WesternBright™ Quantum

Quantify chemiluminescent Western blots over a wide dynamic range

WesternBright Quantum is a new chemiluminescent reagent specially formulated for CCD imaging. This novel Horseradish peroxidase (HRP) substrate provides a strong, long-lasting signal, the broadest useful linear range and high sensitivity for the most quantitative chemiluminescent Western assays.

Chemiluminescence is the method of choice for sensitive Western blot detection, but has not been considered quantitative, primarily because of the limited linear dynamic range of film, and of commercially available substrates. WesternBright Quantum HRP substrate overcomes the limitations of other substrates, showing no substrate depletion at high protein loads. WesternBright Quantum is specially formulated for quantitative chemiluminescent Western blotting, producing a linear signal over a broad range of protein concentrations spanning 3 orders of magnitude. Combined with CCD imaging, which provides a much greater linear dynamic range than film, WesternBright Quantum allows highly quantitative data to be obtained from chemiluminescent Western blots.

The broadest linear range for the most powerful quantitation

Accurate comparison of the intensities of different protein bands requires that the bands be within the linear dynamic range of detection, which is the range of concentrations from the faintest band that can be detected to the most intense band for which the signal is not saturated. When used to detect a Western blot containing a serial dilution of transferrin protein

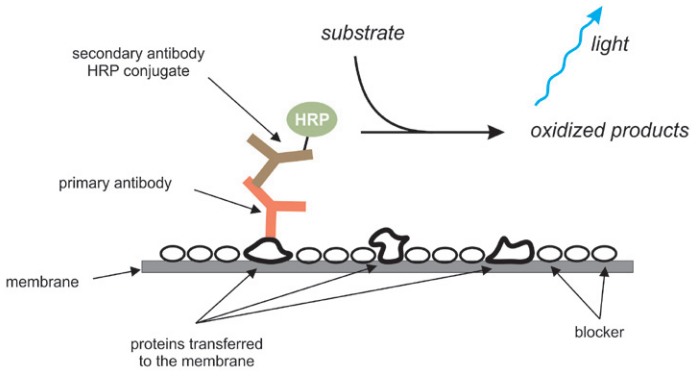

Figure 1. The principle of chemiluminescent Western blotting. A secondary antibody, conjugated to horseradish peroxidase (HRP) binds to a primary antibody directed towards the protein of interest. The blot is incubated with a chemiluminescent substrate, which is converted by HRP into a light emitting luminescent molecule.

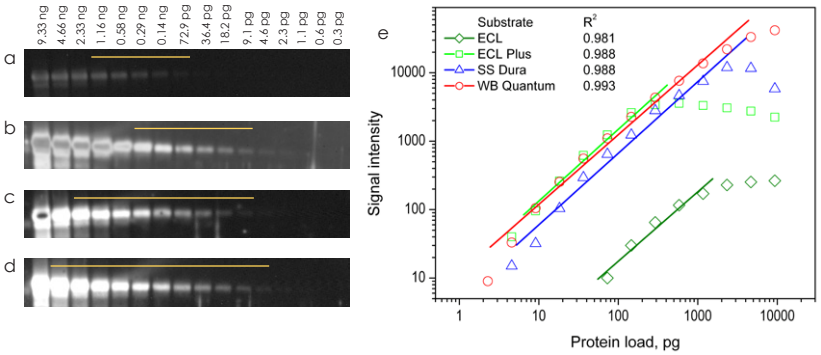

Figure 2. Linear dynamic range of WesternBright Quantum. Identical Western blots containing serial dilutions of transferrin were probed with a rabbit-anti-transferrin primary antibody, and a goat-anti-rabbit secondary antibody conjugated to Horseradish peroxidase. The blots were incubated with chemiluminescent substrates as recommended by each manufacturer. All blots were simultaneously imaged for 2 minutes on a CCD imager and all display parameters are identical across all images shown in the figure. WesternBright Quantum shows the largest dynamic range out of all four substrates with the highest R^2 value. a. Amersham™ ECL™ (GE Healthcare); b. Amersham™ ECL Plus™ (GE Healthcare); c. SuperSignal® West Dura (Thermo Scientific); d. WesternBrights Quantum; e. Signal intensity vs protein amount plot showing best fit linear regression for all four substrates. Bands that fall on the linear part of the curve are indicated.

