A Guide to Western Blot Detection: Chemiluminescence Versus Fluorescence

Picking the appropriate detection method for your Western blot

Introduction

Since the introduction of Western blotting in the late 1970s, it has become a ubiquitous lab technique, enabling the analysis of protein expression in a wide variety of biological sample types. Whether you're a Western blot expert or just getting started, understanding the pros and cons of the available chemiluminescence- or fluorescence-based signal detection methods will let you make the right choice for your Western blot experiments. The entire sample preparation, running and antibody probing procedures require a considerable amount of effort; therefore, you definitely want to have a visualization method in place at the finish line suitable for your protein(s) of interest. In this technical note, we review common detection methods used in Western blotting to help you understand your options, improve your workflow and maximize your data.

Chemiluminescence: Sensitive Protein Detection

Chemiluminescent detection has become the prevalent detection method for Western blotting. Popular methods involve labeling a protein of interest with a primary antibody, followed by a horseradish peroxidase (HRP)-conjugated secondary antibody. A substrate, typically luminol-peroxidase, is added that then produces a chemical reaction measured in the form of light emission. Keep in mind, though, that detection is based on an enzymatic reaction, and the signal is transient, which means that a membrane must be imaged quickly following the detection reaction. Additionally, because the enzymatic reaction is time-dependent, care must be taken to avoid saturating the signal, which may result in over-exposed bands that will negatively impact the ability to accurately detect variations in protein expression.

This reaction can be measured with X-ray film or digital imaging systems. Although X-ray film can be quite sensitive for qualitative purposes, it also requires the use of hazardous materials, a dark room and a scanner for densitometry analysis. This manual scanning process and the limited dynamic range associated with film pose obvious challenges for the accurate quantitation of protein expression. Chemiluminescent detection is a very sensitive approach for the detection of proteins across a wide dynamic range (>5 logs), if using a digital imaging workstation equipped with a charge-coupled device (CCD) camera.

Digital imaging systems with CCD technology have been optimized for sensitivity and can improve image resolution by providing a wider dynamic range of band signal intensity detection than film. **Figure 1** compares chemiluminescent detection of ERK1/2 expression in HeLa cell lysate with 5-second and 1-minute exposures using a FluorChem E Imaging System versus film. Digital imaging of the chemiluminescent signal resulted in the detection of ERK1/2 across an increasing concentration range (10 ng to 1.0 µg) of cell lysate without signal saturation. This wide detection range, characteristic of digital imagers, enables researchers to load less protein, use smaller quantities of antibodies and detect even faint signals.

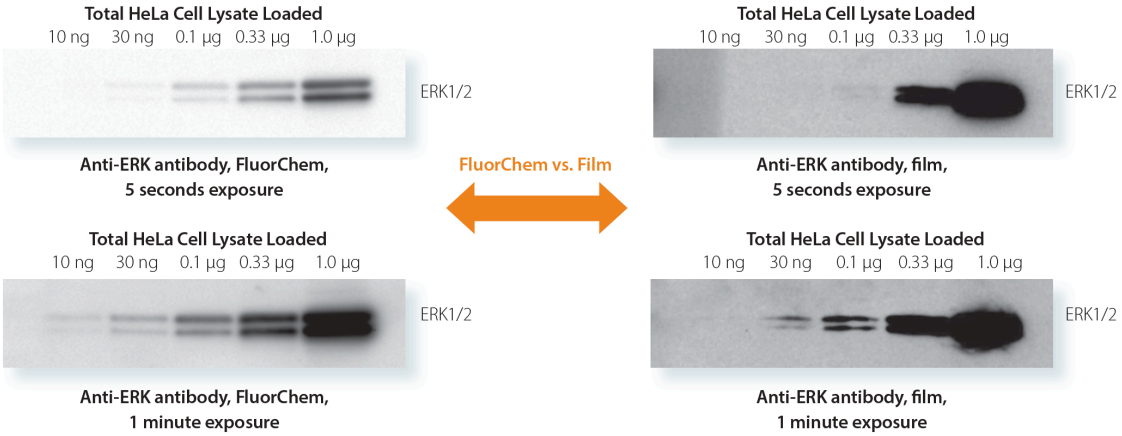

FIGURE 1. Chemiluminescent Detection of ERK in HeLa Cell Lysate. Serial dilutions of HeLa cell lysate were transferred onto a blotting membrane and detected using anti-ERK1/2 antibody and an HRP-tagged secondary antibody, followed by chemiluminescent detection. Blots were imaged with FluorChem Digital Imaging System and film using 5-second and 1-minute exposure times.

