WESTAR HYPERNOVA

the Brightest Substrate

WESTAR HYPERNOVA is the brightest substrate for Horseradish Peroxidase (HRP) available to date. Enhanced chemiluminescence (ECL) is the Western blotting detection method commonly used in most laboratories, as it provides the greatest sensitivity and convenience for detection with film or digital imaging equipment. 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 regarding performance/ price ratio. WESTAR HYPERNOVA is the brightest substrate available to date. Due to its extremely high signal intensity and stable light output, WESTAR HYPERNOVA is suitable for the detection of trace amounts of proteins.

Benchmarking

WESTAR HYPERNOVA exhibits superior performance if compared to the top performers currently on the market, such as WESTAR SUPERNOVA - Cyanagen, SuperSignal™ West Femto - Thermo Scientific™, Amersham™ ECL Select™- GE Healthcare and Clarity Max™ - Bio-Rad.

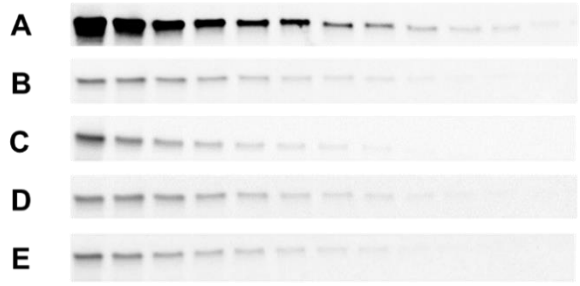

Figure 1. **Western blotting detection of purified IKBα.** Sample: 1.5-fold dilution series of purified IKBα (abcam®) from 667 pg to 5 pg of protein. Membrane: Trans-Blot® Turbo™ Mini Nitrocellulose Transfer Packs (Bio-Rad) Blocking: 2% ECL™ Blocking Agent (GE Healthcare) in PBS-T Primary antibody: Rabbit-anti IKBα (abcam®) 1:1000 Secondary antibody: Goat anti-rabbit IgG HRP (2mg/ml) (abcam®) 1:500000. ECL substrates used are: A) WESTAR HYPERNOVA (Cyanagen); B) WESTAR SUPERNOVA (Cyanagen); C) Clarity Max™ (Bio-Rad); D) Amersham™ ECL Select™(GE-Healthcare); E) SuperSignal™ West Femto (Thermo Scientific™). Imaging: ImageQuant™ LAS 4000 (GE Healthcare). Exposure time: 10 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 HYPERNOVA offers a wide linear dynamic range with an excellent R2 value > 0.99 (Figure 2), allowing the user to accurately quantify protein bands.

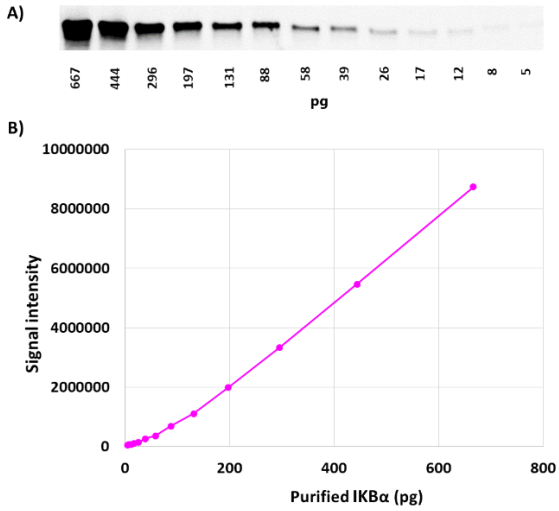

Figure 2. Enhanced precision in Western blotting with WESTAR HYPERNOVA. A) Purified IKBα detection with WESTAR HYPERNOVA. Triplicate blots containing 1.5-fold dilutions of purified IKBα were incubated with primary antibody (Rabbit anti- IKBα) 1:1000 and secondary antibody (Goat anti Rabbit-HRP) 1: 500000 and imaged for 10 seconds with ImageQuant™ LAS 4000 (GE Healthcare). B) Integrated signal intensity. Data show that WESTAR HYPERNOVA delivers a linear signal response, over a wide range of protein levels.

