WESTAR SUPERNOVA: Ultra-Sensitive 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 SUPERNOVA is an ultra-sensitive substrate with an outstanding signal intensity and stable light output at the low-femtogram level.

Benchmarking

WESTAR SUPERNOVA exhibits top-level performance for the most challenging Western blotting application and can substitute without any change in protocol the top performers currently on the market such as SuperSignal™ West Femto - Thermo Scientific™, Amersham™ ECL Select™ - GE Healthcare and Clarity Max™- Bio-Rad.

With its very high signal-to-noise, this substrate is capable of detecting very low amounts of protein (Figure 1).

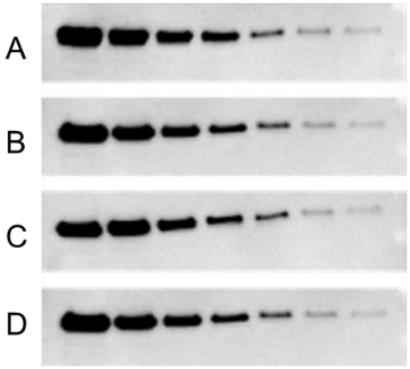

Figure 1. **Western blotting detection of HDAC-1 on HeLa cell lysate with WESTAR SUPERNOVA and other chemiluminescent reagents in the same sensitivity range.** Sample: 2-fold dilution series of HeLa whole cell lysate (abcam®) from 2.5µg to 0.039 µg of total protein. Membrane: Trans-Blot® Turbo™ Mini Nitrocellulose Transfer Packs (Bio-Rad). Blocking: 2% ECL™ Blocking Agent (GE Healthcare) in PBS-T. Primary antibody: Rabbit-anti Human HDAC-1 (abcam®) 1:15000. Secondary antibody: Goat anti-rabbit IgG HRP (2mg/ml) (abcam®) 1:300000. ECL substrates used are: A) WESTAR SUPERNOVA (Cyanagen); B) Clarity Max™ (Bio-Rad); C) Amersham™ ECL Select™ (GE-Healthcare): D) SuperSignal™ West Femto (Thermo Scientific™). Imaging: ImageQuant™ LAS 4000 (GE Healthcare). Exposure time: 120 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 SUPERNOVA offers a wide linear dynamic range with an excellent R^2 value of 0.992, (Figure 2), allowing the user to accurately quantify protein bands.

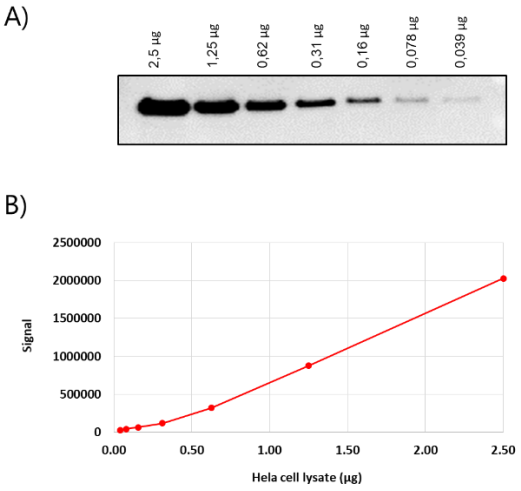

Figure 2. Enhanced precision in Western blotting using WESTAR SUPERNOVA.

A) Western blotting detection of HDAC-1 on HeLa cell lysate with WESTAR SUPERNOVA. Triplicate blots containing 2-fold dilutions of HeLa whole cell lysate were incubated with Rabbit-anti Human - HDAC-1 1:15000 and Goat anti Rabbit-HRP 1:300000 and imaged for 120 seconds with ImageQuant™ LAS 4000 (GE Healthcare).

B) Integrated signal intensity. Data show that WESTAR SUPERNOVA delivers a linear signal response, over a wide range of protein levels.

(Dynamic range = DR; linearity = R^2).

WESTAR SUPERNOVA ultra-sensitive detection and low background results in an excellent high signal-to-noise ratio. This ECL substrate is ideal in detecting low-abundance proteins and when working with highly diluted primary and secondary antibodies.

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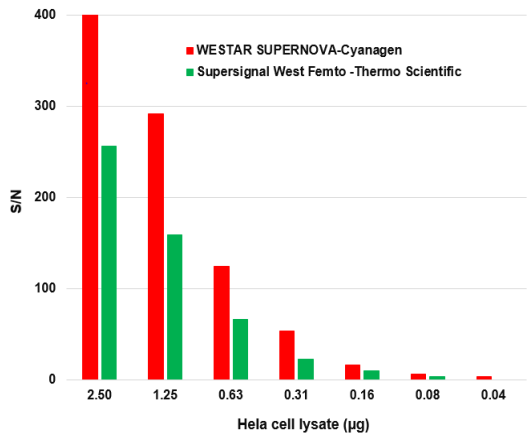

Figure 3. Low background for high sensitive detection with WESTAR SUPERNOVA. Western blotting detection of HDAC-1 on HeLa cell lysate with WESTAR SUPERNOVA compared to SuperSignal™ West Femto - Thermo Scientific™. Triplicate blots containing 2-fold dilutions of HeLa whole cell lysate were incubated with Rabbit-anti Human HDAC-1 1:15000 and Goat anti Rabbit-HRP 1: 300000 and were simultaneously imaged for 120 seconds with ImageQuant™ LAS 4000 (GE Healthcare). In a Signal-to-noise ratio (S/N) analysis, WESTAR SUPERNOVA displays the best combination of sensitivity and signal with low background.

