Multiplex western blotting with fluorescent antibody probes

Long-wavelength Qdot® and Alexa Fluor® conjugates for sensitive and quantitative detection.

The breakneck pace of cell biology research demands more sensitive and quantitative protein analysis methods, capable of dissecting increasingly complex protein expression and interaction patterns. Western blot detection methods have evolved from radioisotope-based detection and colorimetric methods to those based on enhanced chemiluminescence (ECL), which are at least 10 times more sensitive than colorimetric assays. With recent advances in Qdot® probe technology, near-IR–emitting fluorescent dyes, and fluorescence imaging platforms, researchers are now able to obtain detection sensitivities from fluorescence-based detection comparable with those obtained from ECL (Figure 1). Furthermore, unlike ECL techniques, fluorescence-based detection methods provide the capability to detect multiple antigens on a single blot without stripping and reprobing.

Advantages of fluorescent probes for western blots

Qdot® and Alexa Fluor® secondary antibody conjugates are revolutionizing western blot detection. When paired with a fluorescence imager, these detection reagents provide sensitive and quantitative analysis of proteins blotted or spotted onto membranes. Importantly, probes with long-wavelength (red to near-IR) emission allow signal collection beyond the typical autofluorescence emitted by standard nitrocellulose and PVDF membranes, resulting in low background fluorescence and superior signal-to-noise ratios.

Tremendous flexibility in experimental design is afforded by the availability of several different long-wavelength Qdot® and Alexa Fluor® fluorophores, facilitating the detection of multiple proteins on a single western blot (Figures 2 and 3). A multicolor western blot saves time and sample by eliminating the need to produce duplicate gels and blots or to strip and reprocess a single blot with a different antibody probe. Moreover, data analysis is greatly simplified because you can directly compare signals from protein bands that have been exposed to identical electrophoresis and transfer conditions.

Multiplex westerns with Qdot® antibody conjugates

Qdot® secondary antibody conjugates provide a powerful alternative to traditional methods for western blot detection [1–3]. Qdot® probes are exceptionally bright and photostable fluorophores that can be excited at any wavelength below their emission (though most efficiently by UV, violet, or deep-blue light). Qdot® probe fluorescence can

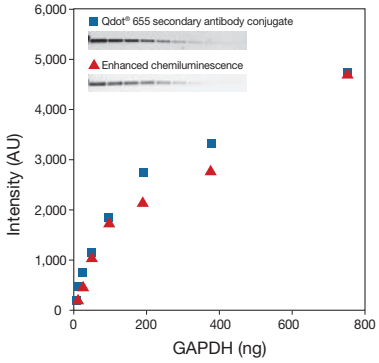

Figure 1. Comparison of enhanced chemiluminescence (ECL)– and Qdot® fluorescence–based western blot protein detection. Serial dilutions (750–3 ng protein) of GAPDH were run on NuPAGE® Novex® 4–12% Bis-Tris precast gels (Cat. No. NP0321BOX) and transferred to iBlot® nitrocellulose membranes (Cat. No. IB3010-32) using the iBlot® Gel Transfer Device (Cat. No. IB1001). The membranes were then probed with mouse anti-GAPDH antibodies followed by either ECL detection using a horseradish peroxidase goat anti–mouse IgG conjugate or fluorescence detection using Qdot® 655 goat anti–mouse IgG conjugate (Cat. No. Q11021MP). Images were collected using the Fujifilm® LAS-4000 gel imager. Partial blot images show GAPDH detection, and the graph shows the detection sensitivities of the two techniques.

