Five Western Blot Problems and How to Troubleshoot Them

1 Unusual or Unexpected Bands If you detect bands at positions lower than expected or are experiencing multiple bands at or below the expected position, there is possible protein degradation occurring. Try to use a newly prepared sample, keep on ice during sample preparation and make sure to add proper and freshly prepared protease inhibitors. If bands are at a higher than expected position, reheat sample to break quaternary protein structure. Alternatively, blurry or incomplete bands are usually an indication of a problem with the transfer process. Lowering transfer voltage, keeping the transfer solution cool during the transfer process, and ensuring there are no air bubbles between the gel and membrane will help resolve these issues. Also, increasing the acrylamide gel percentage may help with diffuse bands. This decreases the gels pore size and reduces diffusion of the protein.

2 No Bands If you are using a PVDF membrane, remember to activate it by soaking it into methanol prior to use. Make sure to use the appropriate lysis buffer and protocol for sample type and protein of choice. Ponceau staining is a rapid, reversible, and non-specific protein stain that can be used to check if proteins were transferred to the membrane. Check that the correct primary antibody and corresponding secondary antibody were used. Also, check the manufacturer's specification sheet to ensure the primary antibody has been validated for Western blots. Next step is to increase the concentration of antibody used or increase the amount of antigen loaded. Additionally, check all buffers for contamination and, when in doubt, make new buffers and new detection reagents. The antigen may also be masked by the blocking buffer or buffers containing azide as preservative may inhibit HRP.

3 Faint Bands Similar to the solution for no bands, increase the concentration of the antibody or increase the amount of antigen loaded. Also, if you are using non-fat dry milk as a blocking agent, try using a lower concentration or switch to BSA. Non-fat dry milk may mask the antigen. Alternatively, increasing exposure time or switching to more sensitive detection reagent may enhance the signal. Although Western blotting is a sensitive technique, more sample may need to be loaded to detect your protein of interest.

4 High Background This is typically a result of using too high of a concentration of antibody. The antibody will begin to non-specifically bind to the membrane. Increase washing time and add extra washes to decrease background signal. Lowering the exposure time may also help. There may be insufficient blocking of non-specific sites. Compare different blocking buffers. Lastly, decrease the concentration of the primary antibody to reduce non-specific binding.

5 Patchy or Uneven Spots This may be related to the transfer process and/or incomplete incubation with the antibodies. Ensure all air gaps between the gel and membrane are removed before the transfer process. Additionally, during incubation with the primary and secondary antibodies, use a rotator or shaker to evenly incubate with the antibody during the incubation periods. When using chemiluminescence make sure to eliminate static charges from your exposure chamber. Lastly, blocking agents may be binding with the antibody. Therefore, switching between nonfat dried milk and BSA may also resolve this issue.