WesternBright® ECL

Save antibody with the most sensitive HRP substrate for film-imaged Western blots

WesternBright ECL is an HRP substrate formulated for chemiluminescent Western blots imaged using film. More sensitive than other HRP substrates developed for routine use, WesternBright ECL saves time and money by allowing you to conserve precious antibody, load less sample, and reduce exposure times.

Western blotting is a prevalent technique to detect the presence of a protein of interest in a complex mixture, or to detect protein modifications such as phosphory-lation. Chemiluminescence (Figure 1) is the most common method of detecting Western blots, with the light signal frequently imaged using X-ray film. WesternBright ECL is an HRP substrate developed for film-imaged Western blots, providing an optimal combination of sensitivity, signal longevity, and low background for high-quality film images.

WesternBright ECL produces a strong signal for high-sensitivity detection. Compared to other HRP substrates, WesternBright ECL can allow exposure times to be shortened, less protein to be loaded onto gels, or less primary and secondary antibodies to be used, adding up to substantial savings of time and money. Add to this the convenience of a substrate so stable that a blot can be exposed to film one hour after substrate incubation with no loss of sensitivity, and WesternBright ECL is simply the best choice for routine film detection of Western blots.

Save antibody

Since WesternBright ECL produces a stronger signal than other substrates, less antibody is needed to achieve a similar image. Duplicate slot

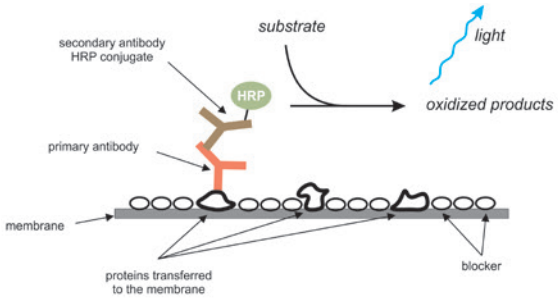

Figure 1. The principle of chemiluminescent Western blotting. A secondary antibody, conjugated to horseradish peroxidase (HRP) binds to a primary antibody directed towards the protein of interest. The blot is incubated with a chemiluminescent substrate, which is converted by HRP into a light emitting luminescent molecule that is detected by exposure to film. WesternBright ECL is specially formulated to produce a strong signal for sensitive film imaging.

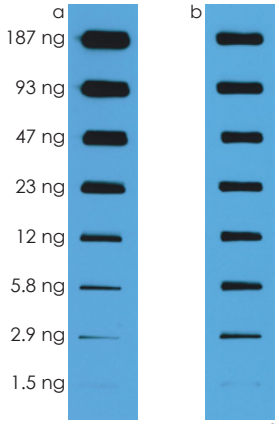

Figure 2. WesternBright ECL detects equal amounts of protein with up to ten times less antibody. Duplicate slot blots on nitrocellulose membrane containing serial dilutions of transferrin were probed with the antibody dilutions shown, and detected with either WesternBright ECL or Pierce® ECL (Thermo Scientific) according to the manufacturers' instructions. The blots were imaged simultaneously on the same piece of film with a 30 second exposure. WesternBright ECL produced a comparable image with ten times less primary (rabbit anti-transferrin, Abcam ab1223-1) and five times less secondary antibody (goat anti-rabbit, Advansta).

blots were conducted using the same primary and secondary antibodies at the dilutions indicated in Figure 2, and detected using either WesternBright ECL (Figure 2a) or Pierce ECL (Figure 2b). The two substrates demonstrated similar sensitivities, while 5-times less primary and 10-times less secondary antibody were used with WesternBright ECL than Pierce ECL. The ability to use less antibody can generate substantial savings over time.

Faster exposure times

Another advantage to WesternBright ECL is that exposure times can be reduced, saving time and increasing productivity. Duplicate Western blots were detected using identical antibody dilutions and either WesternBright ECL (Figure 3a) or Amersham™ ECL™ (GE Healthcare) (Figures 3b and c) as HRP substrate. WesternBright ECL produced a stronger signal and more sensitive detection (73 pg) after a 30 second exposure than Amersham ECL in a 5 minute exposure (292 pg) (Figure 3c).

Higher sensitivity

The stronger signal obtained with WesternBright ECL translates into higher sensitivity when all other aspects of the experiment are the same. Triplicate slot blots containing serial dilutions of transferrin protein were detected using identical antibody concentrations and either WesternBright ECL (Figure 4a), Amersham ECL (Figure 4b) or Pierce ECL (Figure 4c) as HRP substrate according to the manufacturers' instructions. All three blots were simultaneously exposed to the same piece of film for 15 seconds. WesternBright ECL provided the highest sensitivity, with a detection limit of 23 pg, compared to Amersham ECL (182 pg) or Pierce ECL (91 pg).

