G: BOX iChemi Image Analyser and KPL Chemiluminescent Substrates Make Detecting Proteins in Picogram and Femtogram Range Quick and Easy

Introduction

Western blotting is one of the most commonly used applications in protein research. Recent advances in chemiluminescent substrate design have meant when chemiluminescent blots are scanned using CCD image analysis systems, they can generate real-time images, in which smaller amounts of protein can be detected than on blots labelled with traditional colorimetric or fluorescent dyes.

Another advantage of using chemiluminescent substrates is that the blots can be stripped and re-probed, which saves scientists gel running time, as well as time with transferring proteins from gels to blots. The disadvantage of using chemiluminescent substrates is that substrates can produce background noise if left for long exposure times, especially when using film for capturing blot images. Additionally, to be able to visualise results requires either a dark room and film or an imaging system. Even using an imaging system, capturing the optimum images of chemiluminescent blots can often be very daunting as there are so many variables to set to detect bands of protein in the picogram and femtogram range.

Since chemiluminescence is still the best choice for scientists who need to see very small amounts of proteins and want to re-probe the blot with many different antibodies, this application note discusses how to address the issues of background noise and how to set up an imager to achieve optimum imaging results of picogram and femtogram amounts of proteins.

G:BOX iChemi, an Intelligent System for Imaging Chemiluminescent Westerns

To overcome the difficulties of how to capture perfectly exposed chemiluminescent blots, Syngene has developed the G:BOX iChemi intelligent chemiluminescent imaging system (Figure 1). Unlike many chemiluminescence imaging systems, the G:BOX iChemi's processor automatically assesses the sample, sets its own exposure time and sensitivity and auto-scales the captured image. Using these features users can produce high quality images with perfect contrast, without any of the guess work or trial and error imaging usually associated with capturing images of chemiluminescent blots.

To demonstrate the excellent quality and range of imaging a G:BOX iChemi system can achieve, this system was utilised by a major reagent manufacturer, KPL in Gaithersburg USA for imaging their LumiGLO® and LumiGLO Reserve™ Peroxidase Chemiluminescent Substrates. LumiGLO detects low picogram quantities of target protein on blots with signal linearity across a broad dynamic range of detection, with a superior signal to noise profile. LumiGLO Reserve allows detection levels as low as the femtogram range, with little non-specific background signal. The data will show that while many different substrates may be used with the G:BOX iChemi image analyser, KPL's substrates can provide researchers with optimal sensitivity and signal: noise when using the G:BOX iChemi XR system.

Figure 1. Syngene's G:BOX iChemi XR Image Analyser

Method

Generating Chemiluminescent Labelled Western Blots
Five SDS-PAGE gels were run. Two were loaded with 5 dilutions of Human IgG (Lampire, USA) (500ng, 50ng, 5ng, 500pg and 50pg) and three were loaded with 5 dilutions of Human IgG (5ng, 500pg, 50pg, 500fg and 50fg). Each gel was also loaded with a set of pre-stained protein markers (Blue Protein Molecular Weight Marker, ThermoFisher). The proteins were transferred from acrylamide gels onto nitrocellulose membranes using a standard electroblotting method. All the membranes were incubated overnight in 1X Detector Block solution (KPL, Cat. No. 71-83-00) with 1% Detector Block Powder. The blots were incubated (1 hour, 25ºC) in 1X Detector Block containing an HRP Goat anti-Human IgG (KPL Cat. No. 04-18-06 [diluted 1:10,000]). The membranes were washed in 1X Wash Solution (KPL Cat. No. 50-73-00) and LumiGLO® (KPL Cat. No. 54-61-00) was applied to one blot (1 minute, 25ºC), ECL Plus™ (GE Healthcare Life Sciences, Little Chalfont,

Buckinghamshire, UK) was applied to the second blot (5 minutes, 25ºC), LumiGLO Reserve™ (KPL), SuperSignal® West Femto and SuperSignal® West Dura (Thermo Fisher Scientific, Rockford, Ill, USA) were applied to the third, fourth and fifth blot respectively (1 minute) all according to the manufacturer's instructions.

Imaging Chemiluminescent Blots

Each membrane was placed inside the G:BOX iChemi XR darkroom and the focus was set while the darkroom door was open, using the coloured pre-stained protein markers as a focusing guide. The system was then set up without any light source or filters as excitation or emission is not necessary when using chemiluminescent substrates. The system's GeneSnap software was programmed to "capture series" at 60 seconds intervals for 15 minutes. The G:BOX iChemi XR then automatically picked and displayed the image of each of the five blots which showed the correct exposure with minimum background noise after 1 minute and 15 minutes exposure times.

