made gels or use precast gels within their expiry dates.

d) Refer to numbers 3,8-11 and 13.

2. Run taking unusually long time

a) Buffers too concentrated. Check buffer protocol; dilute buffer if necessary.

b) Current too low. Increase voltage by 25-50%.

3. Run too fast, poor resolution

a) Buffers too dilute. Check buffer protocol; concentrate buffer if necessary.

b) Current too high. Decrease voltage by 25-50%.

4. More bands than expected observed for a purified protein

a) Proteolysis. Minimize the time between sample preparation and electrophoresis.

b) Sample partially oxidized or not fully reduced. Increase ß-mercaptoethanol or DTT concentration in the sample buffer. Heat sample in SDS sample buffer 3-4 min. at 98^{o}C to improve dissociation of protein subunits.

5. Fewer bands than expected with a heavy band at the dye front

a) Gel percentage is too low for the molecular weight range of the protein sample. Use a higher percentage acrylamide gel (increase % T in resolving gel).

6. Doublets observed where a single protein band is expected on SDS-PAGE

a) A portion of the protein sample may have re-oxidised during the run, or may not have been fully reduced prior to run. Prepare fresh sample solution using fresh β-mercaptoethanol or dithiothrietol (DTT). Increase β-mercaptoethanol or DTT concentration in the sample buffer.

b) Uneven heating of the gel. Turn down voltage or current setting by 25-50%. Fill the tank / lower buffer chamber to its maximum level. Pre-chill the running buffer.

c) Over heating of the gel. This can occur in large gels. Conduct electrophoresis in a cold room or use direct cooling if your apparatus allows the recirculation of cold tap water in the cooling core. Pre-chill the running buffer.

7. Artifact band observed at approx. 67kDa in reduced samples, especially with silver staining

a) Excess reducing agent (β-mercaptoethanol). The addition of iodoacetamide to the equilibration buffer just before applying the sample to the gel has been shown to eliminate these artifact bands.

b) Skin protein contaminants. Use new electrophoretic solutions and wear gloves when handling and loading the gel. More common when highly sensitive stains are used.

8. Skewed or distorted bands

a) Poor polymerization around sample wells. Increase ammonium persulfate and TEMED concentrations by 25%.

b) High salt concentration in sample. Remove by dialysis, Sephadex G-25 or any other desalting column or by Amicon concentrators.

c) Excessive pressure applied to the gel plates when the gel is placed into the clamp assembly. Do not overtighten the screws on the clamp assembly.

d) Uneven gel interface. Use a spirit level to make sure the gel apparatus is even. Overlay separating gel with water carefully.

e) Uneven heating of the gel. Either use a cooled apparatus or reduce the current at which electrophoresis is performed.

f) Insoluble material in the gel or inconsistent pore size throughout gel. Filter gel reagents before use and ensure that the gel mixture is well mixed and degassed before pouring the gel.

g) Proteins with several cys residues can reoxidize during electrophoresis creating "smears". Large thiol rich proteins (such as serum albumin) are prone to reoxidation. Reducing with DTT (see advice) and alkylating with iodoacetamide or N-ethylmaleimide prevents this.

9. Lateral band spreading

a) Diffusion of sample out of the wells before the power was turned on. Minimize the time between sample application and power start-up.

b) Diffusion during migration through the stacking gel. Increase voltage by 25% during stacking gel or increase %T of stacking gel by 1%.

c) Low salt concentration in sample. Use sample buffer of the same ionic strength as the gel.

10. Vertical streaking of protein

a) Sample precipitation. Centrifuge all samples before loading wells. If problem still persists decrease %T of separating gel.

b) Sample overload. Dilute sample or reduce voltage by about 25% to minimize streaking.

11. Protein band curves upward at both sides of the gel "Smile effect".

a) Center of the gel running hotter than either ends. Decrease power setting. Check buffer protocol to ensure it is properly formulated.

b) Insufficient buffer volume. Make sure the gel wells are completely covered with buffer. Check for leaks in the upper buffer chamber that might have occured during the run.

c) Electrophoresis buffer was too warm. Use cold electrophoresis buffer.

d) Poor quality gel. Bubbles and particles in the gel could cause the smiling effect. Mix and pour all gel preparation solutions carefully to avoid formation of bubbles. If particles are visible in solutions, remove them by filtration.