(Figure 2), WesternBright Quantum provides the broadest linear dynamic range when compared to several other commercially available chemiluminescent substrates (Figure 2e, Table 1). Notably, no substrate depletion is seen at any protein loads with WesternBright Quantum, while substrate depletion interferes with detection of high amounts of protein by both ECL Plus and SuperSignal West Dura (seen as reverse intensity bands in Figures 2b and 2c). The ability to detect high amounts of protein without substrate depletion contributes to the increased dynamic range provided by WesternBright Quantum.

For determining linear range, best fit data analysis was performed using linear regression. Data points corresponding to high protein amounts were excluded from datasets one by one as outliers to obtain the broadest range with R^2 value above 0.98. Using this method, WesternBright Quantum produced the broadest linear range with the highest R^2 value.

Long lasting signal

CCD exposure times with WesternBright Quantum are as quick as film exposures with other substrates. In addition, WesternBright Quantum provides greater signal stability than the competition, allowing long-term CCD camera exposures to be conducted, if desired, to detect faint bands and low-abundance proteins. The long-lasting signal also means there is no need to rush to image a blot, since the signal will remain strong for several hours. To follow signal strength over time, GAPDH was detected on quadruplicate Western blots containing serial dilutions of HeLa cell lysate (Figure 3). A band which was determined to be within the linear range of all four chemiluminescent substrates tested was quantified at several time points over the next 10 hours. WesternBright Quantum maintained signal strength, with signal declining only approximately 30% over a 60 minute span, while the signal from ECL, ECL Plus and SuperSignal West Dura each decayed by almost 90%, to barely detectable levels, over the same period (Figure 3a). In fact, the WesternBright Quantum signal is still detectable in a 2 minute exposure 10 hours later (inset, Figure 3a and 3d).

Substrate	Linear Dynamic Range
WesternBright Quantum	4.6 pg – 4.7 ng
SuperSignal West Dura	9.1 pg – 2.3 ng
ECL Plus	9.1 pg – 0.29 ng
ECL	73 pg – 1.2 ng

Table 1. Linear dynamic range for four chemiluminescent substrates. The linear dynamic range includes data points that produce the best possible linear regression fit (Figure 2e). Analysis of the experiment depicted in Figure 2.

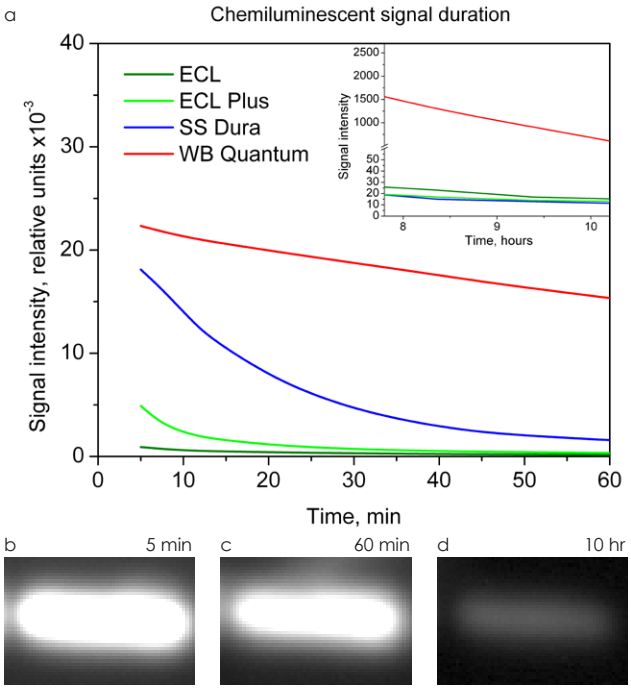

Figure 3. Signal duration of WesternBright Quantum. Blots detected using WesternBright Quantum and three other chemiluminescent substrates were imaged simultaneously at several times over a 10 hour period. Each exposure was 2 min long for all blots and all substrates. Intensities of bands containing the same amount of protein are plotted for each substrate in 3a. Images of this band on the WesternBright Quantum blot obtained after 5 min (3b), 60 min (3c) and 10 hr (3d) are shown.

Low background for high sensitivity

WesternBright Quantum produces an exceptionally strong signal with little to no background, for high signal to noise ratio and excellent sensitivity. The low background is especially apparent when WesternBright Quantum is is used with film detection. To demonstrate the relative lack of background with WesternBright Quantum compared to other chemiluminescent substrates, quadruplicate Western blots were detected using various HRP substrates as described in Methods. The four blots were simultaneously exposed to a single film. After a 20 minute exposure, the blot detected using WesternBright Quantum remains clear of background (Figure 4d) compared to ECL Plus (Figure 4b) or SuperSignal West Dura (Figure 4c).