However, if you want to detect multiple proteins and they are of similar size and co-migrate in your gel, or are phosphorylated and total protein isoforms, only one protein can be analyzed at a time using chemiluminescence-based imaging methods because chemiluminescence emits in a single wavelength. Stripping and reprobing your membrane can enable the detection of multiple proteins of interest. However, this process can be time-consuming. Additionally, the process can result in protein loss, which can impact data integrity and the ability to perform accurate protein expression comparisons.

Fluorescence: Multiplexed Protein Detection

The application of fluorescent detection methodologies to Western blotting can increase experimental flexibility, with the ability to detect multiple targets and co-migrating proteins simultaneously. Protein(s) of interest are labeled with a primary antibody followed by fluorescently conjugated secondary antibodies. Digital imaging systems measure the emission of the fluorescent tag(s), enabling the detection of multiple overlapping targets or closely resolved targets without stripping and reprobing. **Figure 2** illustrates the detection of phosphorylated and total ERK isoforms in a single blot using fluorescence multiplexed detection. An added infrared channel expands multiplexing capabilities, as illustrated in **Figure 3**. The ability to detect multiple proteins simultaneously, without the need to strip and reprobe, allows researchers to save time and precious samples.

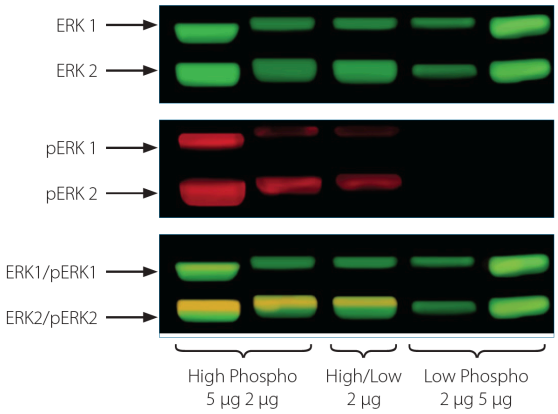

FIGURE 2. Fluorescent Detection of ERK1/2 and pERK1/2 in HeLa Cell Lysate. HeLa lysates with anti-ERK1/2 and anti-pERK1/2 primary antibodies followed by MultiFluor Green and MultiFluor Red secondary antibodies. ERK1/2 labeled with MultiFluor Green; green color shown (Top). pERK1/2 labeled with MultiFluor Red; red color shown (Middle). Overlaid image of ERK1/2 and pERK1/2 (Bottom).

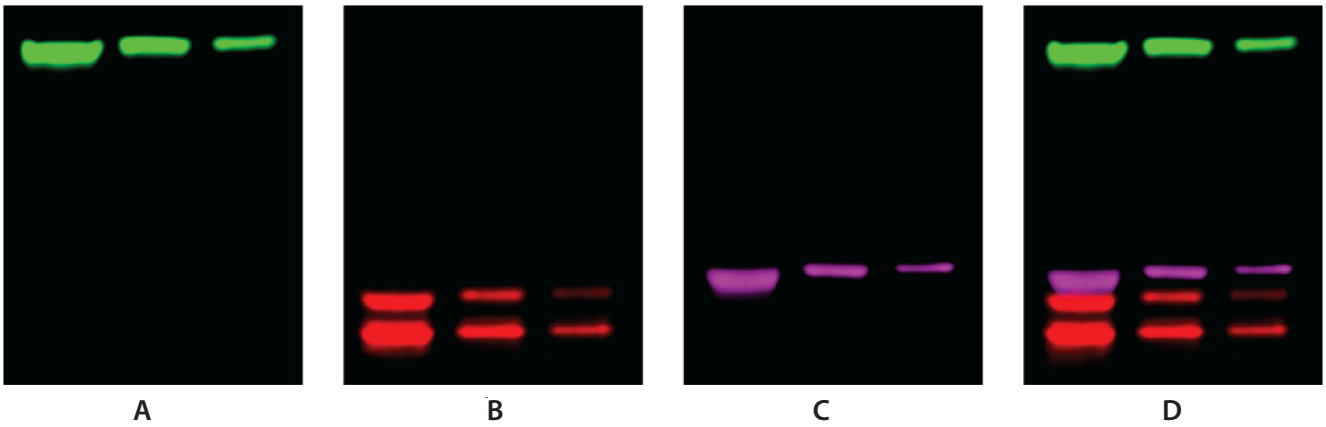

FIGURE 3. Fluorescent and Infrared Detection of HSP70, ERK1/2 and β-actin in HeLa Cell Lysate. Multiplex detection of HSP70 (A), ERK1/2 (B) and β-actin (C) in HeLa lysates with visible fluorescence (A, B) and infrared (C) fluorescence. Independent images for each fluorescent channel were obtained sequentially, and a composite image (D) was generated by overlaying the individual images. All three colors were imaged in a single scan using Digital Darkroom acquisition software on the FluorChem R system.

