Quantitative imaging of chemiluminescent western blots: Comparison of digital imaging and x-ray film

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Introduction

Chemiluminescent western blotting is the most frequently used method for detecting proteins. Traditionally, the chemiluminescent signal is detected and recorded by exposure of the membrane to x-ray film. The introduction of digital imaging systems that use charged coupled device (CCD) cameras to capture the luminescent signals on western blots has provided the user with many more options such as greater dynamic range, higher sensitivity for the detection and quantification of western blots.

Recently there has been a trend towards chemifluorescence which provides the user with many advantages including shorter exposure times and also means the signal is stable for weeks.

This application note will compare the performance of x-ray film with that of Syngene's G:BOX Chemi IR^6 imaging system for the visualisation and quantification of chemiluminescent and chemifluorescence signals on western blots.

Materials and Methods

Materials

Pre-cast Invitrogen gels (4-12% Bis-Tris, 12 well, 1mm). Running buffer used was MOPS. HeLa cell lysate (2.92mg/ml sample) was loaded on to the pre-cast gel. The marker used was BioRad Precision All Blue.

For transfer semi-wet Invitrogen Sure-Lock system was used. Nitrocellulose membrane (Invitrogen) and transfer buffer (50ml 20x NuPAGE transfer buffer, 100ml methanol and 850 MilliQ water) were used.

Primary antibody used was Anti-actin monoclonal (1:5000) (Abcam,UK) and the secondary antibody used was Anti-mouse HRP (1:10,000) (Vector Laboratories, UK).

Chemiluminescence imaging system

Syngene's G:BOX Chemi IR^6 system and a darkroom.

Method

HeLa cell lysate was diluted 1:2 (ranging from 29.2-0.028µg/well with 10µl of each concentration being loaded on to the gel). 2µl of BioRad Precision All Blue marker was loaded on to the gel. The gel was then run at 60V for 30 minutes.

The gel was transferred to a nitrocellulose membrane using the Sure-Lock system (Invitrogen, UK) at 125V for ~1hr 40 minutes. The blot was blocked in Odyssey block buffer for 1 hour.

The membrane was then incubated with primary antibody Anti-actin overnight at 4^{0}c on a rocker. After the incubation period the membrane was washed 4 times, 5 minutes each wash with PBS and 0.1% Tween (PBST).

The membrane was incubated with the secondary antibody (Anti-mouse HRP) for 1 hour at room temperature in the dark. The membrane was then washed 4 times, 5 minutes each wash with PBST.

The membrane was then incubated with the chemiluminescent reagent ECL Plus (GE Healthcare, UK) for 5 minutes. An image was captured of the blot using the G:BOX Chemi IR^6 imaging system then the blot was exposed to film.

Visualisation

ECL Plus can also be used as a chemifluorescent reagent. The following table shows the recommended lighting and filter combinations that should be used when imaging ECL Plus.

	Lighting	Filter
ECL Plus (Chemi)	No light	No filter
ECL Plus (Fluorescence)	Epi UV	Filt UV

Table 1 - Recommended lighting and filter combinations for visualising ECL Plus on an imaging system.

Results

Sensitivity

Three different methods of visualising an ECL Plus western blot were compared (chemiluminescence, chemifluorescence and x-ray film) (**Figure 1**).

