

## G:BOX iChemi XR for imaging large gold stained immunoblots allows rapid and safe analysis of 200 kD disease associated proteins

### Introduction

Often researchers need to run longer protein gels to separate out and visualise proteins of 200 kD or larger. However, working with longer protein gels or the large Western blots produced using these gels can mean scientists have difficulty finding automated systems to accommodate these gels or blots. To overcome this problem some researchers label Western blots with a  $^{125}\text{I}$  secondary antibody and analyse them with an automated PhosphoImager. This is not ideal, as working with radioactive label poses a safety risk and the label requires stringent disposal methods. Additionally, a PhosphoImager and radioactive label are expensive purchases, which may not be within the budget of some laboratories.

Another solution is to label larger blots with a chemiluminescent substrate such as ECL™ and expose them to X-ray film manually. Again this can be problematic because it is difficult to judge the time required to obtain a correct exposure with X-ray film and with a short time frame for appropriate exposure this can lead to re-probing with antibodies, altering concentrations which can be costly to produce an optimum result. A second alternative is to label larger blots with a secondary antibody conjugated to gold and take images of the blot as it develops. This requires a camera with a large enough focal length to accommodate the entire blot and a number of images of the blot need to be produced to ensure the correct exposure.

To overcome these limitations, Syngene offers the G:BOX iChemi XR automated multi-purpose image analyser. This system is ideal for imaging large blots because its darkroom can accommodate blots of up to 20 cm x 30 cm and its camera has a motorised zoom lens which can capture images of large areas with ease. The system performs well with non-radioactive Western blots as it generates 16 bit images with 65,536 grey levels, resulting in a dynamic range which is more than double that of X-ray film. This allows researchers to detect faint bands and therefore quantify them more accurately and quickly so further images can be generated. The G:BOX iChemi XR also provides hands free automated capture of a series of images and long integration times are possible because its cooled CCD camera is designed to produce images without any unwanted background noise. This means scientists can safely generate images of perfectly exposed blots, without the expense and time in developing many X-ray films or using radioactive labels.

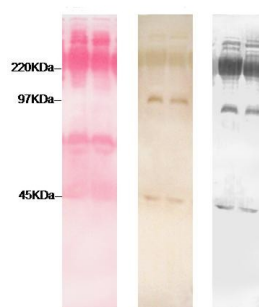
Adiponectin, a 30 kD protein linked with protecting against Type 2 diabetes is a good example of a protein, which can aggregate to form high molecular weight configurations. The following method shows how to produce and visualise large Western blots of the multimeric protein structure.

### Method

Human Serum underwent electrophoresis on a non-reducing, non-heated, denaturing PAGE, (4-12%) continuous gel for 3 hours. The proteins were transferred onto nylon membranes by standard Western blotting. The membranes were probed initially with a Ponceau-S solution (Sigma, Poole, UK) to visualise total protein. The blot image was recorded using the G:BOX iChemi XR. The blot was then washed and probed using a primary anti-adiponectin antibody raised against the human amino-terminal hypervariable region of adiponectin and it was then probed with a secondary antibody conjugated with 10nm gold particles (Amersham-Biosciences, Little Chalfont, UK). The gold bands on the blot were then developed using IntenSE™ BL (Amersham-Biosciences) a silver staining reagent, which intensifies small gold particles and allows visualisation of the higher molecular species on the membrane inside the G:BOX iChemi XR's darkroom. A series of images of the blot were captured with the G:BOX iChemi XR's GeneSnap image acquisition software. When the image with the optimum exposure had been generated, the amount of adiponectin and its aggregate forms were analysed automatically using the G:BOX iChemi XR's GeneTools software.

### Results

Using a G:BOX iChemi XR with gold stained immunoblots researchers have reported being able to visualise and analyse adiponectin proteins of over 200 kD. This has enabled them to run large proteins on longer gels to obtain better separation of the proteins, as well as automatically analyse the results quickly and easily.



*Fig 1: a Western blot of adiponectin and its high molecular weight forms stained with Ponceau S (left) and gold staining (middle) and an image of the blot generated with a G:BOX iChemi XR (right)*

## Conclusions

Syngene's G:BOX iChemi XR provides an effective alternative for detecting large proteins because it can automatically analyse gels or blots of up to 20 cm x 30 cm. This allows researchers the flexibility to run longer gels for the best separation of large proteins without the need to use radioactive labels or manually develop blots. As the G:BOX iChemi XR's software can capture an image series, scientists can more easily determine when they have reached the top end of the dynamic range of their image thus saving time, while generating one correctly exposed blot image. Additionally, since the images generated by a G:BOX iChemi XR have a higher dynamic range than X-ray film and analysing them is fully automated with the system's GeneTools software, scientists can produce accurate quantitative data, something which is difficult to obtain with manual exposures of blots on X-ray film and this removes the need to capture X-ray images from the film into appropriate software.

In summary, using a G:BOX iChemi XR to visualise and analyse large non-radioactive Western blots could lead scientists to produce more accurate information on large protein aggregates, like adiponectin. This may in turn help to speed up the discovery of new drug targets, which could provide more rapid production of novel therapies for debilitating diseases such as Type 2 diabetes.

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*Syngene reserves the right to amend or change specifications without prior notice. This Application note supersedes all earlier versions.*

**Syngene Europe**  
**A Division of the Synoptics Group**  
**Beacon House**  
**Nuffield Road**  
**Cambridge**  
**CB4 1TF**

**Tel: +44 1223 727123**  
**Fax: +44 1223 727101**  
**Email: [sales@syngene.com](mailto:sales@syngene.com)**

**Syngene USA**  
**A Division of the Synoptics Group**  
**5108 Pegasus Court, Suite M**  
**Frederick**  
**MD 21704**

**Tel: 800 686 4407/301 662 2863**  
**Fax: 301 631 3977**  
**Email: [ussales@syngene.com](mailto:ussales@syngene.com)**