

G:BOX iChemi for rapid multiplex analysis

A new method of imaging different proteins using only one blot

Introduction

Multiplex analysis of proteins can be used to determine regulation of specific proteins, to diagnose disease pre-disposition or to accurately quantify protein amounts. Traditionally, multiplex analysis involves scientists producing and comparing a series of Western blots, a task, which is both time consuming and expensive in terms of multiple reagent and blot use. The ideal situation is to analyse a number of proteins on one Western blot, however, many detection methods such as colorimetric probes or chemiluminescence are unsuitable for multiplex analysis of different proteins on the same blot. This is because they produce only one colour or a white chemiluminescent emission so that users cannot distinguish between different proteins, especially when these proteins have the same molecular weight or are very close together.

A new labelling method known as FluoWest™ has been developed by Ozyme, France which uses Qdot® labelled secondary antibodies. Each Qdot can be excited using a single light source and because the emission from each one has a different colour it allows simultaneous detection of a number of different proteins on one blot. To visualise images of Qdot labelled Western blots, scientists can use laser-based scanners but these are generally an expensive and inflexible purchase as they can only be used to image fluorescent dyes.

G:BOX iChemi – an inexpensive alternative for imaging fluorescent Westerns

As a solution to the problem of imaging fluorescently labelled Western blots Syngene has developed the G:BOX iChemi, an affordable CCD-based analyser that can be used for fluorescence and chemiluminescence imaging. The G:BOX iChemi comes with a high quality digital camera inside a light tight cabinet and can be fitted with long wavelength epi-UV illumination and band-pass filters matched to the Qdot emission wavelengths. The epi-UV light combined with the highly cooled cameras ensure visualisation of even the faintest fluorescent signals. G:BOX iChemi features GeneSnap software which allows users to automatically overlay all the different images of each colour to produce one single blot image showing the different colours simultaneously. The system also comes with GeneTools software so that users can analyse images for the molecular weight of proteins and quantify protein amounts. All these features make the G:BOX iChemi a cost-effective alternative to laser-based scanners.

Method

Generating FluoWest™ labelled Western blots

Two PAGE gels were run of protein lysates and the proteins were then transferred from acrylamide gels onto nitrocellulose filters using a standard electroblotting method. Two membranes were labelled using a standard FluoWest Labelling Kit and protocol from Ozyme. The primary antibodies for membrane 1 were a mixture of anti-heat shock protein 70, anti-ubiquitine and anti-O-N-acetylglucosamine and for membrane 2 were a mixture of anti-polypyrimidine tract-binding protein and anti-heterogeneous nuclear ribonucleoprotein. The Qdot secondary conjugates used for membrane 1 were a mix of Qdot 605, goat anti-rat IgG; Qdot 565 goat anti-rabbit IgG and Qdot 705 goat anti-Mouse IgG and for membrane 2 a mixture of Qdot 565 goat anti-rabbit IgG and Qdot 705 goat anti-Mouse IgG.

Imaging fluorescent blots

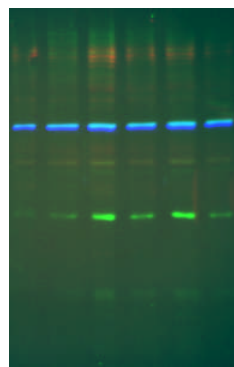
The membranes were placed inside the G:BOX iChemi darkroom and the Qdots were excited by the G:BOX iChemi's overhead epi-UV light. For detecting each Qdot's colour, the special emission filter for each Qdot was selected. Every coloured image was produced by using the G:BOX iChemi's GeneSnap software "capture series" set at 20 seconds. The best image of each Qdot colour was chosen and all the different coloured images were overlaid to create a composite image showing all the Qdot colours.

Results

The image produced by the G:BOX iChemi of membrane 1 shows heat shock proteins in blue, ubiquitinated proteins in green and glycosylated proteins in red (Figure 1).

Figure 1: Fluorescently labelled Western blot image generated by G:BOX iChemi showing from left to right heat shock proteins labelled with blue Qdot 605 (lanes 1-6), ubiquitinated proteins labelled with green Qdot 565 (lanes 1-6) and glycosylated proteins labelled with Qdot 705 red (lanes 3-5).

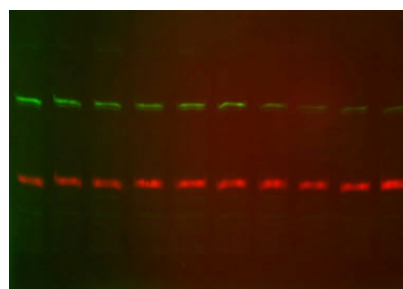
(Figure kindly provided by Ozyme, France)



The image of membrane 2 generated by the G:BOX iChemi shows the simultaneous expression of polypyrimidine tract binding (PTB) protein in green and heterogeneous nuclear ribonucleoprotein in red (Figure 2).

Figure 2: Fluorescently labelled Western blot image captured by the G:BOX iChemi showing from left to right polypyrimidine tract-binding protein labelled with green Qdot 565 (lanes 1-7) and heterogeneous nuclear ribonucleoprotein labelled with red Qdot 705 (track 1-10).

(Figure kindly provided by Ozyme, France)



Conclusion

The Syngene G:BOX iChemi provides a simple, yet accurate method of producing a multiplex image with up to three differently labelled proteins on the same blot. Since there are five different coloured Qdots available, the G:BOX iChemi could potentially be used to detect up to five different proteins on the same Western blot. As the GeneTools software produces precise overlays of the different coloured images, this makes generating the blot images a five-minute task compared to the hours it can take to produce and compare a series of blots. Therefore, the G:BOX iChemi image analysis system guarantees a quick, simple and cost-effective method of detecting and imaging multiple different proteins on Western blots. The G:BOX iChemi's level of flexibility and sensitivity mean it could be used to help save hundreds of research hours and offers an excellent alternative to laser-based scanners.

FluoWest™ is a trademark of Ozyme.

Date: 15th April 2009

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

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