WESTAR HYPERNOVA is specifically designed to provide extreme sensitivity for detection of trace amounts of the target protein. WESTAR HYPERNOVA produces up to 10-fold higher signal-to-noise ratio compared to all the current top-level ECL substrates, resulting in brighter and clearer protein bands and allowing an accurate interpretation of faint bands. Figure 3 shows the performance of WESTAR HYPERNOVA in comparison to one of the top-level ECL substrates (SuperSignal™ West Femto - Thermo Scientific™).

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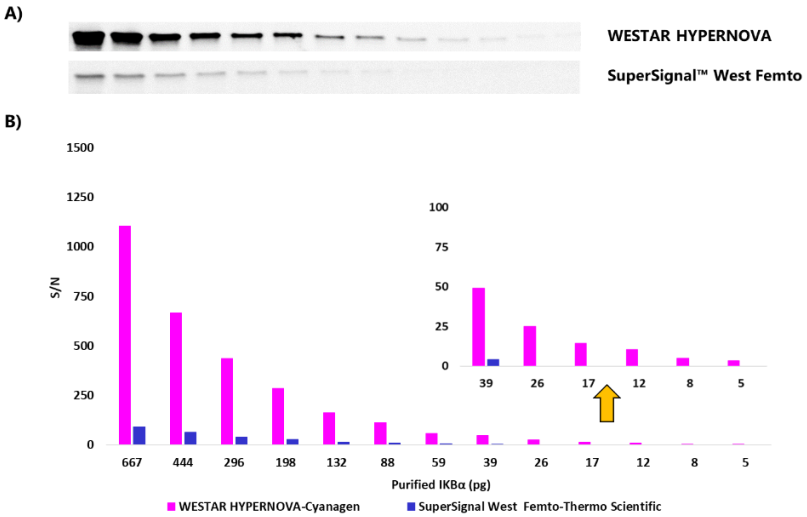


Figure 3. Enhanced sensitivity of WESTAR HYPERNOVA compared to one of the current top-level substrates (SuperSignal™West Femto-Thermo Scientific™).
A) Purified IKBα detection with either WESTAR HYPERNOVA or SuperSignal™ West Femto. Triplicate blots for each substrate containing 1.5-fold dilutions of purified IKBα from 667 pg to 5 pg were simultaneously imaged for 10 seconds with ImageQuant™ LAS 4000 (GE Healthcare).
B) Signal-to-noise ratio (S/N) analysis. The inset enlargement shows the enhanced sensitivity of WESTAR HYPERNOVA.
(LOD = 5 pg) compared to SuperSignal™ West Femto (LOD = 39 pg).
(LOD = Limit of Detection)

Save precious samples and antibodies

Since WESTAR HYPERNOVA generates a much higher signal intensity than current top-performer substrates, considerably smaller protein samples can be loaded onto gels to achieve the same results. Using the same experimental conditions (amount of sample, antibody dilution), WESTAR HYPERNOVA produces the same signal intensity as Amersham™ ECL Select™ – GE Healthcare with 8-times less protein, as shown in Figure 4.

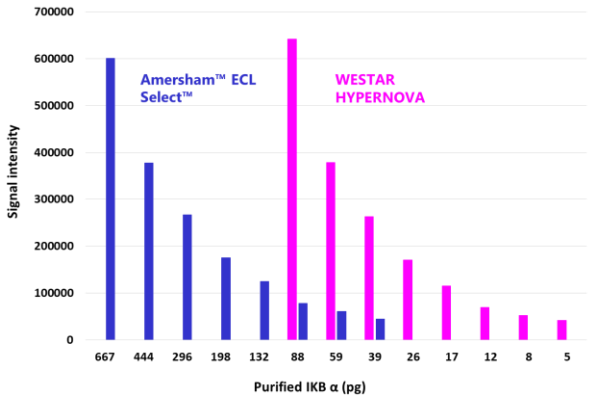


Figure 4. WESTAR HYPERNOVA enables to save protein samples. Triplicate blots for each substrate containing serial dilutions of purified IKBα were incubated with primary antibody (Rabbit-anti IKBα) 1:1000 and secondary antibody (Goat-anti Rabbit-HRP) 1:500000 and were simultaneously imaged for 10 seconds. Bar graph shows that HYPERNOVA produces the same signal intensity as Amersham™ ECL Select™ with up to 8-times less protein.

