WesternBright™ MCF

Multicolor fluorescent Western blotting kit, for more quantitative information from every blot

Multicolor fluorescent Western blotting allows multiple proteins to be assayed in a single experiment, even if the proteins migrate closely together on a gel. A housekeeping protein or loading control can be detected alongside a protein of interest. Phosphorylated and non-phosphorylated isoforms can be assayed simultaneously. Assaying experimental and reference proteins on the same blot improves the quantitative data obtained from an experiment, since stripping and re-probing of the blot, which can affect data quality, is avoided. WesternBright MCF saves time and money relative to alternative detection methods, since no film is required, no incubation with a substrate is needed, and imaging can be conducted immediately after the final wash with no need to wait for blots to dry.

Two-color Western Blots

Two color fluorescent Western blots increase the amount and quality of data obtained from every experiment. Two proteins can be assayed on the same blot, even if they migrate closely together (Figure 1). Expression levels of two proteins can be more accurately compared since no stripping and reprobing, or running of duplicate blots, is required.

The ability to detect two proteins on one blot depends on the use of antibodies coupled to fluorophores that have non-overlapping excitation and emission spectra. Figure 2 depicts the principle of two-color fluorescent Westernblotting;secondaryantibodies conjugated to fluorophores bind to primary antibodies that recognize the protein targets of interest. The blot can then be imaged under the appropriate lighting conditions for each fluorophore.

The WesternBright MCF fluorescent Western blotting kit includes two secondary antibody conjugates, each pre-labeled with a highly fluorescent protein isolated from cyanobacteria and eukaryotic

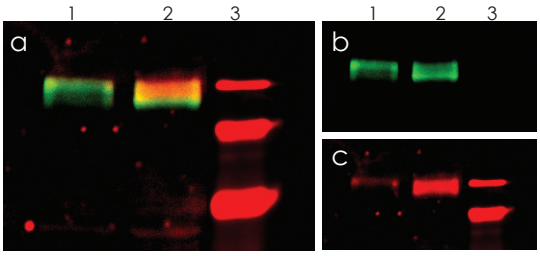

Figure 1. Simultaneous detection of EGFR and phospho-EGFR with WesternBright MCF. The increase in phosphorylation of EFGR in response to EGF was detected. Lysates from A431 cells (lane 1) and from A431 cells treated with EGF (lane 2) were blotted and detected in the green channel (b) and phospho-EGFR detected in the red channel (c). The two channels are superimposed in (a). Lane 3: molecular weight markers.

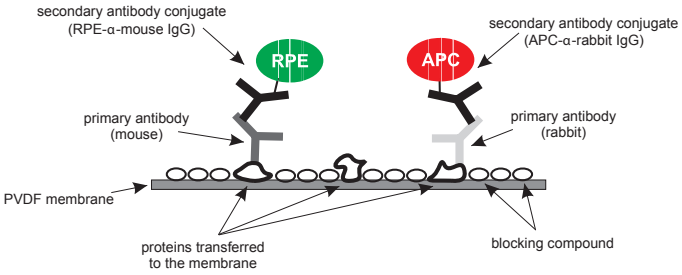

Figure 2. The principle of two-color fluorescent Western blotting. Using secondary antibodies labeled with different fluorophores, two proteins can be detected on a single blot by controlling excitation and detection channels. WesternBright conjugates incorporate the phycobiliproteins allophycocyanin (APC) and R-phycoerythrin (RPE), extremely bright fluorescent proteins from cyanobacteria and eukaryotic algae.

algae. These phycobiliproteins, allophycocyanin (APC) and R-phycoerythrin (RPE) are components of the photosynthetic system of these organisms, and are among the brightest fluorophores known. APC and RPE have excitation and emission spectra compatible with filters used with Cy™5 and Cy3 dyes (Figure 3), allowing them to be imaged with most commercial fluorescence imaging systems.

Quick Results

WesternBright MCF provides data on two proteins in less than 3.5 hours (Figure 4). This is much faster than is possible with chemiluminescent detection, since the WesternBright MCF protocol does not require stripping and re-probing of the blot, or incubation with a substrate to generate signal, or exposure to and developing of film.

Additionally, WesternBright MCF blots can be imaged directly after the final wash, with no need to wait for the blot to completely dry as with other fluorescent dyes.

WesternBright MCF includes AdvanBlock™-PF, a synthetic, non-protein blocking agent that blocks the PVDF membrane in only 10 minutes, greatly shortening the blocking time relative to traditional Western protocols.

