Chemiluminescent Western Blot Detection using the FluorChem® HD2 Imaging System

Abstract

Digital image acquisition using the FluorChem HD2 is compared to film for chemiluminescent Western blot detection. The results show that digital imaging produces higher quality images at equal exposure times and achieves greater dynamic range and detects lower levels of protein than film. With the additional advantages of reduced cost and time expended per image, image archiving, and quantitative image analysis, digital imaging is the method of choice for chemiluminescent Western blot detection.

Introduction

Western blotting is a well characterized and widely used method for quantification of protein abundance in cell culture, tissue, serum and purified protein samples (1). Chemiluminescence is the standard detection technique exploiting the catalytic reaction of an enzyme and a peroxide-based substrate to produce a light signal with very low background as no illumination is required. The enzyme is conjugated to a secondary detection antibody that binds to the primary antibody specific to the protein of interest. Chemiluminescence is widely used by researchers because it is much less hazardous than radioactivity while achieving equal performance.

Detection of chemiluminescence by exposure to film is used commonly, but suffers from the well documented limited linearity response and reciprocity failure of film.

The FluorChem HD2 and FC2 imaging systems apply CCD cameras, fast lenses, and dark enclosures for quantitative chemiluminescent detection and when used optimally are powerful tools for Western blot analysis. Coupled with Cell Biosciences ChemiGlow® chemiluminescent substrate, high quality images and accurate results are obtained with fast imaging times. Furthermore the need for costly film and film developers is eliminated.

Materials and Methods

Film vs FluorChem HD2 detection of chemiluminescent Western Blot

The performance of film and the HD2 were assessed visually by imaging the same blot, first on the HD2 and then by film.

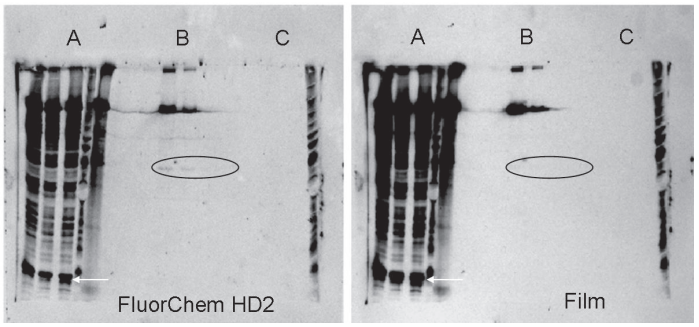

Figure 1: Comparison of HD2 and film images of a Western blot. Chemiluminescent labeled Western blot showing: (A) whole cell bacterial lysates; (B) purified transposase (tnp) protein; and (C) molecular weight standard. Images acquired using a FluorChem HD2 imaging system (left image) and film (right image) using a 5 minute exposure time on each system. FluorChem HD2 system was set on "Normal" resolution. Film image was converted to a digital image by imaging it on the FluorChem HD2 system with white light illumination. Note the higher image quality of FluorChem HD2 image as indicated by resolution of dim bands adjacent to bright bands (arrows) and detection of dim individual bands (ellipses).

A standard SDS-PAGE gel loaded with bacterial whole cell lysates, purified transposase protein and standard protein ladder was resolved and transferred to a blot using a standard transferring technique. The blot was blocked, incubated with rabbit anti-transposase primary antibody, and then labeled with HRP conjugated goat anti-rabbit antibody. The blot was treated with ECL (GE Healthcare) and imaged using a FluorChem HD2 imaging system for five minutes on normal resolution (no binning). The blot was then exposed to film for five minutes and the film developed.

Slot Blot Dilution Series

The capability of the FluorChem HD2 to quantify protein amounts was assessed by imaging and analyzing a protein dilution series.

A dilution series of an HRP conjugated antibody (goat anti-rabbit IgG Horseradish Peroxidase Antibodies Inc, Davis, CA) was prepared and deposited onto the surface of a low autofluorescence blotting membrane (Millipore-FL) using a filtration manifold apparatus (The Convertible, Gibco BRL, Gaithersburg, MD). The concentration of protein in the first band deposited was 1ng of total protein. Additional dilutions were included as a 1:10 dilution series with a final concentration of 100fg/band. The blot was treated with ChemiGlow and imaged on the FluorChem HD2 imaging system.

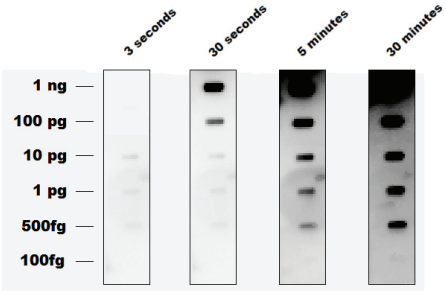

Figure 2: Dilution series showing effect of extending exposure times to 30 minutes on a chemiluminescent slot blot imaged with the FluorChem HD2 system. The capability to detect small quantities of protein (500 fg) increases with increased exposure time when using digital imaging.

Results and Discussion

Image quality, linear dynamic range, and the capability to detect low levels of protein are critical performance criteria for chemiluminescent Western blot detection.

High image quality is required to visually assess the separation of adjacent bands (resolution) and to see the range of protein amounts (contrast). Film also required an additional step of digitization of the original film using white light illumination with a slight loss of contrast. The FluorChem HD2 image of the chemiluminescent Western blot, when judged by the clarity and contrast of the bands as well as visualization of dim bands, was superior to that of film.

Quantitative analysis of protein amounts requires a broad linear measurable dynamic range and the capability to detect low levels of protein. The FluorChem HD2 achieves 3.8 logs of linear measurable dynamic range (2) for longer sustained (and useful) signal collection times compared to film based imaging (3). The signal from 500 fg deposited protein was significantly above background variance. While routine detection of low levels of proteins can also depend on sample preparation and labeling conditions, these results indicate that digital imaging is capable of detecting low levels of protein.

When using the FluorChem HD2 small quantities of protein can be detected simply by extending exposure time while maintaining a broad linear response to signal intensity. The detection of very faint bands by film can require extended exposure times compared to the times optimal for brighter signals due to reciprocity failure of film with a loss of contrast and more rapid saturation of the brighter signals.

In conclusion, detection of chemiluminescent Western blots using the FluorChem HD2 imaging system produces the high quality images and quantitative results required by the most demanding Western blot experiments.

References

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