WESTAR HYPERNOVA enables to save money on antibody costs thanks to a reduction of the amount of antibody required to detect the protein of interest compared to the amounts needed with the current top-level substrates. When blots are incubated with two different antibody dilutions, WESTAR HYPERNOVA and Clarity Max™ - Bio-Rad demonstrate a similar performance (Figure 5), but 10-times less primary antibody is used with WESTAR HYPERNOVA, as shown in the signal-to-noise ratio graph (Figure 6).

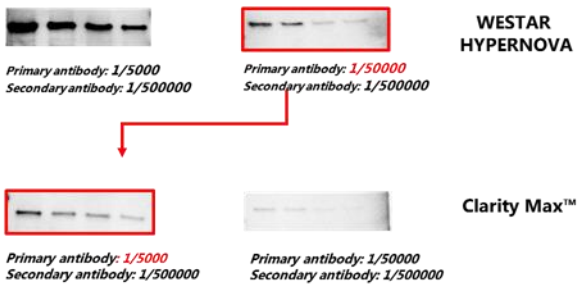


Figure 5. WESTAR HYPERNOVA enables to save expensive primary antibodies. Triplicate blots containing serial dilutions of purified IKBα were probed with 1:5000 and 1:50000 primary antibody (Rabbit anti- IKBα) dilutions and with 1:500000 secondary antibody (Goat anti Rabbit-HRP) dilutions, and detected with either WESTAR HYPERNOVA - Cyanagen or Clarity Max™ - Bio-Rad. Blots were imaged simultaneously with 60 seconds exposure time.

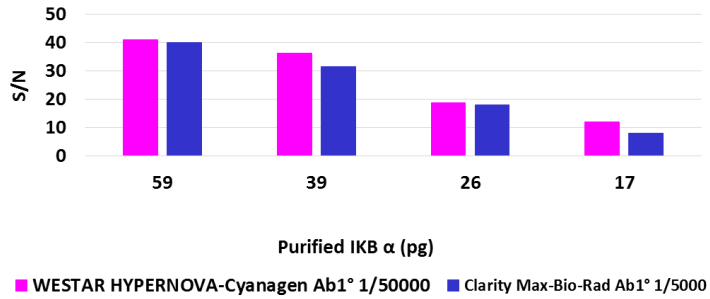


Figure 6. Signal-to-noise ratio comparison between HYPERNOVA and a top-level substrate. WESTAR HYPERNOVA produces a comparable result to Clarity Max™- Bio-Rad with 10-times less primary antibody.

