WESTAR ANTARES: High sensitivity and long-lasting substrate

Enhanced chemiluminescence (ECL) is the method of choice for detecting Western blots, as it provides the greatest sensitivity and convenience. Cyanagen has developed WESTAR, a product line of ECL substrates for Western blotting application. Each WESTAR substrate is at the top of its respective market segment in terms of performance/price ratio.

WESTAR ANTARES is a versatile substrate with stable light output at the mid-femtogram detection level: the perfect ECL formulation combining high sensitivity and extremely long signal duration.

Benchmarking

WESTAR ANTARES exhibits a mid-level performance for everyday experiments, offering a stable signal and a very good sensitivity. WESTAR ANTARES can substitute, without changes in the protocol, the most common substrates, such as Amersham™ ECL Prime™ (GE Healthcare), Clarity™ (Bio-Rad), Supersignal™ West Dura and West PICO PLUS (Thermo Scientific™). WESTAR ANTARES provides an excellent performance in routine Western blotting application, with higher signal intensity and sensitivity than several of its competitors, such as West PICO PLUS (Thermo Scientific™) and Clarity™ (Bio-Rad) (Figure 1).

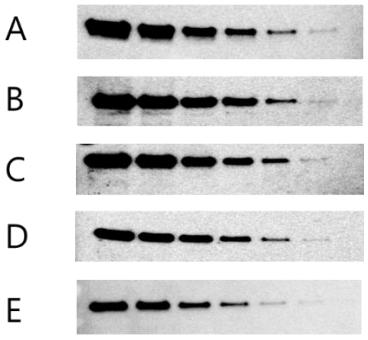

Figure 1. **Western blotting detection of HDAC-1 on HeLa cell lysate with WESTAR ANTARES and other chemiluminescent substrates in the same sensitivity range.** Sample: 2-fold dilution series of HeLa whole cell lysate (abcam®) from 5µg to 0,016 µg of total protein. Membrane: Trans-Blot® Turbo™ Mini Nitrocellulose Transfer Packs (Bio-Rad). Blocking: 2% ECL™ Blocking Agent (GE Healthcare) in PBS-T. Primary antibody: Rabbit-anti Human HDAC-1 (abcam®) 1:5000. Secondary antibody: Goat anti-rabbit IgG HRP (2mg/ml) (abcam®) 1:75000. ECL substrates used are: A) WESTAR ANTARES (Cyanagen); B) SuperSignal™ West Dura (Thermo Scientific™); C) Amersham™ ECL Prime™ (GE-Healthcare); E) SuperSignal™ West Pico Plus (Thermo Scientific™); E) Clarity™ (Bio-Rad); Imaging: ImageQuant™ LAS 4000 (GE Healthcare). Exposure time: 180 seconds.

Sensitivity and Precision

The goal of Western blotting is the detection of a target protein within its linear dynamic range. The linear dynamic range is the region over which the chemiluminescent emission is directly proportional to the concentration of the sample: this is essential for quantitative analysis. WESTAR ANTARES offers a wide linear dynamic range with an excellent R^2 value of 0.998, (Figure 2), allowing the user to accurately quantify protein bands.

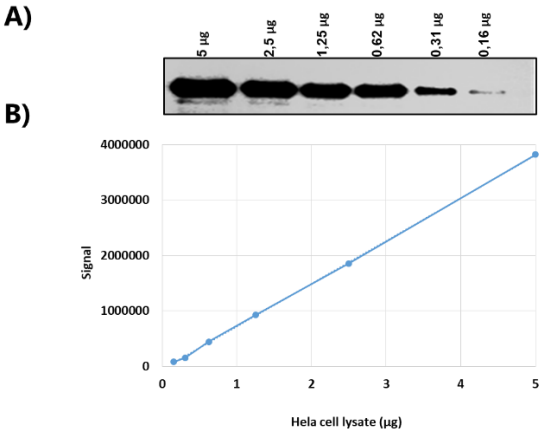

Figure 2. Enhanced precision in Western blotting with WESTAR ANTARES.

A) Western blotting detection of HDAC-1 on HeLa cell lysate with WESTAR ANTARES. Triplicate blots containing 2-fold dilutions of HeLa whole cell lysate were incubated with Rabbit-anti Human HDAC-1 1:5000 and Goat anti Rabbit-HRP 1: 75000 and imaged for 180 seconds with ImageQuant™ LAS 4000 (GE-Healthcare).

B) Integrated signal intensity. Data show that WESTAR ANTARES delivers a linear signal response, over a wide range of protein levels. (Dynamic range = DR; linearity = R^2).

