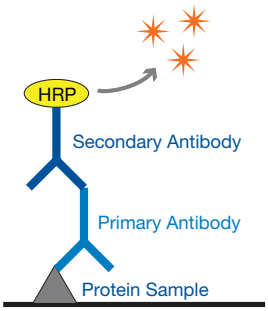
How to Improve Your Chemiluminescent Western Blots

Chemiluminescent Westerns are popular assays for assessing protein expression. In this indirect detection method, chemiluminescent substrates emit light when reacted with an antibody conjugated to an enzyme. The emitted light is captured and archived on x-ray film (traditional), or through digital imaging. Chemiluminescent Western blotting is a highly sensitive assay and can detect femtograms of protein. Although it is only semi-quantitative, it is useful for detecting the presence or absence of a protein. For example, chemiluminescence can be used to detect the induction of exogenous protein expression, to confirm and follow purification of a known protein, or for verification of antibodies during production.



Chemiluminescent Westerns can be difficult to perform. The ultimate goal is to obtain a blot with a high signal-to-noise ratio. However, chemiluminescent Westerns can be plagued with high background, either in the form of an overall background that masks the signal from the protein of interest, or as bright dots and speckles and/or splotches scattered randomly over the blot. The increase in background noise can arise from a variety of factors. It can also be difficult to obtain a strong signal from the protein of interest.

Tips for improving chemiluminescent Westerns

- Optimize the amount of protein to load on the gel. In general, 20-40 µg of total protein can be loaded without overloading the well. However, the total amount of protein should be optimized for each protein:antibody pair. Ideally, enough protein should be loaded to allow for easy capture of the signal without experiencing saturation.
- Choose the correct membrane. Nitrocellulose and PVDF membranes are commonly used, and each has advantages and disadvantages.
- Keep everything clean. Prevent background by thoroughly cleaning all equipment and trays prior to use. Only handle the gel and membrane with gloved hands. Keep trays covered during incubations.
- Ensure that all buffers are well mixed. Particulates in blocking and antibody incubation buffers will stick to the membrane and cause high background. Buffers can be filtered prior to use.
- If experiencing high background, use a larger volume of washing buffer and increase number and duration of washes.
- Try different blocking buffers. Some antibodies react with proteins in blocking buffers, causing a high background. Alternatively, some blocking buffers can mask the protein of interest, preventing detection. Non-fat dry milk and bovine serum albumin are the two most common protein blockers containing multiple proteins. Blocking buffers containing one protein can also be used. Protein-free buffers can be used when the primary antibody reacts with protein components in the buffer.
- Titrate both primary and secondary antibodies. Use a dot blot and checkerboard titration to determine the optimum primary and secondary antibody concentrations.

- Never dilute a horseradish peroxidase-conjugated secondary antibody in buffer with sodium azide. Sodium azide inhibits HRP activity.
- Use enough substrate. Make sure the blot is coated entirely with substrate to prevent local concentration differences.
- Try different substrates to increase sensitivity and signal duration. Different substrates are available with differing sensitivities for detecting high to moderate versus low abundance proteins. Different substrates also have different reaction rates and emit light for different durations of time. This can affect your ability to capture multiple exposures.
- The substrate may need to be equilibrated to room temperature before use to increase the enzyme activity.
- Use a digital imager rather than film. Digital imagers increase the linear dynamic range allowing easier detection of low abundance proteins while limiting saturation when detecting high abundance proteins.

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