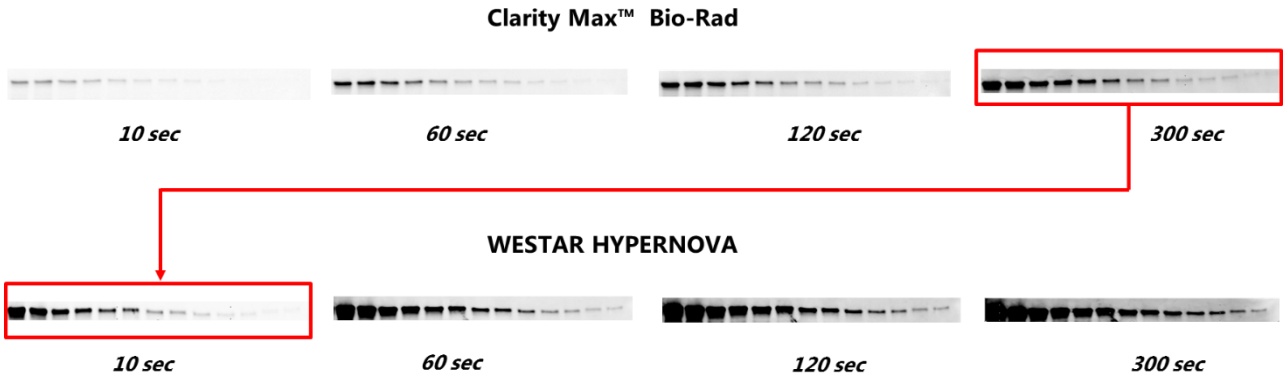


Figure 7. Extremely high sensitivity detection with extra-short exposure time. Triplicate blots containing 1.5-fold dilutions of purified IKBα from 667 pg to 5 pg were probed with 1:1000 primary antibody dilutions and with 1:500000 secondary antibody (Goat anti Rabbit-HRP) dilutions and detected with either WESTAR HYPERNOVA - Cyanagen or Clarity Max™- Bio-Rad. Blots were imaged simultaneously with 10-60-120-300 seconds exposure time.

Shorten the exposure time

Another advantage of using WESTAR HYPERNOVA is the reduction of exposure time, thus saving time and increasing the productivity. As shown in Figure 7, WESTAR HYPERNOVA delivers the detection of a target protein with the same signal intensity and sensitivity as with Clarity Max™ Bio-Rad in just 10 seconds instead of 5 minutes of exposure.

Reproducibility

In western blotting technique, many sources of variability can affect the outcome. Minimization of the variability related to the substrate in the detection step is the key to increase the precision of immunoblot results. WESTAR HYPERNOVA provides a strong reproducibility, as shown in a simplified multiwell assay in which the performance of the chemiluminescent substrate is not affected by sources of variability related to sample loading, transfer efficiency, blocking, antibodies performance, etc.

Well	*R.L.U.*
1	8982123
2	8624526
3	8768416
4	8586783
5	8881792
6	8886633
7	8448543
8	8978920
9	9045379
10	9047719
11	9076586
12	9106944
13	9108654
14	9063764
15	9058510
16	8903861
MEAN	8910572
ST.DEV	203187
CV%	2.28

Table 1. Extremely reduced variability using WESTAR HYPERNOVA. 200 µL of WESTAR HYPERNOVA were 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 Victor^3 microplate reader (Perkin Elmer).

WESTAR HYPERNOVA maximizes reproducibility, thus increasing the significance of experimental results. In triplicate blots, its variability is less than 20%, Table 2.

IKBα (pg)	M1	M2	M3	MEAN	ST.DEV	CV%
667	8142349	8225041	9631108	8666166	836686	10
444	6256439	4639359	5632330	5509376	815522	15
296	2218886	2782659	3172242	2724596	479323	18
198	1529430	1923108	1841358	1764632	207752	12
132	1118374	925260	1003406	1015680	97140	10
88	609959	603648	537624	583744	40065	7
59	390132	337664	309295	345697	41013	12
39	284753	303746	222108	270202	42720	16
26	162146	135260	126275	141227	18665	13
17	89608	101769	93849	95075	6172	6
12	46671	53806	53465	51314	4025	8
8	49771	52794	48563	50376	2180	4
5	37825	54615	44866	45769	8432	18

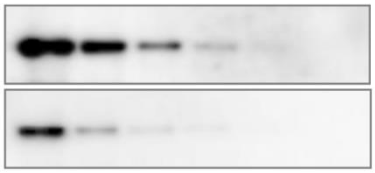
Table 2. Increased reproducibility in Western blotting with WESTAR HYPERNOVA. Standard deviation (ST.DEV) and coefficient of variation (CV%) were calculated on the mean of signal intensities from membranes M1, M2, M3.