High Sensitivity

When used with high quality primary antibodies and optimized assay conditions, low picogram levels of protein can be detected with WesternBright MCF (Figure 5), sensitivity comparable to that obtained with chemiluminescent detection methods.

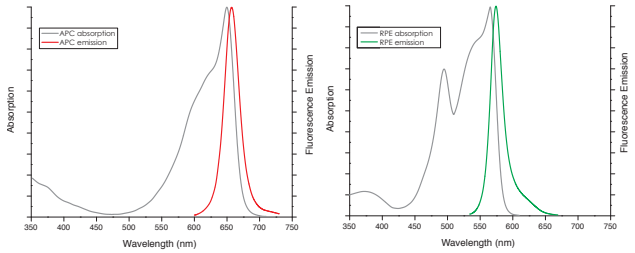

Figure 3. Absorption and emission spectra of the WesternBright conjugates. Absorption (grey lines) and emission (red and green lines) spectra of the WesternBright conjugates are compatible with most commercially available imaging instruments capable of imaging Cy3 and Cy5 dyes.

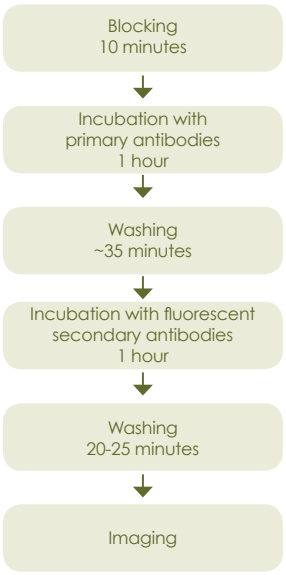

Figure 4. Outline of the WesternBright MCF protocol. Western blotting with WesternBright MCF can be carried out more quickly than alternative detection methods. Blocking with AdvanBlock-PF can be completed in only 10 minutes, no incubation with substrate is required after the final wash, and the blot does not need to be dried before imaging. The entire protocol can be completed in less than 3.5 hours.

Figure 5. Detect low picograms of protein with WesternBright MCF. Serial dilutions of Transferrin were separated by 10 % SDS-PAGE, followed by Western blotting detection with a rabbit-anti-transferrin primary antibody and WesternBright APC-goat-anti-rabbit IgG conjugate. Imaging was conducted with a FluorChem Q (Cell Biosciences). The amount of Transferrin loaded in each lane is indicated; 1.1 pg was detected.

Brighter than Cy Dyes

Duplicate Western blots imaged under identical conditions demonstrate that WesternBright conjugates provide much brighter signals than the secondary antibodies labeled with Cy3 and Cy5 that are included in the ECL Plex™ fluorescent Western blot system (Figure 6). The red channel is four times brighter and the green channel 20 times brighter in the WesternBright MCF blot. The brighter signal provides higher sensitivity, allowing 0,6 ng of AFP to be detected using WesternBright MCF, compared to only 5.5 ng detected using Cy3.

The greater signal from WesternBright MCF can also allow researchers to conserve resources, by loading less precious sample, or using less primary antibody in every experiment.

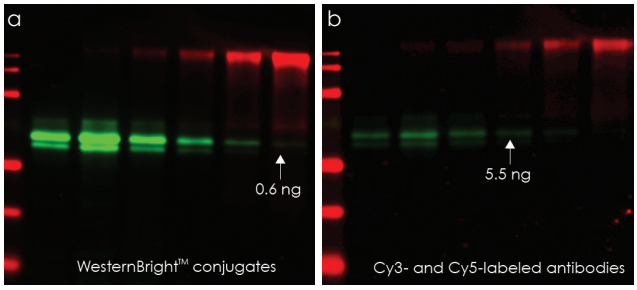

Figure 6. WesternBright MCF conjugates provide a brighter signal than Cy™ Dye labeled secondary antibodies. Duplicate Western blots containing samples of AFP and CEA were treated identically, and probed with the same mixture of mouse anti-AFP and rabbit anti-CEA primary antibodies. One blot was then stained with WesternBright MCF conjugates (Figure 6a) and the other with Cy3 anti-mouse and Cy5 anti-rabbit antibodies (Figure 6b), following the protocol recommended for ECL Plex™. One hour after the final wash, when blots were dry, the blots were imaged on a FluorChem Q (Cell Biosciences), using settings optimized for Cy3 and Cy5 dyes. Exposure times were 8 seconds in the red channel and 2 seconds in the green, and brightness and contrast settings were adjusted identically for both images.

Conduct 3-color Western blots

The excitation and emission spectra of the WesternBright conjugates are compatible with the use of blue fluorescent dyes such as Cy2 and HiLyte Fluor™ 488, allowing 3-color blots to be performed (Figure 7). With three-color fluorescent blots, three proteins can be assayed in one experiment, allowing two proteins to be assayed in addition to a loading control.

