

WesternBright™ MCF and MCF-IR

Quantitative, multi-color fluorescent Western blotting kits

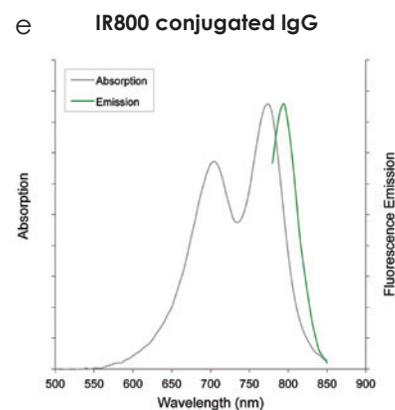
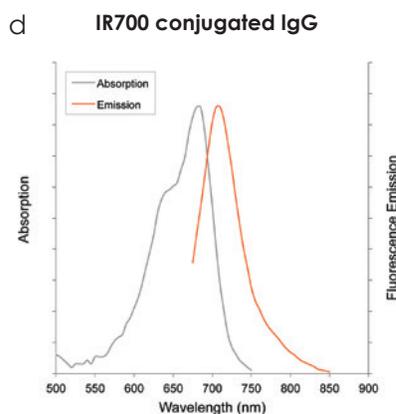
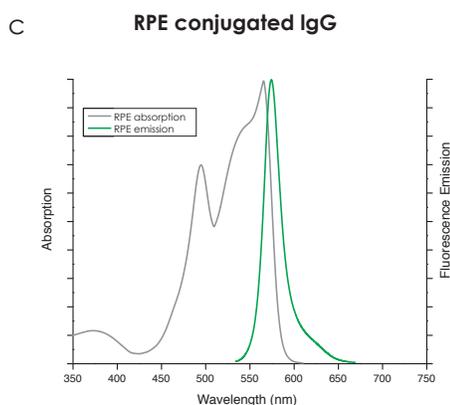
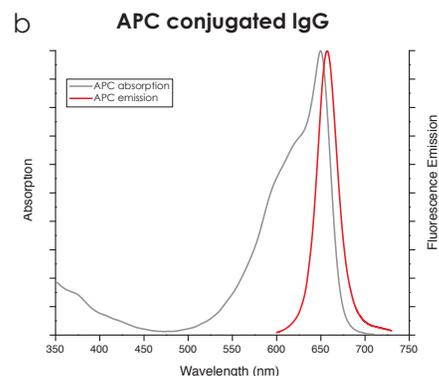
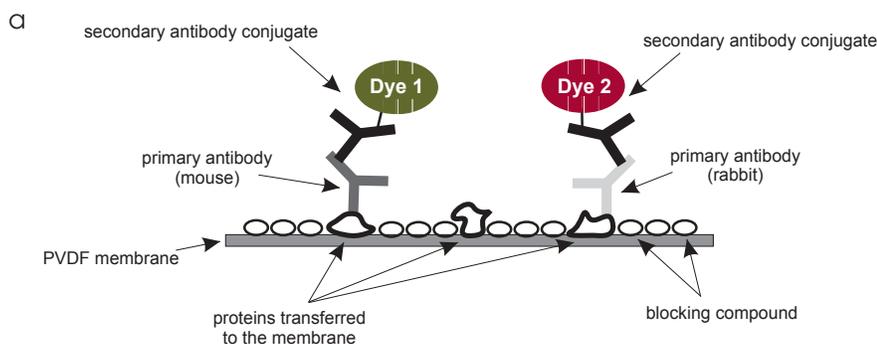


WesternBright MCF visible and near infrared (IR) fluorescent Western blotting kits allow the assay of two proteins at once, increasing the quality and quantity of information that can be gained from a single blot. Assay a control alongside a protein of interest. Assay phosphorylated and un-phosphorylated isoforms of a protein simultaneously. The fluorescent dyes provided with WesternBright MCF and WesternBright MCF-IR outperform Cy™ dyes and allow detection of low pg of protein. The WesternBright MCF protocols save time and money since there is no need for disposable film, and the blot can be imaged immediately, without drying.