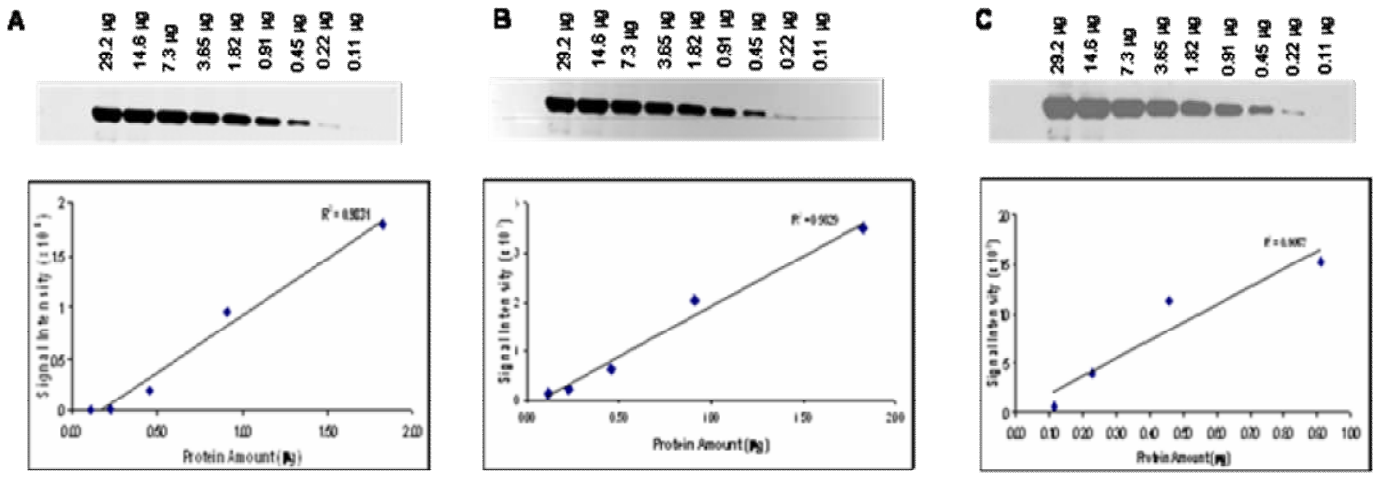
The results demonstrate that all three methods have the same sensitivity and have a limit of detection of 0.11µg (**Figure 1**).

Linearity

The linearity of the data was determined by the square of the correlation coefficient (R^2). The images of each blot were analysed using GeneTools analysis software (Syngene, UK). The signal intensities were plotted against the amount of protein loaded on to the gel. A linear curve fit was then performed (the graphs are displayed below the corresponding blot images in Figure 1).

ECL Plus chemiluminescence and ECL Plus fluorescence had a linear detection range of 1.83-0.11µg and a R^2 value of 0.983 (**Figure 1a**) and 0.982 (**Figure 1b**) respectively.

ECL Plus exposed to x-ray film had a dynamic range of 0.91-0.11µg which is less than that of chemiluminescence and chemifluorescence methods and a R^2 value of 0.905 (**Figure 1c**).



System	Detection Limit (LOD)	Linearity R^2	Dynamic Range	
			Orders of magnitude *	Range
ECL Plus (Chemi)	0.11µg	0.9831	1.2	1.83-0.11µg
ECL Plus (Fluorescence)	0.11µg	0.982	1.2	1.83-0.11µg
ECL Plus (Film)	0.11µg	0.905	0.9	0.91-0.11µg

* calculated over the data range shown.

Figure 1- Comparison of ECL Plus chemiluminescence, ECL Plus fluorescence and x-ray film

HeLa whole cell lysate diluted 2-fold starting at 29.2µg. Blotted on to nitrocellulose membrane and detected with **A)** ECL Plus chemiluminescence, **B)** ECL Plus fluorescence and **C)** ECL Plus x-ray film. Imaged blots (top) and linearity plots based on analysed data in GeneTools software (below). The dynamic range and R^2 values are shown in the table.

Visual assessment

X-ray film is more traditionally used for the visual assessment of relative band intensities. This type of assessment is acceptable in situations when the compared signal intensities differ significantly. However, this method does have limitations and it is not useful for performing precise signal quantification.

In some cases the signal on x-ray film rapidly exposes the film to saturation which will show as strong dark bands on the film. This can lead to the perception that x-ray film is more sensitive than digital imaging, whereas a CCD captured image has a greater dynamic range which will typically have data distributed over a much broader range. Therefore, moderate signals which would typically have reached saturation (strong black bands) on film should be in the middle (grey) range of the dynamic range of the imaging system. This makes the data more quantitative.

Cost savings with digital imaging

There are several costs associated with the use of x-ray film including the purchase and maintenance of the film processor, the cost of film and the chemicals needed to process the film and the waste disposal of chemicals, which are all avoided by the use of a digital imaging system.

The advantage of using a digital imaging system is that even though there is initial investment cost, the lower operational costs over time makes an digital imaging system a less expensive alternative.

Conclusion

Digital imaging systems provide a convenient and practical solution for imaging and performing quantitative analysis of chemiluminescent blots. Syngene's G:BOX Chemi IR^6 system has a broader dynamic range and better linearity than film - 1.2 orders of magnitude (over the data range shown) and R^2 of 0.9831 compared to 0.9 and R^2 of 0.95 respectively.

Syngene's G:BOX Chemi systems not only offer high sensitivity, broad dynamic range and easy set-up but also save you money in the long run.

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