Conclusions

WESTAR HYPERNOVA is the most powerful substrate available to date allowing the detection of trace amounts of proteins. Thanks to its extreme signal intensity and sensitivity, WESTAR HYPERNOVA enhances the accuracy of Western blotting for a clearer interpretation of the faintest bands. Its wide linear dynamic range together with its extreme sensitivity make WESTAR HYPERNOVA suitable for quantitative analysis. WESTAR HYPERNOVA is the best choice to detect minute amounts of proteins, using extremely diluted antibodies or loading limited amounts of cell lysates, thus saving precious samples and expensive primary antibodies. Furthermore, WESTAR HYPERNOVA with its extremely high light emission produces very bright bands in few seconds of exposure.

What our customers say about WESTAR HYPERNOVA

«Hypernova is more sensitive than Supernova. Hypernova is the highest chemiluminescence reagent available to date definitely!»
A Japanese life science company

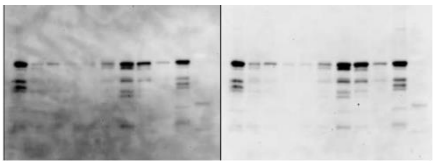


WESTAR HYPERNOVA (Cyanagen)

WESTAR SUPERNOVA (Cyanagen)

Western blotting detection of β-actin in Hela cell
Hela cell lysate, 2-fold dilution
Ab 1° Mouse anti β actin (sc-47778 Santa Cruz) 1:5000 30 min RT
Ab 2° Goat anti Mouse HRP (NB7574 Novus) 1:100000 30 min RT
Imager: Chemidoc touch MP

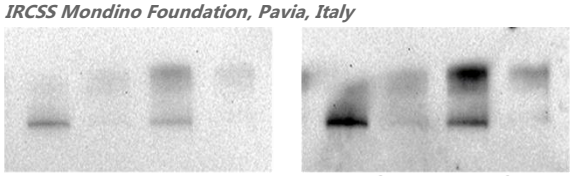
«Significantly higher sensitivity for samples with extremely low antigen content»
Ludwig Maximilian University, Munich, Germany



WESTAR SUPERNOVA (Cyanagen) *WESTAR HYPERNOVA (Cyanagen)*

Western blotting detection of AIF, p-53, cyclin D3 in primary cell culture line
2 µg cell lysate loaded
Ab 1° Mouse anti AIF (E-1) (sc-13116 Santa Cruz) 1:5000 overnight 4°C
Mouse anti p-53 (sc-126 mab Santa Cruz) 1:10000 overnight 4°C
Mouse anti cyclin D3 (#2936 Cell Signaling) 1:2000 overnight 4°C
Mouse anti HAM (Cell signaling) 1:25000 overnight 4°C
Ab 2° Horse anti mouse –HRP 120 min 4°C
Exposure time: 5 minutes
Imager: INTAS CHEMOCAN

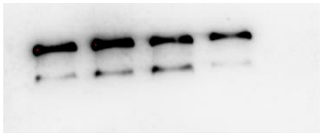
«WESTAR HYPERNOVA is more sensitive than ECL SELECT from GE Healthcare»
IRCSS Mondino Foundation, Pavia, Italy



Amersham ECL Select (GE Healthcare) *WESTAR HYPERNOVA (Cyanagen)*

Western blotting detection of HDAC6 in SHSY5Y cell
30 µg cell lysate loaded
Ab 1° Rabbit anti HDAC6 (#7558 Cell Signaling) 1:1000 overnight 4°C
Ab 2° Goat anti rabbit (NA934V GE Healthcare) 1:8000 60 min RT
Exposure time: 2 minutes
Imager: Kodak Digital Science ™ Image Station 440CF (IS440CF) system

«I obtained a good result with WESTAR HYPERNOVA. Sample was too faint to be detected with Clarity Max from BIO-RAD».
Department of Sciences and Biological Technologies, University of Salento, Italy



WESTAR HYPERNOVA (Cyanagen)

Co-immunoprecipitation assay on Hela cell
20 µg cell lysate loaded
Ab 1° Rabbit anti α-myc (sc-789 Santa Cruz) 1:500 2 hours RT
Ab 2° α rabbit –HRP (BIO-RAD) 1:5000 60 min RT
Exposure time: 30 seconds
Imager: CHEMIDOC BIO-RAD

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