12. Same protein observed in several neighboring lanes

a) Samples from one well has contaminated adjacent wells. Use a Hamilton syringe to load wells and reduce the sample volume.

b) Do not delay while loading wells. A full well left next to an empty well would eventually contaminate the empty well over time.

c) Contamination of the sample buffer. Use new sample buffer.

d) Gel cassette is held in place too tightly. Do not over-tighten the assembly of the gel with the electrophoresis device.

13. Diffuse tracking dye

a) Decomposition of sample solution and/or buffer stock solution. Prepare fresh reagents.

14. Diffuse protein bands

a) Diffusion due to slow migration. Increase voltage by 25-50%. Check buffer to ensure it was properly prepared.

b) SDS or sample buffer too old. Prepare fresh solutions.

c) Protein sample not equilibrated. Equilibrate sample to running conditions.

d) Poor quality acrylamide or by resulting in incomplete catalysis. Use electrophoresis grade reagents.

e) Problems in sample preparation. Check to make sure sample is heated to at least 90°C for 2 min before loading.

15. Inconsistent relative mobilities

a) Incomplete catalysis. Excessive TEMED or ammonium persulfate. TEMED and ammonium persulfate should be 0.05%.

b) The constituents of the gel may vary in quality from batch to batch or with age. Use one batch of a chemical for as long as possible. Replace aged stock solutions and reagents.

c) The protein amounts loaded differ greatly. Do not overload the gel. Keep the loadings roughly similar in size.

d) Excess salt concentration in the sample. Remove excess salts by gel filtration or dialysis.

e) Improper sample preparation.
To ensure proper migration during electrophoresis, protein samples must contain SDS, dithiothreitol (DTT) or 2-mercaptoethanol and must be heated prior to loading.

f) Migration discrepancies due to protein modifications. Protein modifications including phosphorylation and glycosylation, may alter protein mobility.

16. Aggregation of proteins

a) Some samples aggregate on boiling. Treat sample at lower temperature (60°C).

b) Formation of disulfide bonds between protein in a complex mixture because of insufficient reducing agent. Prepare new sample buffer.

17. Band Streaking

a) High salt concentration. Precipitate with TCA and resuspend in lower salt buffer, use desalting columns or dialyze sample.

b) Sample too concentrated. Load a maximum of 10 μg per protein or 100 μg per protein extract.

c) Not enough SDS. Dilute the sample with more SDS solution.

d) High Voltage. Best typical conditions for electrophoresis are at 10-15 V/cm.

e) Protein aggregation. Add 4-8 M urea to the sample.

18. Heavily Stained band at gel origin

a) Gel concentration too high. Use lower gel concentration.

b) Aggregation of protein sample prior to electrophoresis. Refer to no. 16.

c) No SDS in wells. The protein precipitates as it enters the gel.

19. Some samples run as "wide lanes"

a) High salt concentration in these samples. Precipitate and resuspend or Dialyze these samples.

20. Sample floats out of sample well or fails to form a layer at the bottom of the well.

a) Running buffer incorrect. Usually the running buffer is too concentrated. Insure of thorough mixing especially when diluting stock solutions.

b) Glycerol was omitted or is insufficient in sample buffer. Add more glycerol to the sample buffer. Some researchers use sucrose instead of glycerol and insufficient sucrose would also cause this effect.

21. Aberrant Molecular Weight determination High molecular weight

a) Proteins with >10% carbohydrate bind less SDS. Check for carbohydrate.

b) Hydrophilic proteins bind less SDS.

c) Potassium or divalent cations present in sample precipitate SDS. Precipitate sample and resuspend in different buffer.

22. Aberrant Molecular Weight determination Lower Molecular Weight

a) Hydrophobic proteins bind more SDS.

b) Incomplete disulfide bond dissociation because of insufficient reducing agent. Prepare new sample buffer and samples.