Results

The image produced by the G:BOX iChemi XR of membranes 1 and 2 (Figure 2) showed that three bands (500ng, 50ng and 5ng) were visible after a 1 minute exposure with ECL Plus and four bands (500ng, 50ng, 5ng and 500pg) were visible with LumiGLO.

Figure 2: LumiGLO (left) and ECL Plus (right) labelled Western blot images generated by G:BOX iChemi XR after a 1 minute exposure showing from left to right 500ng, 50ng, 5ng, 500pg and 50pg of human IgG.

The image of membranes 1 and 2 (Figure 3) generated by the G:BOX iChemi XR shows 5 bands (500ng, 50ng, 5ng, 500pg and 50pg) are visible with both LumiGLO and ECL Plus after a 15 minute exposure. Therefore both substrates will detect human IgG protein in the 50pg range, if left for a sufficient exposure time and are consistent with the levels of protein detection claimed by the manufacturers.

Figure 3: LumiGLO (left) and ECL Plus (right) labelled Western blot image captured by the G:BOX iChemi XR after a 15 minute exposure showing from left to right 500ng, 50ng, 5ng, 500pg and 50pg of human IgG.

The image produced by the G:BOX iChemi XR of membranes 3, 4 and 5 [Figure 4] shows 4 bands (5ng, 500pg, 50pg and 500fg) are visible with LumiGLO Reserve, while 3 bands (5ng, 500pg and 50pg) are each visible with SuperSignal West Femto and SuperSignal West Dura after a one minute exposure.

Figure 4: LumiGLO Reserve (left), SuperSignal West Femto (middle) and SuperSignal West Dura (right) labelled Western blot image generated by G:BOX iChemi XR after a one minute exposure showing from right to left (5ng, 500pg, 50pg, 500fg and 50fg)

The image of membranes 3, 4 and 5 (Figure 5) generated by the G:BOX iChemi XR after a 15 minute exposure shows 5 bands (5ng, 500pg, 50pg, 500fg and 50fg) are visible with LumiGLO Reserve (KPL Cat. No. 54-71-00) and SuperSignal West Dura, while 4 bands (5ng, 500pg, 50pg, 500fg) are visible with SuperSignal West Femto. All three substrates will therefore detect human IgG protein in the 500-50fg range if left for a sufficient exposure time and again are consistent with the levels of protein detection claimed by the manufacturers.

Figure 5: LumiGLO Reserve (left), SuperSignal West Femto (middle) and SuperSignal West Dura (right) labelled Western blot image captured by the G:BOX iChemi XR after a 15 minute exposure showing from right to left (5ng, 500pg, 50pg, 5pg and 500fg)

A summary of the performance of the five different chemiluminescent substrates with the G:BOX iChemi XR is shown below in Table 1.

Table 1: Comparison of chemiluminescent substrates detection with a G:BOX iChemi XR system.

	LumiGLO	LumiGLO Reserve	ECL Plus	Super Signal West Femto	Super Signal West Dura
Sensitivity	Low pico-gram range with 1 min-ute and 15 minute exposure.	Femtogram range with 1 minute and 15 minute exposure.	Nanogram range with 1 minute expo-sure. Low picogram range with 15 minute exposure	Low picogram range with 1 minute expo-sure. Femtogram range with 15 minute expo-sure.	Low picogram range with 1 minute expo-sure. Femtogram range with 15 minute exposure.
Signal:Noise	+++++	+++++	+++	+++	+++
Signal Duration	1 - 2 hrs	4 - 8 hrs	24 – 48 hrs	8 hrs	24 hrs
Linearity	+++++	+++++	+++	++++	++++

Conclusion

The Syngene G:BOX iChemi XR system provides an easy to set up method of accurately detecting proteins detected with a range of different chemiluminescent substrates. In addition, KPL's LumiGLO and LumiGLO Reserve substrates demon-strates higher sensitivity and linearity than other commonly used chemiluminescent substrates. Since these systems are designed for fully automated set up and walk away image cap-ture, scientists can choose to use substrates such as LumiGLO or LumiGLO Reserve for rapid low picogram and femtogram level detection. Alternatively, they can leave their exposures for longer periods using the G:BOX iChemi sys-tem with dyes such as ECL Plus, SuperSignal West Dura and SuperSignal West Femto to produce optimum results in the picogram and femtogram range.

These results demonstrate the level of flexibility and sensitiv-ity that this innovative image analyser can achieve and makes the G:BOX iChemi XR chemiluminescent imaging system an excellent tool for detecting poorly expressed protein.

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