Normalization of a protein of interest to a loading control is made simple and straightforward with multicolor Western blotting. In Figure 7b, the intensities of two proteins (CEA and AFP) are reported relative to a loading control (Transferrin) that was detected in the blue channel using a Cy2-labeled primary antibody.

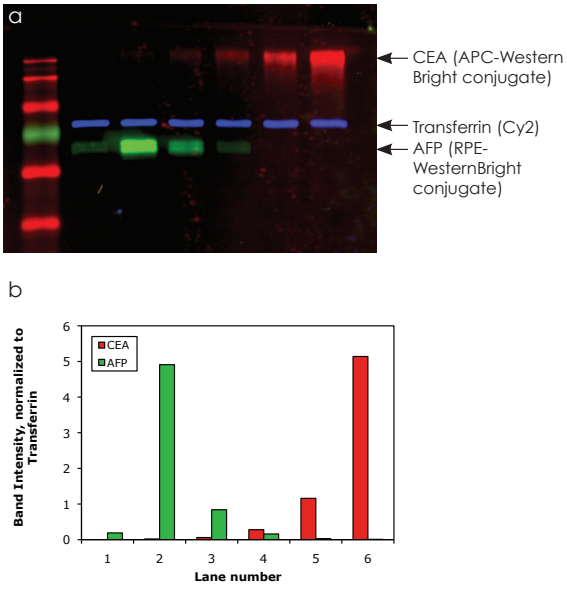

Figure 7. Multicolor fluorescent WesternBright blots provide quantitative data. With multicolor fluorescent Westerns, simultaneous detection of loading controls allows protein amounts to be accurately quantified, and different samples to be accurately compared. WesternBright conjugates are compatible with common blue dyes, allowing 3-color blots to be conducted. In Figure 7, a Cy2-labeled primary antibody was used to detect the loading control, Transferrin, while WesternBright conjugates were used to detect CEA (red) and AFP (green) proteins. The intensities of the CEA and AFP bands, normalized to the loading control, are shown (Figure 7b).

Complete Kit

The WesternBright MCF kit (Figure 8) provides all the reagents necessary to conduct two-color fluorescent Westerns, including secondary fluorescent antibody conjugates, a novel blocking solution, AdvanBlock-PF, AdvanWash™ washing solution, pre-cut, low-background fluorescent PVDF membranes, and background quenching sheets. WesternBright MCF is designed to work with mouse and rabbit primary antibodies.

AdvanBlock-PF is fast-acting, quickly blocking non-specific protein binding sites on the PVDF membrane to reduce any background due to non-specific antibody binding. AdvanBlock-PF also stabilizes the WesternBright MCF conjugates, preserving their fluorescence.

Finding the optimal blocking agent for an antibody-antigen pair can be a process of trial and error. AdvanBlock-PF contains no protein, so can reduce the background observed with some primary antibodies that have a high degree of cross-reactivity to protein blockers such as BSA, casein or milk protein. For low-quality primary antibodies where protein-based blocking agents may be required, BSA or nonfat dry milk can be dissolved directly in AdvanBlock-PF solution and used to dilute the primary antibody.

The pre-cut PVDF membranes provided with WesternBright MCF are optimized to have very low autofluorescence, maximizing the fluorescent signal that can be detected.

Over time, sample holders, support covers and other components of imaging instrumentation may become contaminated with random fluorescent materials. The background quenching sheets included with the WesternBright MCF kit have virtually no autofluorescence across the visible spectrum, and can be used to block unwanted fluorescence from the imaging environment to dramatically increase signal to noise ratios.

Figure 8. The WesternBright MCF Western Blotting Kit contains all the reagents needed to carry out 10 two-color blots.

Ordering Information

Catalog Number	Product	Size
K-12021-010	**WesternBright™ MCF fluorescent Western blotting kit** (Contains: secondary antibodies, washing solution, blocking solution, pre-cut PVDF membranes and background quenching sheets)	**10 blots**

Related Products

R-05051-250	APC-goat-anti-rabbit IgG conjugate	250 µl
R-05052-250	RPE-goat-anti-mouse IgG conjugate	250 µl
R-03023-D20	AdvanBlock™-PF protein-free 5x blocking solution	200 ml
R-03024-D50	AdvanWash™ 10x washing solution	500 ml
L-07001-010	Background quenching sheets	10 sheets
L-08001-010	Low-fluorescence PVDF membrane, 9x7 cm	10 sheets

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