23. Gelling time too long

a) Too little ammonium persulfate or TEMED. Increase both by 50%.

b) Temperature too low. Cast at room temperature.

c) Old ammonium persulfate and TEMED. Use fresh ammonium persulfate and new TEMED.

d) Poor quality acrylamide or bis. Use electrophoresis grade acrylamide and bis.

e) High concentration of thiol reagents. High concentrations of thiol reagent inhibit polymerization. Use less thiol reagent.

f) Omission of a reagent from the gel mixture. Have a list of all the reagents required and thick off reagent when utilized.

g) Incorrect concentrations of prepared reagents. Check protocol. Make up new reagents.

h) Degassing the acrylamide solution leads to a more rapid polymerization. However I have found that this step is cumbersome and unnecessary for most applications.

24. Gel too soft

a) Poor quality acrylamide or bis. Use electrophoresis grade acrylamide and bis.

b) Acrylamide is too old. Acrylamide becomes partially hydrolyzed as it ages.

c) Too little crosslinker. Make sure proper %C.

25. Gel does not polymerize

a) Temperature too low. Cast at room temperature.

b) Too little ammonium persulfate or TEMED. Increase both by 50%.

c) Poor quality acrylamide or bis. Use electrophoresis grade acrylamide and bis.

d) Ammonium persulfate or TEMED are old. Use fresh ammonium persulfate and new TEMED.

26. Gel is gooey and sticks to glass.

a) Crosslinker is missing or is too little.

27. Swirls in gel

a) Excessive catalysis. Gel polymerizes in less than 15 minutes. Reduce ammonium persulfate and TEMED by 25% each.

b) Gel inhibition. Polymerization time should be >1 hour. Increase ammonium persulfate and TEMED by 50%.

28. Gel brittle

a) Too much crosslinker. Check protocol for %C. Recheck solution and weights.

29. Gel turns white

a) Bis concentration too high. Recheck solution or weights used.

30. Upper buffer chamber leaks

a) Upper buffer chamber over filled.

b) Improper assembly of the upper buffer chamber. Check assembly.

31. Leaking during gel casting

a) Chipped glass plates. Check glass plates for flaws. If minor flaws at bottom of glass plate parafilm could be used to properly seal the glass plates.

b) Improper alignment of gel plates. Check to ensure that the spacers and plate bottoms are flush.

32. Gel cracking during polymerization

a) Excess heat generation. Use cooled reagents.

33. Samples do not sink to bottom of well

a) Insufficient glycerol in the sample buffer. Recheck protocol.

b) Combs removed before stacking gel properly polymerized. Let stacking gel polymerized for 30 minutes before removing combs.

34. Sample preparation yellow in colour

a) Solution is acidic. Add NaOH until the solution turn blue.

b) Too little bromophenol blue in sample buffer. Add bromophenol blue (from a concentrate in double distilled water) to sample or make fresh sample buffer with bromophenol blue concentration of 0.005-0.01%.

35. Detachment of slab gels from glass plates during gel electrophoresis.

a) Inadequately cleaned glass plates. After being rinsed with distilled water, they must drain cleanly without water spots.

36. Base of sample well appears to be dragged downwards in the direction of electrophoresis.

a) Could be due to trapping of high molecular mass, high charged species at the gel surface. This is very common when a high concentration of nucleic acid is present in the sample.

b) Check sample for nucleic acid and remove if present in significant quantities in the sample.

37. Poor Sample Wells

a) Distorted or broken wells are formed when the comb is not removed carefully. Comb should be removed only in a vertical manner.

b) When stacking gel resists the removal of the comb use a gel of lower % T.

c) When the wells contain a loose webbing of polyacrylamide it is likely that the comb fits loosely or the gelling rate is too fast. Replace the comb with a tighter fitting one and check the amount of TEMED and ammonium persulfate being added.

38. Gel cracking during electrophoresis

a) The running conditions are too warm. This is especially common with high percentage gels.

39. Bands on part of the slab do not move down the gel

a) This is usually due to air bubbles between the plates underneath the affected lanes. Make sure no bubbles are present in the gel when pouring.