WESTAR ANTARES produces a strong signal in the presence of a very low background level, resulting in a high signal-to-noise ratio and high sensitivity, comparable to Amersham™ ECL Prime™ (GE Healthcare) and Supersignal™ West Dura (Thermo Scientific™) and significantly better than Clarity™ (Bio-Rad) and Supersignal™ West PICO PLUS (Thermo Scientific™) (Figure 2). The high sensitivity, combined with its wide linear range, allows an excellent quantitation of low and high abundance proteins on the same blot, with a single exposure.

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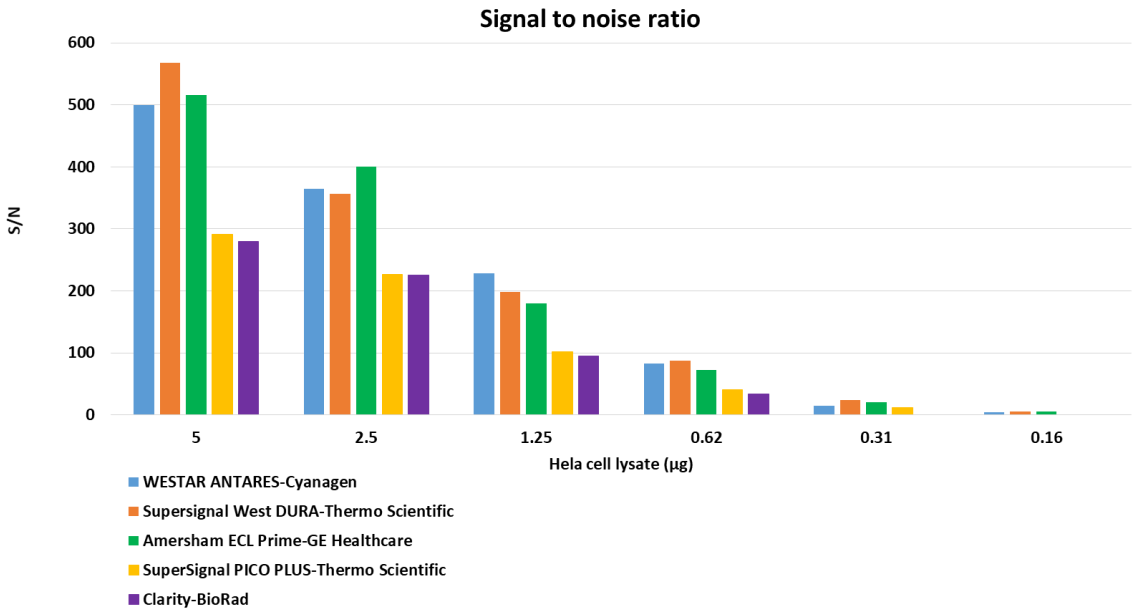

Figure 3. Signal-to-noise ratio (S/N) analysis of WESTAR ANTARES and its competitors. Triplicate blots for each substrate containing 2-fold dilutions of HeLa whole cell lysate from 5 to 0.016 µg were incubated with Rabbit-anti Human HDAC-1 1:5000 and Goat anti Rabbit-HRP 1: 75000 and were simultaneously imaged for 180 seconds with ImageQuant™ LAS 4000 (GE Healthcare).

Signal Duration

Since WESTAR ANTARES provides an extremely extended signal duration when compared to most mid-level range ECL substrates. The HDAC-1 signal intensity variation over time was analyzed using WESTAR ANTARES and its competitors (Figure 4).

