TROUBLESHOOTING SODIUM DODECYL SULFATE-POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE)

Troubleshooting *This troubleshooting document gives the problem, possible cause and suggested solution for problems during the SDS-PAGE application:*

Problem: Weak of missing protein bands

The protein/antigen quantity is below the detection level of the stain	Increase the sample concentration. Use a more sensitive stain.
The proteins are not fixed in the gel	Use a stain which will fix the proteins. Use a gel fixing solution.
Proteins have ran off the gel	Use a SDS-PAGE gel with a higher % acrylamide.
Proteins are degraded	Make sure there is no protease contamination. Ensure the samples did not freeze-thaw.
The small-peptides (<4 kDa) did not fix in the gel	Fix the gel with 5% glutaraldehyde. Rinse the gel well with water before staining.

Problem: Poor band resolution

The concentration of the protein is too high	Decrease protein concentration.
Sample volume is too large	Increase protein concentration.
Gel concentration is not correct	If the size of the protein is unknown, use a 4%-20% gradient gel.
The gel is too old	Order fresh precast gels or cast a fresh gel.
There is excess micelle formation	Do not exceed 200 µg SDS/30 µl sample.
The run is too fast because buffers are too diluted	Increase the buffer concentration.
The run is too fast because the current is too high	Decrease the voltage by 25-50%.
The protein bands are not sufficiently resolved	Insufficient electrophoresis has taken place, prolong the run. The gels pore size is not correct for the proteins that need to be separated. Use a gel with a different % acrylamide.

Problem: Band smearing

The voltage used is too high	Decrease the voltage by 25-50%.
The concentration of the protein is too high	Reduce the amount of protein loaded on the gel.
The salt concentration is too high	Dialyze the sample, precipitate the protein with trichloroacetic acid (TCA) or use a desalting column.

Problem: Bands are skewed or disorted

The salt concentration is too high	Dialyze the sample, precipitate the protein with trichloroacetic acid (TCA) or use a desalting column.
The polymerization around the sample wells is poor	Increase the amount of ammonium persulfate and TEMED.
Excessive pressure has been applied to the gel plates when the gel is placed into the clamp assembly	The screws on the clamp should not be too tight.
Material in the gel is insoluble or the pore size are inconsistent throughout the gel	Filter the gel reagents, ensure that the gel mixture is well mixed and degassed before pouring the gel.
The gel interface is uneven	With a spirit the gel apparatus can be made even. Overlay the separating gel carefully with water.
Heating of the gel is uneven	Use a cooled apparatus or reduce the current.

Problem: Running defects and gel casting

Time that the gel polymerizes is too long	Increase ammonium persulfate or TEMED or use fresh ammonium persulfate and new TEMED. The temperature is too low, cast at room temperature. Quality of the acrylamide or bis is poor. The concentration of the thiol reagent is too high which inhibits

	polymerization. A more rapid polymerization can be accomplished by degassing the acrylamide solution.
The gel does not polymerize	TEMED and ammonium persulfate are left out of the gel mixture. Increase ammonium persulfate or TEMED. Use fresh ammonium persulfate and new TEMED. The temperature is too low, cast at room temperature. Quality of the acrylamide or bis is poor.
The gel is too soft	Quality of the acrylamide or bis is poor. There is too little crosslinker, increase the amount of bisacrylamide.
The gel turns white	The bis concentration is too high, recheck the amount that is used.
The gel is brittle	There is too much crosslinker, decrease the amount of bisacrylamide.
The upper buffer chamber leaks	The upper buffer chamber is overfilled. Assemble the casting apparatus correctly.
There is leaking during gel casting	Glass plates might be chipped. The glass plate might not be aligned well. Use Vaseline at the spacers.
The gel cracked during polymerization	There is an excess of heat generation, use cooled reagents.
The samples do not sink to the bottom of the well	There is insufficient glycerol in the sample buffer. Comb is removed before the stacking gel has properly been polymerized. Let the gel polymerize 30 minutes before removing the combs.
SDS is not added to sample	There are no net negative charges on proteins, the protein will not move down the gel, ensure SDS has been added to the sample.
Sample preparation is yellow in color	The solution is acidic, add NaOH until the solution turns blue. There is too little bromophenol blue in the sample buffer.
The run takes an unusual long time	The buffers are too concentrated, dilute the buffer if necessary. The current is too low, increase the voltage.
Some bands do not move down the gel	There might be air bubbles in the gel under the affected lanes. Make sure there are no air bubbles in the gel.
The gel detaches from the glass plates	The glass plates are not clean.
Gel has cracked during electrophoresis	The running conditions are too warm, it happens faster with high percentage gels.
The sample wells are poor	When the comb is not removed carefully , the wells can be broken or distorted. When stacking gel resists the removal of the comb, use a gel with lower % acrylamide.
The base of the sample well appears to be dragged downwards	High molecular mass might be trapped. Check the sample for nucleic acid and remove them if they are in significant quantities.

Problem: The protein has aggregated

Proteins have aggregated	The salt concentration is too high, precipitate and resuspend in lower salt buffer. Disulfide bonds are formed by the proteins in the complex mixture because of insufficient reducing agent. Prepare new sample buffer. Some samples aggregate on boiling, threat them at a lower temperature (60°C).
There is protein precipitation in the well	The proteins are hydrophobic, add 4-8 M urea to the sample.
Band streaking	The sample is too concentrated or there is not enough SDS. Dilute the sample with more SDS solution.

Problem: There are artifact bands

There are fewer bands than expected and there is a heavy band at the dye front	The gel percentage is too low, increase the % acrylamide in the gel.
There is lateral band spreading	Before the power was turned on the sample diffused out of the well. The time between sample application and power start up should minimized.
There is vertical streaking of	Sample precipitation. Centrifuge all the samples before they are loaded

protein	in the wells. The sample is overloaded, dilute the sample or reduce the voltage by about 25% to minimize streaking.
'Smile effect'	The center of the gel is running hotter than either ends. Decrease the power setting and check the buffers.
An approximate 67 kDa band is observed in reduced samples	This band is from the excess of the reducing agent (β-mercaptoethanol). By adding iodoacetamide to the equilibration buffer just before applying the sample to the gel artifact bands will be eliminated.
There are doublets observed where a single protein band is expected	A portion of the protein sample may have re-oxidized during the run, or may not have been fully reduced prior to the run. Prepare fresh sample solution using fresh β-mercaptoethanol or dithiothrietol (DTT), or increase the amount in the sample buffer.

Helpful links / references

http://www.molecularstation.com/wiki/SDS-PAGE_protocol

http://www.omx-online.com/calculator.html

www.protocol-online.org