40. Formation of a sticky top on the gel

a) Penetration of the gel by butan-2-ol. Overlay the gel with butan-2-ol without mixing them. Do not leave butanol-2-ol to stand overnight on a polymerized gel or use water instead of butan-2-ol.

41. Protein bands are not sufficiently resolved

a) Insufficient electrophoresis. Prolong the run.

b) The separating gel's pore size is incorrect for the proteins that need to be separated. Alter the %T and/or %C of the separating gel appropriately.

42. Protein bands are not of uniform thickness

a) The sample was loaded unevenly. Check that the sample well bottoms are straight and horizontal.

43. Faint or missing protein bands

a) Proteins were not fixed in the gel. Either use a gel fixing solution (such as 25% isopropanol, 8% acetic acid) or a stain which also fixes the proteins.

b) Small peptides (<5 kDa) did not fix in the gel. Fix the gel with 5% glutaraldehyde. Rinse the gel well with water before staining.

c) Proteins are degraded.

d) Protein ran off the gel. Use a higher concentration gel. Use a 4-20% gel if the size of the protein is unknown.

e) Excessive electrophoresis run time. Stop the electrophoresis run as soon as the tracking dye front reaches the bottom of the gel. In low percentage gels (4-8%), small proteins (10-15 kDa) migrate with the tracking dye during electrophoresis and may be not visible. Use high percentage or gradient gels to resolve low molecular weight proteins.

f) Amount of protein loaded was below the detection limit of the staining method used.

g) Failed to load the well.

h) Connected the electrodes the wrong wayresulting in the gel running the wrong way (always check the tracking dye as it begins to migrate).

44. Protein precipitation in the sample

a) Most likely SDS precipitation due to the presence of potassium or guanidine salts in the sample.

45. Protein precipitation in the well

a) High content of hydrophobic proteins in sample. Add 4-8 M urea to the sample will prevent this precipitation.

46. Diffuse tracking dye.

a) Bromophenol Blue concentration is too high. Make fresh sample buffer with Bromophenol Blue concentration of 0.005-0.01%.

b) Sample Buffer solutions are old. Prepare fresh sample buffer. Maximum shelf life of the sample buffer depends on how it is stores- 30 days at 4°C; 6 months at -20°C; > 1 year at -80°C;

47. Bromophenol blue doesn't sharpen into a concentrated zone in the stacking gel.

a) High salt concentration in sample. Dialyze or precipitate and resuspend the protein in lower slat solutions.

48. Protein bands collect near the bottom of the gel

a) Gel pore size is too large. Choose a higher concentration of acrylamide (increase % of resolving gel).

b) Sample protein degradation. Minimize the time between sample preparation and electrophoresis. When running denaturing gels, make sure the samples are being heated for 3 min. at >95°C in the presence of agents such as SDS. Store on ice after heating. Store sample to be frozen in aliquots to prevent repeated freezing and thawing (store at –20°C to –80°C). Store sample on ice before it is denatured and use protease inhibitors like PMSF to prevent proteolytic degradation of sample.

49. Power supply current reading on power supply is zero when swiched on.

a) Connection to power supply is not complete. Check connections between lid of apparatus and the electrode assembly jacks. Check connections between lid of apparatus and power supply.

b) Insufficient buffer level. Make sure the upper buffer chamber doesn't leak. Verify that the assembly was properly done.

c) Ionic strength of the buffer is too high.

d) Power supply is operating at a current close to its limit. Use a power supply with higher limits.

e) Broken electrode. Check wire electrodes to make sure they are not broken.

f) Blown fuse on power supply. Check fuse and replace, if necessary.

50. Protein bands collect near the top of the gel when the dye front has reached the bottom of the resolving gel.

a) Gel pore size is too small. Choose a lower concentration of acrylamide (decrease % of resolving gel).

b) Presence of precipitate/aggregate in sample prior to electrophoresis. Decrease the temperature at which the sample is prepared to 70° C or less and limit exposure to heat to 1-2 min. Store sample on ice before it is denatured and minimize the time between sample preparation and electrophoresis. Store sample to be frozen in aliquots to prevent repeated freezing and thawing (store at –20°C to –80°C). Use protease inhibitors like PMSF to prevent proteolytic degradation of sample.