Figure 5 demonstrates the superior sensitivity of WesternBright Quantum on film; duplicate blots were detected using WesternBright Quantum or SuperSignal West Pico. In a short exposure, WesternBright Quantum (Figure 5a) detects a band of a truncated protein not visible with the other reagent (Figure 5b) unless a longer exposure is used (Figure 5c).

Accurately quantify low and high abundance proteins

WesternBright Quantum's high sensitivity, low background and broad linear range combine to allow the accurate quantitation of low and high abundance proteins in a single experiment. Low abundance proteins can be detected due to the excellent sensitivity, and the broad linear dynamic range allows high abundance proteins to be detected with the same exposure, without saturation of signal. Figure 6 demonstrates the superior performance of WesternBright Quantum when probing for either a high abundance (actin) or low abundance (STAT-1) protein. Serial dilutions of extracts from A431 cells were assayed by replicate Western blots, probed for actin and STAT-1, and visualized using WesternBright Quantum or other commercially available chemiluminescent substrates as described in Methods. For quantitative detection on the linear part of the intensity vs protein curve, WesternBright Quantum proved to be 8-times more

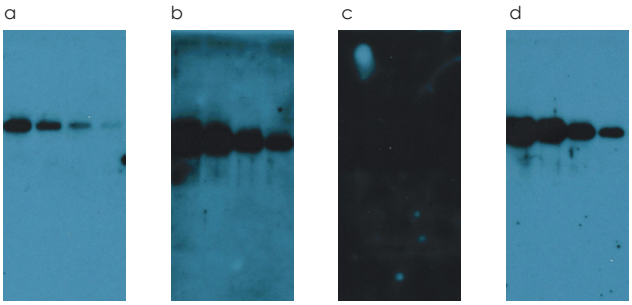

Figure 4. Extremely low background with WesternBright Quantum. Duplicate Western blots were developed using (a) ECL, (b) ECL Plus, (c) SuperSignal West Dura, or (d) WesternBright Quantum as substrates. After a 20 minute exposure to film, WesternBright Quantum displays the best combination of sensitivity and signal with low background. All display parameters are identical across all images shown in this figure.

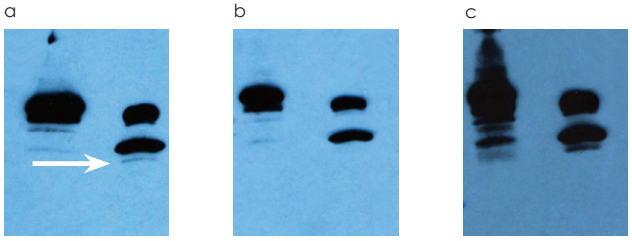

Figure 5. Superior sensitivity of WesternBright Quantum with film detection. WesternBright Quantum (a) or SuperSignal West Pico (Thermo Scientific) (b, c) were used to detect duplicate Western blots. A band (arrow in panel a) is detected by WesternBright Quantum in a brief exposure, while a much longer exposure is needed to detect the same band with SuperSignal West Pico (c).

sensitive than ECL Plus in detecting STAT-1 (Figure 6g), and twice as sensitive as ECL in detecting actin (Figure 6c).

Simply the best choice for CCD imaging

WesternBright Quantum outperforms other enhanced chemiluminescent reagents, providing superior sensitivity, signal duration, and linear dynamic range. Specially formulated for CCD imaging, WesternBright Quantum maintains a detectable signal in a two-minute exposure for at least 10 hours, long after the signals from other substrates have decayed to levels undetectable without exposures many times longer.

Methods

Gel electrophoresis

Proteins were separated on self-made 12 % polyacrylamide gels using a Laemmli buffer system. Gels were 10 cm wide and 0.8 mm thick.

Transfer

Proteins were transferred to PVDF membrane using a tank (Idea Scientific) and buffers developed by Bolt and Mahoney (1). Transfers were conducted for 25 min at 24 V.

Blocking, Primary and Secondary Antibodies, and Washing

Membranes were blocked with 2% non-fat dry milk in AdvanWash buffer for 1 hour at room temperature (RT). Primary and secondary antibodies were diluted as described in Table 2 in blocking buffer, and applied to membranes for 1 hour at RT. Washing was conducted with AdvanWash buffer according to the WesternBright user manual.