In a second experiment, WesternBright ECL was compared to Amersham ECL, Pierce ECL and SuperSignal® West Pico. Identical slot blots containing serial dilutions of an HRP-conjugated antibody were detected using each HRP substrate according the manufacturers' instructions. The blots were exposed

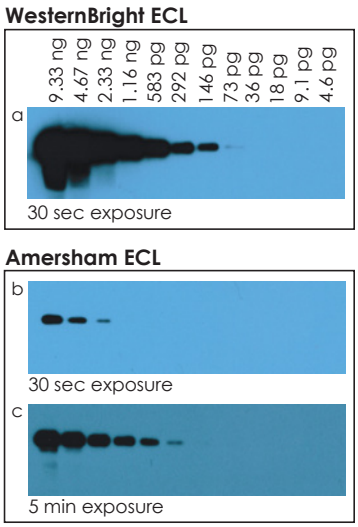

Figure 3. Higher sensitivity detection and shorter exposure times with WesternBright ECL. Samples of serial dilutions of transferrin protein were separated by PAGE in duplicate gels and transferred to PVDF membranes. The resulting blots were detected using WesternBright ECL or Amersham ECL according to the manufacturers' instructions. The blots were exposed simultaneously to the same piece of film. In a 30 second exposure, WesternBright ECL produced a much stronger signal than a 5 minute exposure of the blot detected with the other substrate. Primary antibody dilution was 1:10,000 (rabbit anti-tranferrin, Abcam ab1223-1), and secondary antibody dilution was 1:20,000 (goat anti-rabbit, Advansta).

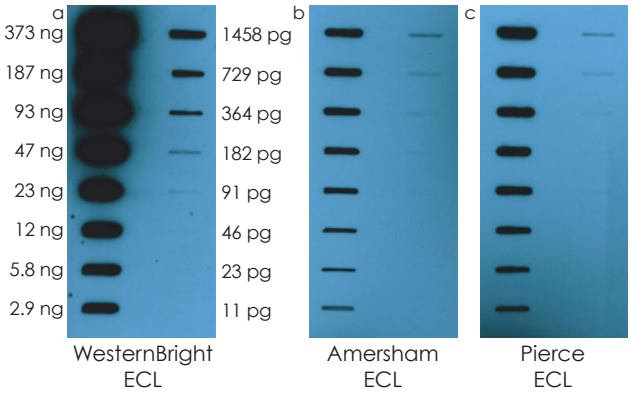

Figure 4. WesternBright ECL provides the highest sensitivity. Triplicate slot blots containing serial dilutions of transferrin protein were detected using WesternBright ECL or one of two other substrates according to the manufacturers' instructions. The blots were simultaneously exposed to the same piece of film. In a 15 second exposure, a 23 pg band can be seen on the WesternBright ECL blot, approximately 8 times more powerful than the blots conducted using other substrates. Primary antibody dilution was 1:10,000 (rabbit anti-tranferrin, Abcam ab1223-1), and secondary antibody dilution was 1:20,000 (goat anti-rabbit, Advansta).

simultaneously to the same piece of film for 5 seconds (Figure 5). WesternBright ECL outperforms all the other substrates, producing a substantially stronger signal for greater sensitivity.

Long-lived signal for convenient imaging

The signal from WesternBright ECL is very stable, allowing for more convenient imaging. The image of a slot blot obtained 1 hour after incubation with WesternBright ECL (Figure 6b) is similar to that obtained 5 minutes after WesternBright ECL incubation (Figure 6a). In fact, substantial signal remains 6 hours after substrate incubation (Figure 6c). With such signal stability, there is no need to rush to the darkroom, or worry about signal loss, resulting in much more flexible imaging.

The signal stability of WesternBright ECL allows more signal to be captured by long-term exposures than is possible with shorter lived substrates. Figure 7 demonstrates that a 60 minute exposure of a blot detected using Amersham ECL is very similar to a 10 minute exposure of the same blot (Figure 6a and b), since the majority of the signal has disappeared by the time the 60 minute exposure has completed. A 60 minute exposure with WesternBright ECL, however, visualizes a band in the lane containing 1.25 µg of cell lysate (Figure 7d) that was previously undetected.

WesternBright ECL is the best choice for film-imaged chemiluminescent Western blots. With a strong, long-lived signal, WesternBright ECL provides high sensitivity and the ability to conserve precious samples and antibodies.

