Weak or no Signal

Possible Causes	What can you do?
Protein not expressed in the sample used	Make sure that the protien of interest in sufficiently induced in the sample used. Fractionation might be necessary for some proteins expressed in particular organelles.
Inadequate/incomplete transfer	Be sure that the transfer is adequate, especially for high molecular weight proteins. Make sure that the transfer is complete by staining the membrane with Ponceau S solution or using a pre-stained marker as indicator.
Antibody issue	Make sure that primary antibody dilution and the incubation condition is optimal. Compatible secondary antibody should be used.
Poor activity of ECL	Prepare ECL solution freshly prior to detection.
Sodium Azide interference	Make sure that there is no Sodium Azide in the antibody dilution buffer. Wash the blot thoroughly before adding ECL.

High Background

Possible Causes	What can you do?
Antibody concentration too high	Optimize the dilution factor of primary and secondary antibody.
Insufficient washing	Make sure that the blot is washed in sufficent washing buffer. Increase washing time or the percentage of Tween-20 if necessary.
Insufficient blocking	Make sure that the blot is sufficiently blocked. Increase the percentage of skimmed milk up to 5% if necessary.
Improper blocking buffer used	For the detection of phospho-proteins, use BSA instead of milk as blocking agent.
Membrane dried out	Make sure that the membrane is moist throughout the whole process of western blot.

Smeary Staining

Possible Causes	What can you do?
Poor gel preparation	Mix gel completely before pouring. If SDS-PAGE should be run the day after preparation, make sure that the gel is prepared at RT and stored in moist chamber at 4°C.
Protein overloaded	Make sure that total amount of protein loaded into each well is between 20-50 μg.
High membrane protein concentration in samples	If membrane fraction is used, make sure that sample is sufficiently diluted before loading into SDS-PAGE.

Band Artifacts (White bands, smile effect, streaks)

Possible Causes	What can you do?
Poor gel preparation	Mix gel completely before pouring. If SDS-PAGE should be run the day after preparation, make sure that the gel is prepared at RT and store in moist chamber at 4°C.
Voltage, temperature too high, field effect	Keep the voltage and temperature low while running SDS-PAGE. If necessary, run gel in the cold room. Remove bubbles trapped at the bottom of gel to ensure even electrophoresis.
High salt concentration in samples	Make sure that the salt concentration of lysis buffer is kept between 0.15M to 0.5M.
Protein overloaded	Make sure that total amount of protein loaded into each well is between 20-50 μg.
Antibody concentration too high	Optimize the dilution factor of primary and secondary antibody.

Black Dots

Possible Causes	What can you do?
Reagent contaminated	Prepare all reagents freshly.
Blocking agent insufficiently dissolved	Make sure that the blocking agent such as milk or BSA is completely dissolved before use. Alternatively, filter the blocking solution with 0.45 μm filter before use.

Multiple bands

Possible Causes	What can you do?
Protein post-translationally modified or alternatively spliced	Check if the protein of interest is post-translationally modified or alternatively spliced and produce other isoforms.
Protein degradation	Add enough protease inhibitor to lysis buffer throughout all steps of sample preparation. Avoid frequent freeze-thawing of samples.
Protein Multimerization	Freshly add DTT or β-Mercaptoethanol to sample buffer, or adequaltely boil samples to ensure complete bond breakage between peptides.
Antibody concentration too high	Optimize the dilution factor of primary and secondary antibody.
Antibody issue	Some antibodies use common epitopes as immunogen. Blast the immunogen region of the antibody to make sure that the immunogen does not cross-react with epitopes from other proteins.
Interference from secondary antibody	While performing Immunoprecippitaiotn (IP) experiment, make sure that the secondary antibody used to detect the protein of interest is derived from a species different from that of antibody used to pull down protein. Alternatively, use a secondary antibody that recognize only the native form of IgG to detect IP protein.

Molecular Weight Different from Predicted

Possible Causes	What can you do?
Protein post-translationally modified or alternatively spliced	Check if the protein of interest is post-translationally modified or alternatively spliced and produce other isoforms.
Incomplete protein denaturation	Freshly add DTT or β-Mercaptoethanol to sample buffer, or adequaltely boil samples to ensure complete bond breakage between peptides.
Membrane protein issue	If a membrane protein is to be detected, try low temperature (~65 ° C) or avoid boiling which might cause aggregation of membrane proteins.