WesternBright MCF and MCF-IR allow the assay of two or more proteins on one blot with the sensitivity of chemiluminescence. Save time and money relative to chemiluminescence detection by avoiding the need to run duplicate blots or to strip and re-probe a blot to study multiple targets. WesternBright MCF and MCF-IR are quick, easy to use kits that provide everything necessary to conduct two-color Westerns.

Two-Color Western Blotting

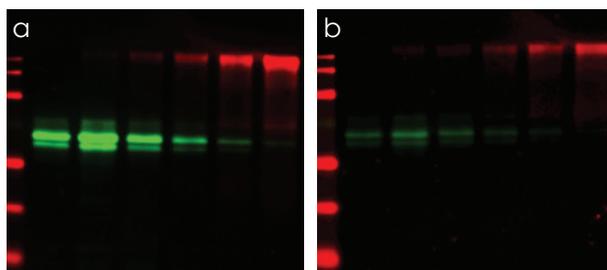
The principle of two-color Western blotting. Using secondary antibodies labeled with different fluorophores, two proteins can be detected on a single blot by controlling excitation and detection channels (a). WesternBright MCF conjugates incorporate the phycobiliproteins allophycocyanin (APC) and R-phycoerythrin (RPE), extremely bright fluorescent proteins from cyanobacteria and eukaryotic algae (b,c). WesternBright MCF-IR conjugates incorporate IR700 and IR800 fluorescent dyes with near-infrared excitation and emission wavelengths (d,e).



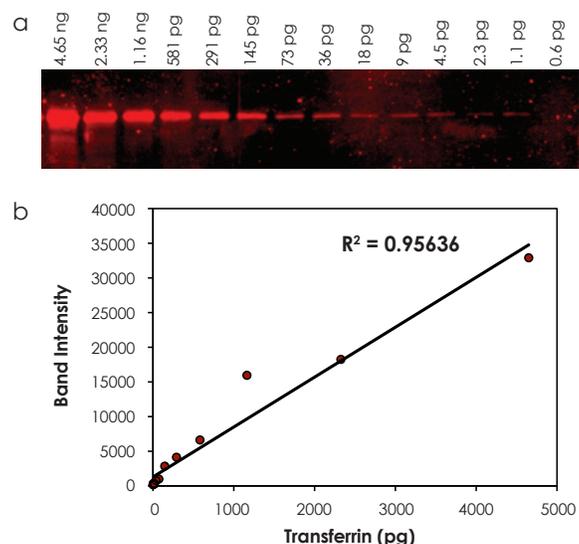
WesternBright™ MCF

Brighter than Cy Dyes

WesternBright conjugates provide a brighter signal than the ECL Plex Western blotting system. Duplicate Western blots containing samples of AFP and CEA proteins 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 conjugates and the other with an identical concentration of Cy3 anti-mouse and Cy5 anti-rabbit antibodies following the protocol recommended for ECL Plex. Under identical imaging conditions, WesternBright MCF provides a brighter signal, and 10x greater sensitivity.



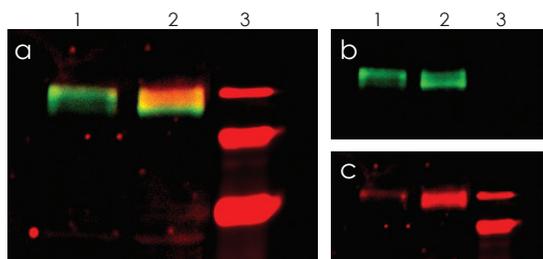
Picogram Detection Levels, Linear Data



Detect picograms of protein with WesternBright MCF and MCF-IR. WesternBright MCF and MCF-IR are as sensitive as chemiluminescence, while providing more quantitative data than chemiluminescence. The fluorescent signal is directly related to the amount of bound antibody, and not dependant on an enzymatic reaction as is a chemiluminescent signal. A blot containing a serial dilution of Transferrin was probed with rabbit-anti-transferrin primary antibody, and detected using WesternBright conjugate APC-goat-anti-rabbit IgG. As little as 1.1 pg of transferrin can be visualized in the resulting image (a). The signal is linear with respect to protein concentration over 3 orders of magnitude (b).