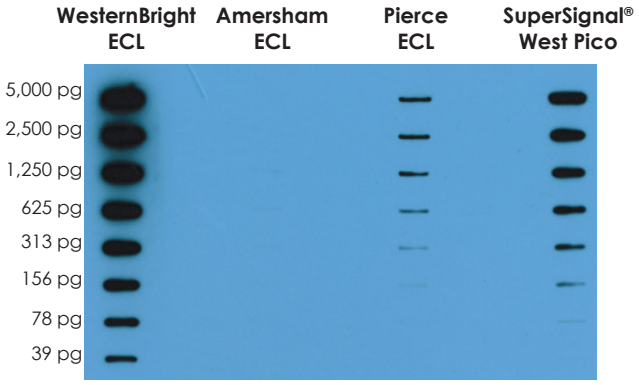

Figure 5. WesternBright ECL outperforms even high-sensitivity substrates. Slot blots containing serial dilutions of HRP-conjugated antibody (Advansta R-05072-500) were detected using WesternBright ECL or three other HRP substrates as indicated in the figure. All blots were simultaneously exposed to the same piece of film for 5 seconds. WesternBright ECL produces the strongest signal and highest sensitivity.

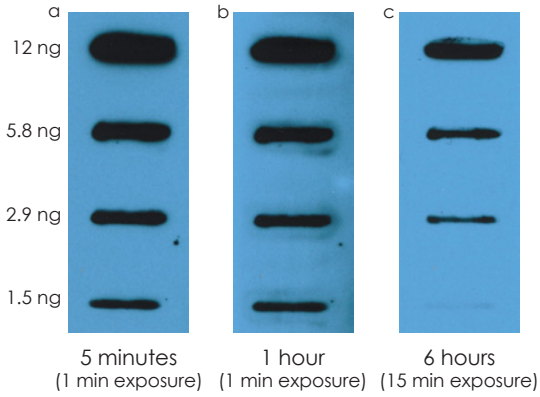

Figure 6. WesternBright ECL produces a long-lasting signal. A blot detected with WesternBright ECL can be imaged six hours after substrate incubation. There is no need to rush to the darkroom to image a blot.

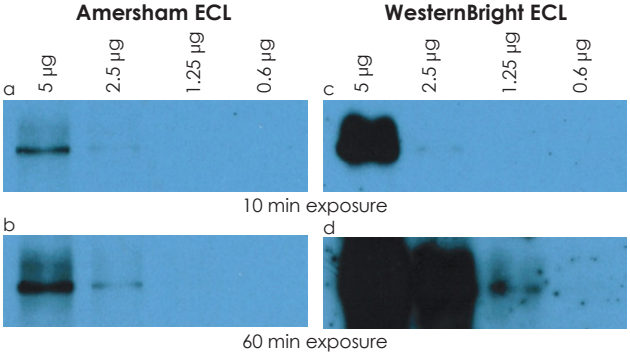

Figure 7. The greater stability of WesternBright ECL allows greater signal to be accumulated during long exposures, increasing the ability to detect very low abundance proteins. Duplicate Western blots of serial dilutions of total protein lysate obtained from HepG2 cell line were probed for ATP1A1 protein and detected using WesternBright ECL or Amersham ECL. In a 10 minute exposure, only 2 bands can be detected with either substrate. With a 60 minute exposure, a band is visible in the 1.25 µg lane of the WesternBright ECL blot, while signal decay results in little difference between a 10 minute or 60 minute exposure of the Amersham ECL blot.

Ordering Information

Catalog Number	Product		Size
K-12045-C20	WesternBright® ECL Western Blotting HRP Substrate	Trial size kit	20 ml
K-12045-D20	WesternBright® ECL Western Blotting HRP Substrate	For 2000 cm^2 membrane	200 ml
K-12045-D50	WesternBright® ECL Western Blotting HRP Substrate	For 5000 cm^2 membrane	500 ml

Related Products

K-12049-D50	WesternBright® ECL Spray		500 ml
L-07014-100	LucentBlue™ X-ray film	8 x 10" sheets	100 sheets
L-08001-010	Low-Fluorescence PVDF Transfer Membrane	7x9 cm	10 sheets
L-08002-010	Nitrocellulose Transfer Membrane 0.45 µm	8x10 cm	10 sheets
L-08003-010	Nitrocellulose Transfer Membrane 0.22 µm	8x10 cm	10 sheets
R-03024-D50	AdvanWash™ 10x washing solution		500 ml
R-05071-500	Goat-anti-mouse HRP-conjugated secondary antibody		500 µl
R-05072-500	Goat-anti-rabbit HRP-conjugated secondary antibody		500 µl

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