Substrate incubation

After washing, blots were incubated with chemiluminescent substrates as recommended by manufacturers. Incubation with WesternBright Quantum substrate was done for 2 minutes.

Imaging

After incubation, blots were placed on a plastic sheet, covered with Saran wrap, and imaged using a FluorChem Q (Cell Biosciences). Images were analyzed using AlphaView® software (Cell Biosciences).

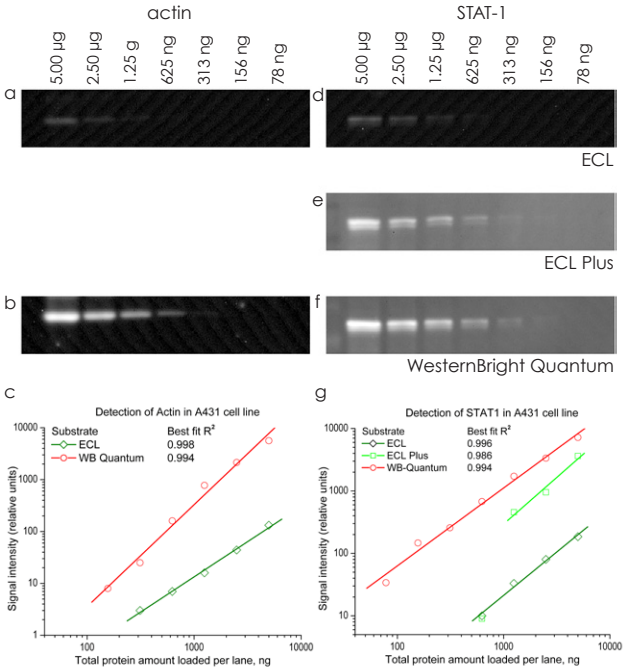

Figure 6. Detect high and low abundance proteins with WesternBright Quantum. Identical blots containing serial dilutions of cell A431 lysates were probed with a mixture or primary antibodies to actin and STAT-1. The blots were then detected with WesternBright Quantum (panels b and f) or one of two other chemiluminescent substrates and imaged simultaneously with a 2 minute exposure using a CCD imager. The intensities of the bands were plotted against total protein loaded (panels c and g), demonstrating that WesternBright Quantum provided the highest sensitivity and the broadest linear dynamic range for both the high and low abundance proteins.

Experiment	Primary antibody	Dilution	Secondary antibody	Dilution
Linear dynamic range	Rabbit anti-transferrin (Abcam, cat. no. ab122301)	1:10,000	HRP-Goat-anti-rabbit (Advansta, cat. no. R-05072-500)	1:20,000
Signal duration	Mouse anti-GAPDH (Millipore, cat. no. MAB374)	1:2,000	HRP-Goat-anti-mouse (Advansta, cat. no. R-05071-500)	1:2,500
Cell lysates	Mouse anti-actin (Sigma, cat. no. A4700)	1:1,000	HRP-Goat-anti-mouse (Advansta, cat. no. R-05071-500)	1:2,500
	Mouse anti-STAT 1 (BD, cat. no. 10116)	1:250	HRP-Goat-anti-mouse (Advansta, cat. no. R-05071-500)	1:2,500

Table 2. Antibodies and dilutions used.

References

1. Bolt M.W. and Mahoney P.A. 1997. Anal Biochem. 247. 185-192

Catalog Number	Product	Size
K-12042-C20	WesternBright™ Quantum Western Blotting HRP Substrate Trial kit size	20 ml (200 cm^2)
K-12042-D10	WesternBright™ Quantum Western Blotting HRP Substrate (for 1000 cm^2 membrane)	100 ml (1000 cm^2)
K-12042-D20	WesternBright™ Quantum Western Blotting HRP Substrate (for 2000 cm^2 membrane)	200 ml (2000 cm^2)
L-07014-100	LucentBlue™ X-ray film	100 sheets
R-03024-D50	AdvanWash™ 10x washing solution	500 ml
R-05072-500	Goat-anti-rabbit HRP-conjugated secondary antibody	500 µl
R-05071-500	Goat-anti-mouse HRP-conjugated secondary antibody	500 µl
L-08001-010	Low Fluorescence Western Membrane (PVDF) 7x9 cm	10 sheets
L-08002-010	Nitrocellulose Transfer Membrane 0.45 µm 7x9 cm	10 sheets
L-08003-010	Nitrocellulose Transfer Membrane 0.22 µm 7x9 cm	10 sheets

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