Why Two-Color Westerns?

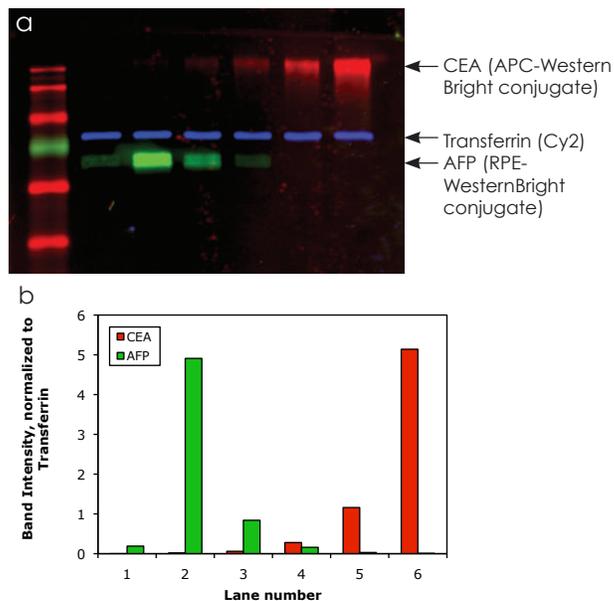
Simultaneously Detect Closely Migrating Proteins



Simultaneous detection of EGFR and phospho-EGFR with WesternBright MCF. The increase in phosphorylation of EGFR in response to EGF was detected. Chemiluminescence detection of the same experiment would require stripping and reprobing the blot, which can introduce errors due to protein loss, or incomplete stripping of primary antibody. Lysates from A431 cells (lane 1) and from A431 cells treated with EGF (lane 2) were blotted and EGFR detected in the green channel (b) or phospho-EGFR detected in the red channel (c). The two channels are superimposed in (a). Lane 3: molecular weight markers.

Get More Data from Every Blot

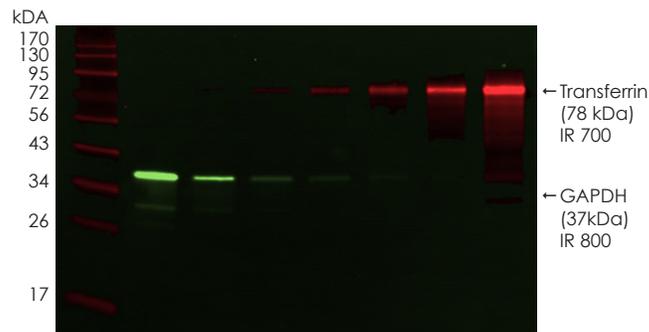
Multicolor fluorescent WesternBright blots provide quantitative data. With multicolor fluorescent Westerns, simultaneous detection of loading controls allows protein amounts to be accurately quantified, and different samples to be accurately compared. WesternBright conjugates are compatible with common blue dyes, allowing 3-color blots to be conducted. In the figure, 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 (b).