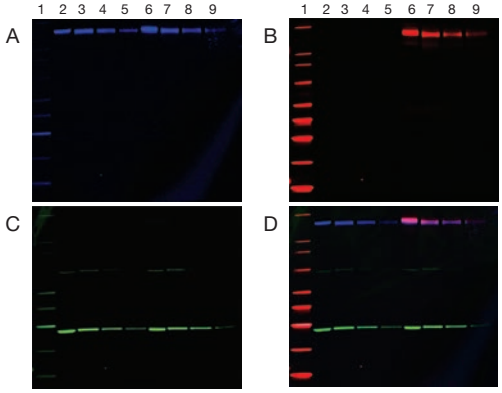

Figure 2. Simultaneous detection of three proteins on a single blot using Qdot® secondary antibody conjugates. A western blot containing serial dilutions (20–3 µg protein) of lysates from unstimulated (lanes 2–5) and hEGF-stimulated (lanes 6–9) A431 cells was probed with mouse anti-EGFR, rabbit anti–phospho-EGFR, and chicken anti-GAPDH antibodies, followed by **(A)** Qdot® 800 goat anti-mouse (pseudocolored blue), **(B)** Qdot® 605 goat anti-rabbit (pseudocolored red), and **(C)** Qdot® 655 goat anti-chicken (pseudocolored green) conjugates. **(D)** The merged image shows overlaid red and blue bands as purple. The blot contains MagicMark™ XP Western Protein Standard (lane 1, Cat. No. LC5603) and was imaged using the Fujifilm® LAS-4000 gel imager.

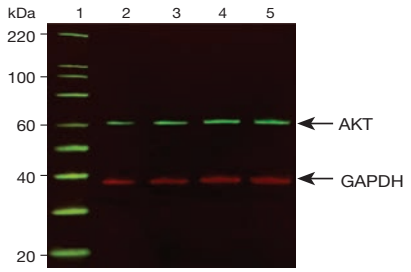

Figure 3. Western blot detection of AKT and GAPDH using Alexa Fluor® antibody conjugates. A western blot containing serial dilutions of Jurkat cell lysate (lanes 2–5) was probed with mouse anti-GAPDH and rabbit anti-AKT antibodies followed by Alexa Fluor® 790 goat anti–mouse IgG (pseudocolored red, Cat. No. A11375) and Alexa Fluor® 680 goat anti–rabbit IgG (pseudocolored green, Cat. No. A21109) conjugates, and then imaged using the LI-COR® Odyssey® Imaging System. Lane 1: MagicMark™ XP Western Protein Standard.

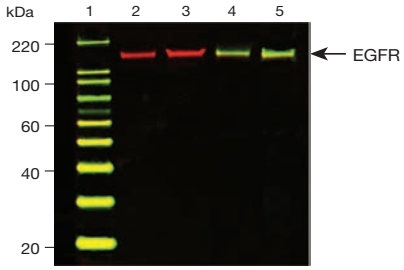

Figure 4. Simultaneous detection of EGFR and phospho-EGFR using Alexa Fluor® antibody conjugates. A western blot containing lysates from unstimulated (lanes 2, 3) and hEGF-stimulated (lanes 4, 5) A431 cells was probed with mouse anti-EGFR and rabbit anti–phospho-EGFR antibodies followed by Alexa Fluor® 790 anti–mouse IgG (pseudocolored red) and Alexa Fluor® 680 anti–rabbit IgG (pseudocolored green) conjugates, and imaged using the LI-COR® Odyssey® Imaging System. Lane 1: MagicMark™ XP Western Protein Standard.

be detected on many commercially available fluorescence imagers, including relatively inexpensive gel imagers equipped with a UV light box and appropriate emission filters—e.g., the E-Gel® Imager with the Qdot® 625 filter. More advanced fluorescence imagers, such as the Fujifilm® LAS-4000 gel imager, enable higher-order multiplexing and quantitation.