Signal duration

WESTAR SUPERNOVA exhibits a significantly longer signal duration than its competitor SuperSignal™ West Femto - Thermo Scientific™. HDAC-1 was detected over time (0-11 h) on quadruplicate Western blots, containing 2-fold dilution series of HeLa cell lysate with WESTAR SUPERNOVA and SuperSignal™ West Femto - Thermo Scientific™ (Figure 4).

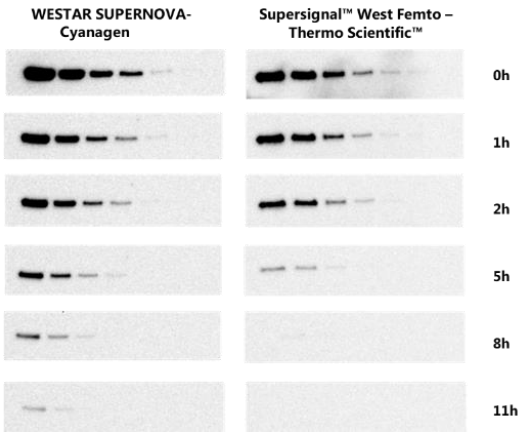

Figure 4. Signal duration of WESTAR SUPERNOVA compared to SuperSignal™ West Femto-Thermo Scientific™. Quadruplicate blots for each substrate containing 2-fold dilutions of HeLa whole cell lysate were incubated with Rabbit-anti Human HDAC-1 1:15000 and Goat anti Rabbit-HRP 1:300000 and were simultaneously imaged with ImageQuant™ LAS 4000 (GE Healthcare) at time points up to 11 hours post substrate addition.

The first band (2.5 µg of total protein) was quantified at different time points up to 11 hours. WESTAR SUPERNOVA signal is still detectable in a 120 seconds exposure 11 hours later (Table 1). The long-lasting signal enables flexible imaging. Long signal duration combined with ultra-sensitivity allows detection of extremely low abundant proteins.

Time points (hours)	WESTAR SUPERNOVA Cyanagen	SuperSignal™ West Femto Thermo Scientific™
0	100	100
1	52	50
2	37	28
5	11	5
8	5	
11	2	

Table 1. Remaining signal after time points up to 11 hours post substrate incubation. Band 2.5 µg was analyzed and exposure time is 120 seconds for each time points.

Reproducibility

In Western blotting, many sources of variability can affect the outcome. Reducing variability is thus the key to maximize precision of immunoblot results.

WESTAR SUPERNOVA provides high reproducibility, as shown in a simplified multiwell assay (Table2).

Well	R.L.U.	Well	R.L.U.
1	1530407	10	1592844
2	1527239	11	1591315
3	1545548	12	1540140
4	1469865	13	1491912
5	1561004	14	1555670
6	1538439	15	1553452
7	1508285	16	1552781
8	1586679	17	1579881
9	1564677	18	1596721

Table 2. Reduced variability using WESTAR SUPERNOVA. 200 µL of WESTAR ANTARES was added to 18 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: 1549270; Standard Deviation: 35173; Coefficient of Variation (%): 2.3%.

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WESTAR SUPERNOVA maximizes reproducibility, thus increasing the significance of experimental results. When WESTAR SUPERNOVA is used in independent experiments of Western Blot the variability is very low (< 20 %, Figure 5).

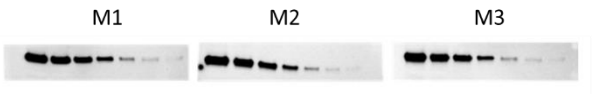

Figure 5. Increased reproducibility in Western blotting using WESTAR SUPERNOVA. Detection of HDAC-1 in a triplicate blots using WESTAR SUPERNOVA. Blots containing serial dilutions of HeLa cell lysate were incubated with Rabbit-anti Human HDAC-1 1:15000 and Goat anti Rabbit- HRP 1:300000. WESTAR SUPERNOVA was applied to all blots in parallel, which were detected simultaneously for 120 seconds with ImageQuant™ LAS 4000 (GE Healthcare).

Conclusions

WESTAR SUPERNOVA is Cyanagen ultra-sensitive substrate with low-femtogram detection level. Thanks to its excellent signal intensity and sensitivity, WESTAR SUPERNOVA detects very low amounts of proteins, using less antibodies in immunoblot experiments.

Signal intensity is directly proportional over a wide range of protein concentration. Its wide linear dynamic range together with its ultra-high sensitivity make WESTAR SUPERNOVA eminently suitable for quantitative analysis. Furthermore, WESTAR SUPERNOVA long signal duration results in superior reproducibility ease of use, and less of a chance for creating artifacts.

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