STAINING AND DESTAINING

51. Non-specific Coomassie blue staining

a) Decomposition of undissolved dye. Filter dye solution.

52. Protein bands not seen properly

a) Coomassie stain not sensitive enough. Gel can be rinsed and subsequently silver stained.

b) Not enough protein loaded onto the gel. For Coomassie blue stained gels each protein band needs at least 0.5 microgram of protein to be sufficiently stained.

c) Volume of Coomassie Blue too little. Increase the volume of staining solution to dilute out the SDS present in the gel.

d) Use a more concentrated staining solution and longer staining time.

e) Check the concentration of methanol (which strips SDS from the protein) used in the staining solution. Increase the methanol concentration if necessary.

53. Uneven staining of gels

a) Incomplete penetration of the dye. Leave gel in stain for a longer time.

b) Not enough dye.

c) Agitation was insufficient. Agitate when staining.

d) High concentration of SDS may interfere with coomassie blue staining.

54. Metallic sheen on gels after staining with Coomassie Blue

a) Solvent was allowed to evaporate causing the dye to dry on the gel.

b) Precipitated Coomassie Blue R 250. Rinse the gel for 15 seconds in methanol and immediately return to water or destain.

55. A thin layer of Coomassie Blue on gel surface after destaining

a) Can easily be removed by a quick rinse in 50% methanol or by gently swabbing the gel surface with destain-soaked tissue paper.

56. Blotches near gel borders and over gel

a) Could be due to gel handling without gloves

57. Continuous stained region from the gel origin to near the buffer front.

a) Contamination of sample buffer. Make fresh sample buffer.

58. High silver staining background

a) Acrylic acid contamination in the acrylamide and/or bis-acrylamide. The highest quality reagents should be used.

b) Backgroung staining that is associated with silver detection of proteins in polyacrylamide gels has been shown to be due mostly to the amide groups in the crosslinker bisacrylamide (4). If the background staining is a serious problem use diacrylylpiperazine as the crosslinker. This crosslinker provides improved electrophoretic separation of proteins together with reduced background.

59. Silver color development is very slow.

a) Add more formaldehyde to developer or use fresh formaldehyde (Formaldehyde oxidizes and is light sensitive).

60. Diffuse background smear after silver staining.

a) Insufficient washing steps during silver staining procedure. Increase number of washing steps.

61. Uneven staining after silver staining.

a) Volume of stain too small. Increase volume of stain.

b) Insufficient agitation. Staining requires sufficient agitation.

62. Stained bands become decolourized

a) Over-destained gel. Restain the gel and reduce the destaining time.

63. Stained bands lost after destaining

a) Gel left too long in destain. Restain the gel and reduce the destaining time.
b) Improper fixative. Use a different fixative.

64. Low or high molecular weight proteins missing
a) Low molecular weight proteins not adequately fixed after SDS-PAGE.; Use 20% TCA or glutaraldehyde as fixative instead of 40% alcohol and 10% acetic acid.

DRYING GELS

65. Cracking of gels during drying under vacuum

a) Vacuum released before gel is properly dry.

b) Slab gels > 1.5mm are being used.

c) Gel was allowed to swell before drying.

66. Cracking of gels during drying at room temperature

a) Air bubble was present between the cellophane sheets.

b) Excess water was present between the cellophane sheets.

c) Depending on the % acrylamide in the gel the gel may require incubation in 10% glycerol, 8% acetic acid for 30 minutes before drying.

Electrophoresis Links

American Electrophoresis Society
(known as The Electrophoresis Society): http://www.aesociety.org/
British Society for Proteome Research (formely the British Electrophoresis Society):
http://www.bspr.org/
Swiss Proteomics Society: http://www.swissproteomicsociety.org/
Japanese Electrophoresis Society (JES): http://wwwsoc.nii.ac.jp/jes1950/index-e.html
French Electrophoresis Society: http://sfeap.free.fr/
German Electrophoresis Society: http://www.dgpf.org/dgpf-set.htm
SWISS-2DPAGE Protein Database: http://www.expasy.ch/ch2d/