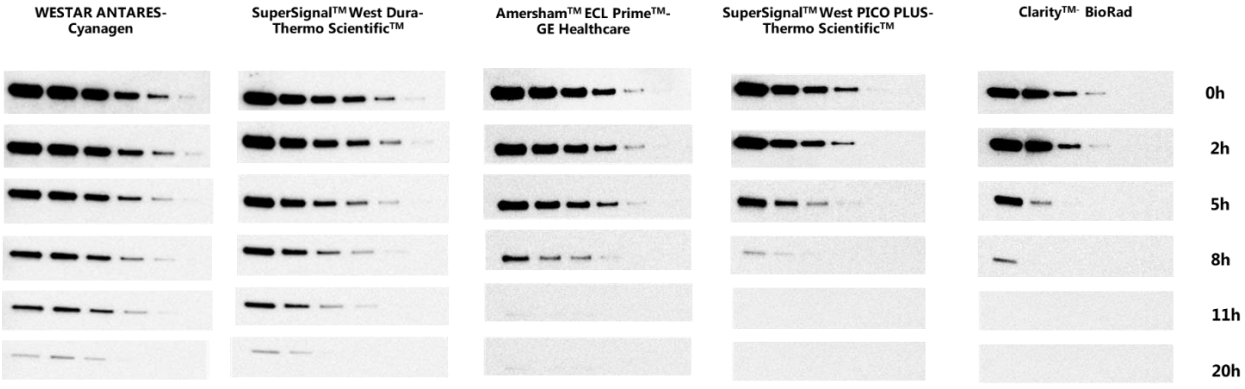

Figure 4. Signal duration of WESTAR ANTARES and its competitors. Quadruplicate blots for each substrate containing 2-fold dilutions of HeLa whole cell lysate were incubated with Rabbit-anti Human HDAC-1 1:5000 Goat anti Rabbit-HRP 1: 75000 and were simultaneously imaged with ImageQuant™ LAS 4000 (GE Healthcare) at time points up to 20 hours post substrate addition.

The second band (2.5 µg of total protein) was quantified at different time points, up to 20 hours. After 5 hours, the remaining signal for WESTAR ANTARES is about half the initial signal, while for most of the other competitors the remaining signal is less than 10% (Table 1). WESTAR ANTARES signal is still detectable after 20 hours of incubation. The long signal duration enables repeated exposures and both handling of several blots in the same experimental run and long-term CCD camera exposures. The latter allows higher sensitivity to detect faint bands, which may be not detected, in a single short exposure.

Time points (hours)	WESTAR ANTARES - Cyanagen	SuperSignal™ West Dura -Thermo Scientific™	Amersham™ ECL Prime™ - GE Healthcare	SuperSignal™ West PICO PLUS – Thermo Scientific™	Clarity™-BioRad
0	100	100	100	100	100
2	71	67	57	50	48
5	42	30	8	8	5
8	27	20	1	1	
11	18	5			
20	4	0,8			

Table 1. Remaining signal after time points up to 20 hours post substrate incubation. Band 2.5 µg was analyzed and exposure time is 180 seconds for each time points.

Reproducibility

Several variables can affect Western blot outcome. Thus, the reduction of the variability is the key to maximize precision of immunoblot results. WESTAR ANTARES provides a strong reproducibility, as shown in a simplified multi-well assay, where its performance is not affected by sources of variability related to sample loading, transfer efficiency, blocking, antibodies performance, etc. (Table 2).

Well	R.L.U.	Well	R.L.U.
1	603618	***9***	569630
2	586657	***10***	558443
3	581649	***11***	567103
4	609454	***12***	582138
5	582014	***13***	628081
6	570619	***14***	596527
7	573486	***15***	607258
8	573788	***16***	628938

Table 2. Reduced variability using WESTAR ANTARES. 200 µL of WESTAR ANTARES was added to 16 wells of a 96-well black plate, furthermore adding HRP enzyme at a final concentration of 0.8 ng/mL and reading with Victor3 micro plate reader (Perkin Elmer). MEAN: 588713. ST.DEV. 21443; CV %: 3.64.

WESTAR ANTARES maximizes reproducibility, thus increasing the significance of experimental results.
When WESTAR ANTARES is used in triplicate blots of the same experiment, the variability is less than 15%, as shown in Figure 5:

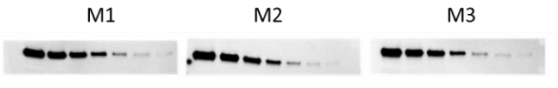

Figure 5. Detection of HDAC-1 in triplicate blots using WESTAR ANTARES. Blots containing serial dilutions of HeLa cell lysate were incubated with primary antibody (Rabbit-anti Human HDAC-1) 1:5000 and secondary antibody (Goat anti Rabbit-HRP) 1:75000. WESTAR ANTARES was applied to all blots in parallel, which were detected simultaneously for 180 seconds with ImageQuant™ LAS 4000 (GE Healthcare).

Conclusions

WESTAR ANTARES is the best choice for mid-femtogram detection level. Extremely versatile, WESTAR ANTARES enables the detection of the protein of interest when immunoblotting conditions are not yet optimized. Its high sensitivity combined with a broad linear dynamic range allows an accurate quantification of both low and high abundance proteins in the same experiment. Furthermore, WESTAR ANTARES extremely long signal duration results in superior reproducibility, ease of use, and less of a chance for creating artifacts.

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