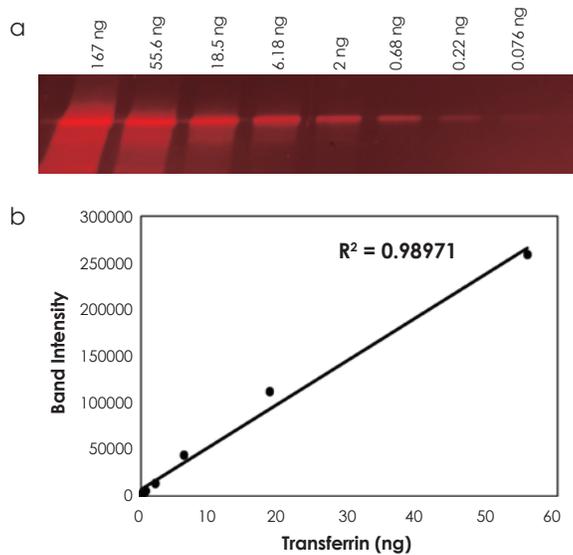
WesternBright™ MCF-IR

Two-Color Near Infrared Blots

Detect two proteins in one experiment with WesternBright MCF-IR. Near IR dyes are ideal for Western blot applications because autofluorescence from membranes decrease with wavelengths near-IR spectra giving you cleaner western blots. A blot of serial dilutions of transferrin and GAPDH was probed simultaneously with rabbit-anti-transferrin primary antibody and mouse-anti-GAPDH and detected with WesternBright goat-anti-rabbit IgG IR700 and WesternBright goat-anti-mouse IgG IR800.



Broad Linear Range



Choose WesternBright MCF and MCF-IR for quantitative blotting experiments. Both kits provide sensitive detection and a broad linear range. A blot containing a serial dilution of Transferrin was probed with rabbit-anti-transferrin primary antibody, and detected using WesternBright goat-anti-rabbit IgG IR700. As little as 25 pg of transferrin can be visualized in the resulting image without saturation of a high concentration band containing 55 ng of protein (a). The signal is linear with respect to protein concentration over 3 orders of magnitude (b).

WesternBright™ MCF and MCF-IR

Quantitative, multi-color fluorescent Western blotting kits

WesternBright MCF and MCF-IR Western blotting kits provide all materials needed to conduct 2-color Western blots: fluorescent labeled secondary antibodies, blocking solution, washing solution, pre-cut low-autofluorescence PVDF membranes, and background quenching sheets.



Features

- **FLUORESCENT DETECTION** – of two proteins, with sensitivity 10x greater than Cy dyes
- **ADVANBLOCK™-PF** – blocking solution for fast, protein-free blocking and greater signal-to-noise ratios
- **HIGH SENSITIVITY** – detect picograms of protein
- **FAST RESULTS** – entire protocol can be completed in 3.5 hours
- **WESTERNBRIGHT MCF** – compatible with imaging systems that detect Cy3 and Cy5 or similar dyes
- **WESTERNBRIGHT MCF-IR** – compatible with near-IR imaging systems
- **ADVANWASH and ADVANWASH-IR** – washing solutions optimized for chemiluminescent and fluorescent or for near-IR fluorescent Western blots

Ordering Information

Catalog Number	Product		Size
K-12021-010	WesternBright™ MCF fluorescent Western blotting kit	Goat-anti-rabbit IgG APC/ Goat-anti-mouse IgG RPE	10 blots
K-12022-010	WesternBright™ MCF-IR fluorescent Western blotting kit	Goat-anti-rabbit IgG IR700/ Goat-anti-mouse IgG IR800	10 blots
K-12023-010	WesternBright™ MCF-IR fluorescent Western blotting kit	Goat-anti-mouse IgG IR700/ Goat-anti-rabbit IgG IR800	10 blots

Related Products

R-03090-D25	FLASHBlot™ Transfer Buffer, 50x concentrate	Sufficient for up to 50 blots	250 ml
R-03090-D50	FLASHBlot™ Transfer Buffer, 50x concentrate	Sufficient for up to 100 blots	500 ml
L-07045-060	Pre-cut Blotting Paper	7 x 9 cm, vertical gels	60 sheets
L-07046-060	Pre-cut Blotting Paper	8 x 10 cm, vertical gels	60 sheets

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