Using protein-specific primary antibodies and Qdot® secondary antibody conjugates, we have probed a single western blot for two proteins of interest (EGFR and phosphorylated EFGR), as well as a third protein (GAPDH) for normalizing signals (Figure 2). In this example, multiplex detection facilitates the normalization of fluorescent signals and allows us to distinguish proteins that co-migrate in a single gel lane, enabling the identification and quantitation of posttranslational modifications when specific primary antibodies for these modifications are available. This type of multicolor analysis not only reduces workflow significantly by minimizing the need for multiple blots, but also makes the quantitation of multiple proteins much more reliable. To see what's new in western blot detection with Qdot® probes, visit **lifetechnologies.com/qdotbp69**.

Multiplex westerns with Alexa Fluor® antibody conjugates

The near-IR–emitting Alexa Fluor® secondary antibodies are also valuable immunodetection tools that can be imaged and quantitated using near-IR fluorescence scanners (Figures 3 and 4). Figure 4 shows the detection of EGFR and phosphorylated EGFR with Alexa Fluor® 790 and Alexa Fluor® 680 antibody conjugates, respectively. Because the two proteins co-migrate in the lysates from hEGF-stimulated cells, the overlaid red and green bands appear yellow in the merged image. Although not resolved by molecular weight, each labeled protein can be individually detected and quantitated by the fluorescence imager.

An increasingly fluorescent future

As researchers migrate from ECL to multiplex fluorescence-based western detection, the number of fluorescent probes and imaging platforms continues to increase. We are developing and testing our reagents on the latest platforms so that beginners and experts alike can get the most out of their assays. To learn more about our western blot products, visit **lifetechnologies.com/westernbp69**. ■

References

1. Buttigieg J, Pan J, Yeger H et al. (2012) *Am J Physiol Lung Cell Mol Physiol* 303:L598-L607.
2. McGovern S, Pan J, Oliver G et al. (2010) *Lab Invest* 90:180–195.
3. Blumenstein M, McMaster MT, Black MA et al. (2009) *Proteomics* 9:2929–2945.

Product	Quantity	Cat. No.
Alexa Fluor® 680 Goat Anti–Mouse IgG (H+L) Antibody	500 µL	A21057 A21058*
Alexa Fluor® 680 Goat Anti–Rabbit IgG (H+L) Antibody		A21076 A21109*
Alexa Fluor® 790 Goat Anti–Mouse IgG (H+L) Antibody	500 µL	A11375 A11357*
Alexa Fluor® 790 Goat Anti–Rabbit IgG (H+L) Antibody		A11367 A11369*
Qdot® 585 Goat F(ab´)$_2$ Anti–Mouse IgG (H+L) Antibody	200 µL	Q11011MP
Qdot® 585 Goat F(ab´)$_2$ Anti–Rabbit IgG (H+L) Antibody		Q11411MP
Qdot® 605 Goat F(ab´)$_2$ Anti–Mouse IgG (H+L) Antibody	200 µL	Q11001MP
Qdot® 605 Goat F(ab´)$_2$ Anti–Rabbit IgG (H+L) Antibody		Q11401MP
Qdot® 625 Goat F(ab´)$_2$ Anti–Mouse IgG (H+L) Antibody	100 µL	A10195
Qdot® 625 Goat F(ab´)$_2$ Anti–Rabbit IgG (H+L) Antibody		A10194
Qdot® 655 Goat F(ab´)$_2$ Anti–Mouse IgG (H+L) Antibody	200 µL	Q11021MP
Qdot® 655 Goat F(ab´)$_2$ Anti–Rabbit IgG (H+L) Antibody		Q11421MP
Qdot® 705 Goat F(ab´)$_2$ Anti–Mouse IgG (H+L) Antibody	200 µL	Q11061MP
Qdot® 705 Goat F(ab´)$_2$ Anti–Rabbit IgG (H+L) Antibody		Q11461MP
Qdot® 800 Goat F(ab´)$_2$ Anti–Mouse IgG (H+L) Antibody	200 µL	Q11071MP
Qdot® 800 Goat F(ab´)$_2$ Anti–Rabbit IgG (H+L) Antibody		Q11471MP

*Highly cross-adsorbed antibodies.