Unlike chemiluminescence, visible fluorescent signals are stable over an extended period and can be detected with a high degree of accuracy and linearity. To enable normalization, one fluorescent channel can be used to detect a sample loading control, so inconsistencies in protein loading can be detected, and band intensities for your protein of interest can be normalized to the loading control for more accurate data comparison. However, fluorescent detection is less sensitive than chemiluminescent detection, and background autofluorescence can impact the ability to detect proteins with low levels of expression.

Picking the Appropriate Detection Method for Your Assay

Which detection method is right for you? Although chemiluminescent detection offers excellent sensitivity, it lacks the ability to multiplex without the need to strip and re-probe. Fluorescent detection allows researchers to measure co-migrating proteins accurately and to analyze multiple proteins simultaneously without the additional assay time and signal loss. Above all, it's important to remember that your experimental needs may change over time. **Figure 4** outlines key considerations for each detection method.

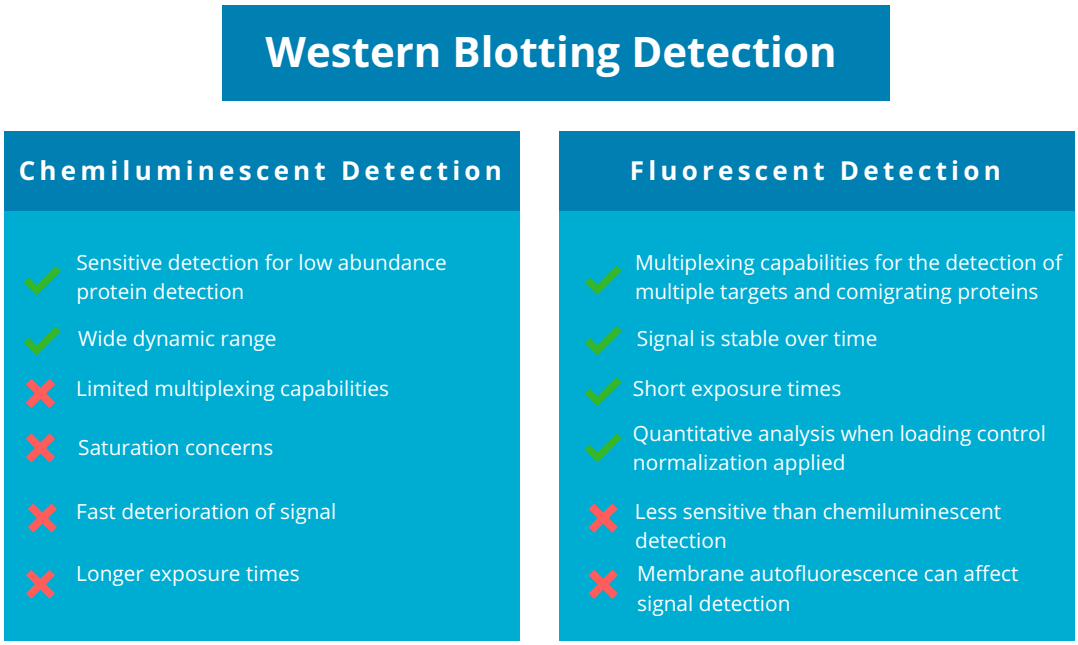

FIGURE 4. Considerations for Western Blotting Detection

How Can FluorChem Help You?

FluorChem digital imagers are platforms that evolve to meet your experimental needs, enabling chemiluminescent and fluorescent imaging of Western blots, giving you the ultimate application flexibility. With a 5-log dynamic range enabled by the large, high-resolution CCD sensor, FluorChem imagers let you detect bands and bright bands in a single exposure without oversaturating your image. The 8.3-megapixel CCD cameras ensure that you'll get the best image quality and the resolution you need to perform accurate analyses and differentiate between bands that are close together. The FluorChem Q, M and R also enable multiplex fluorescent imaging, letting you analyze overlapping protiens in the same blot.

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