

Chemiluminescent and Fluorescent Westerns: Choose the Best Assay for Your Experiment

Chemiluminescent Westerns

Chemiluminescent Western blotting is an indirect method for detecting proteins bound on a membrane. The method relies on an enzyme-substrate reaction that emits light, which is traditionally detected on x-ray film.

Chemiluminescent Westerns are widely used across a variety of laboratories, and many facilities provide the necessary darkroom and developer for documentation with x-ray film. The technique is popular because it is relatively easy to perform and can be extremely sensitive; substrates can be purchased that detect proteins in the femtogram range. Chemiluminescence is a convenient chemistry to use when the proteins being detected differ significantly in molecular weight, so as to be resolved easily through gel electrophoresis. For example, chemiluminescence is often used to detect the induction of exogenous protein expression in transfected cell lines, to confirm specific purification of a known protein, or for verification of antibodies during production.

Chemiluminescent drawbacks

Since the chemiluminescent reaction emits light in a broad spectra, emission wavelength cannot be used to differentiate signal from individual proteins. Thus, protein differentiation necessitates probing for proteins that are easily resolved during the electrophoresis process. This can become problematic when detecting co-migrating proteins, specifically when visualizing small molecular weight post-translational modifications. Additionally, normalization/loading controls must be performed by stripping and reprobing (not quantitative), running a separate blot (not a true loading control), or may be limited to using proteins that are sufficiently resolved. Furthermore, inherent in the enzyme-substrate reaction, is the varying rate of reaction kinetics; this contributes to the semi-quantitative nature of

chemiluminescence as a detection chemistry. Finally, the traditional use of x-ray film as a method of visualization suffers from dynamic range limitations that can often lead to signal saturation.

Benefits of Chemiluminescence

- High sensitivity – detect protein in the femtogram range
- Good for detecting a single protein
- Assay for presence/absence of protein
- Can use x-ray film or a digital imager

Fluorescent Westerns

Fluorescent Western blotting uses secondary antibodies directly conjugated to fluorescent dyes. Unlike chemiluminescent Westerns, which are limited by the varying kinetics of the enzyme-substrate reaction, the amount of light emitted from fluorophores is consistent, and directly proportional to the amount of protein on the membrane. This allows for a truly quantitative analysis of the proteins in question. Fluorophores can be chosen based on their specific excitation and emission spectra, thus providing another variable to differentiate proteins on the membrane (contrasted to the non-specific, broad light emission of chemiluminescence). Because of this, the major advantage of using fluorescent chemistry rather than chemiluminescence is the ability to multiplex more than one antibody per assay. This allows detection of a normalization/loading control on the same blot, as well as convenient visualization of post-translational modifications.

Fluorescent Westerns are typically visualized using a digital imager rather than x-ray film. The newer generation of imaging systems often contain sophisticated cameras that typically exhibit a broader dynamic range than film, thus not saturating the signal in question as easily. Finally, fluorescent

dyes are relatively stable; blots can be archived and imaged months after the initial experiment as long as precautions are taken to avoid photo-bleaching of the fluorophores. Because of the reasons presented, fluorescent Westerns are experiencing a rapid growth trend in the lab. For instance, the prevalence of multiplex [*fluorescent*] Westerns appearing in the scientific literature has seen an estimated 5-fold increase from 2000 to 2012.

Fluorescent drawbacks

Fluorescent detection can be less sensitive than chemiluminescent detection; chemiluminescent Westerns can be 10-100 times more sensitive, depending on the protein in question. Reagents (e.g. bromophenol blue) or supplies (e.g. certain membranes) can autofluoresce leading to high background, thus reducing the limit of detection of the assay. When switching from a chemiluminescent assay, all primary and secondary antibodies need to be titrated individually to find the highest signal-to-noise ratio. Lastly, fluorescent Westerns blots are visualized using digital imagers rather than the customary x-ray film and developer paradigm established with chemiluminescent detection.

Benefits of Fluorescence

- Sensitive – detect proteins in the picogram range
- Easily detect, differentiate and visualize multiple proteins
- Assay loading and normalization controls on one blot
- Detect post translational modifications
- The digital imaging advantage
- Best assay for quantitative Westerns

Choosing the best assay

A laboratory should not be a “chemiluminescent Western laboratory” or a “fluorescent Western laboratory”. Fluorescent detection can complement, enhance and provide new insight when coupled with chemiluminescent detection.

Therefore, the best assay should be chosen for each experiment:

Use Chemiluminescence to:

- Detect a single protein
 - Assay for presence/absence of a protein
 - Measure antibody responses
 - Follow protein purification
- Detect low abundance proteins

Use Fluorescence to:

- Detect multiple proteins simultaneously
 - Study posttranslational modifications
 - Have same-blot loading control
 - Have in-lane normalization
- Perform quantitative Westerns

References

- <http://www.biocompare.com/Editorial-Articles/41740-Fluorescent-Western-Blotting/>