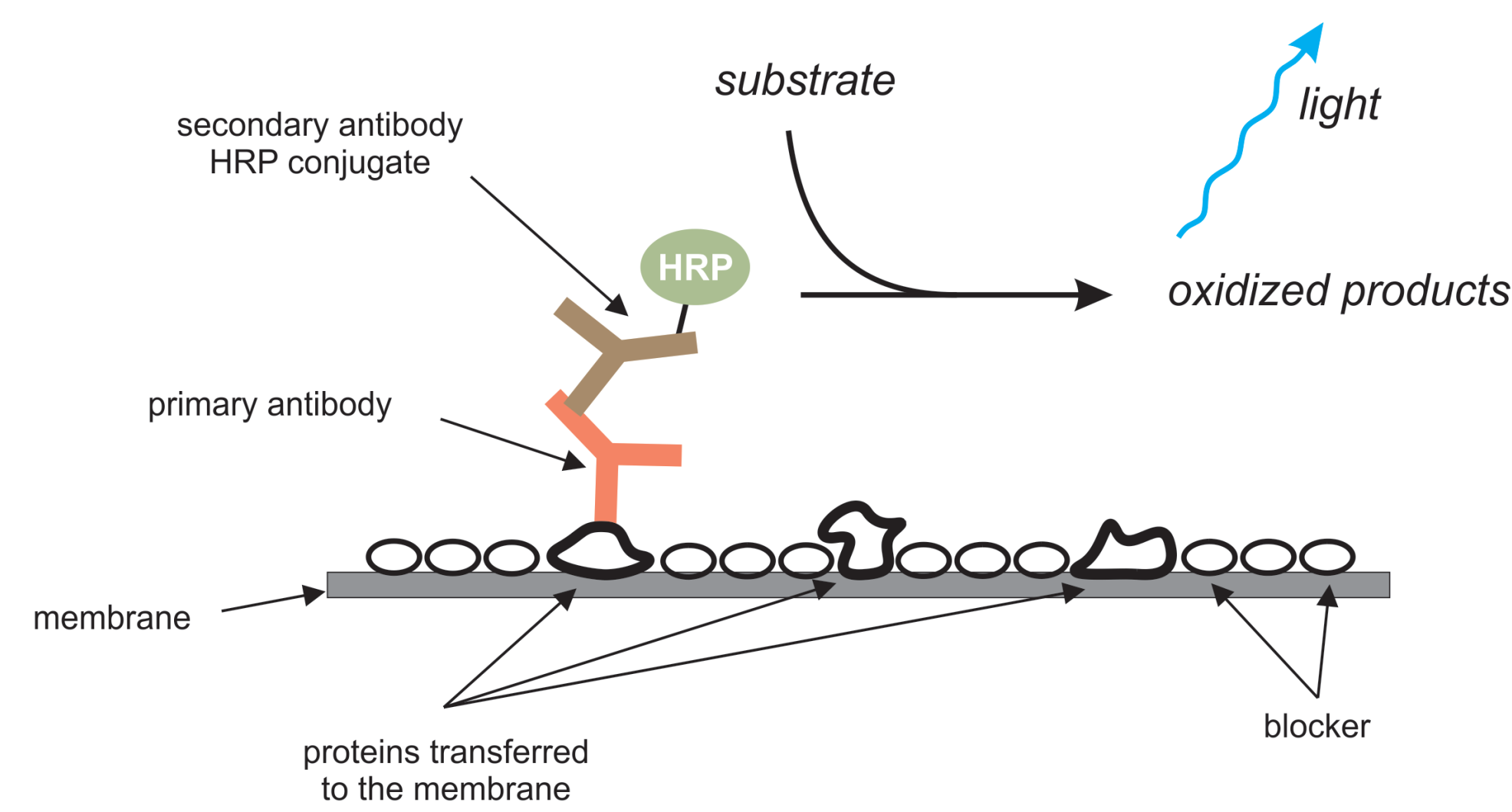


Enhanced ECL for Quantitative Westerns and ELISA's

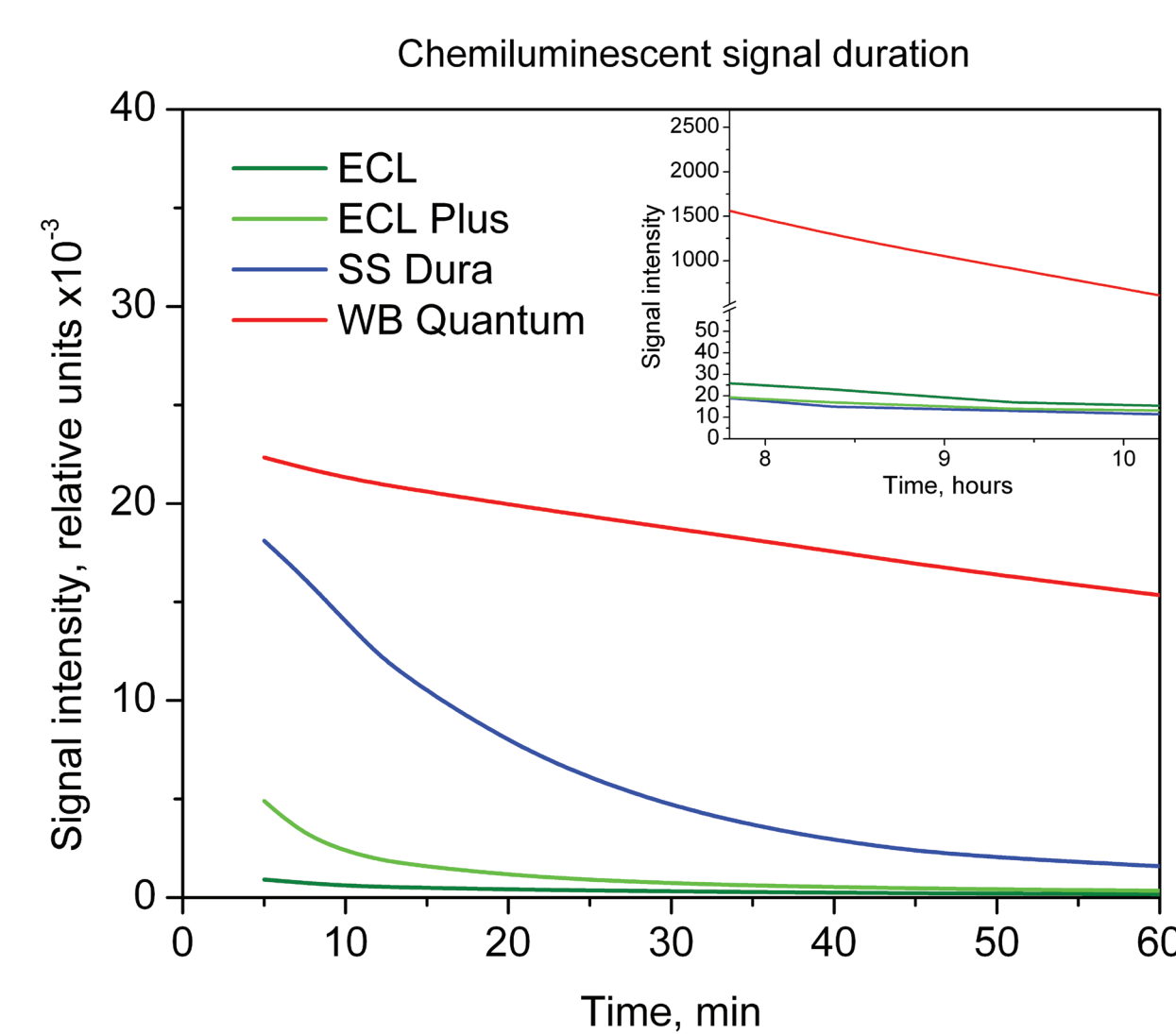
Advansta, Inc., Menlo Park, CA

Abstract

WesternBright™ Quantum and WesternBright Sirius are enhanced chemiluminescent substrates designed for quantitative and high-sensitivity Western blotting. WesternBright Quantum has a linear range of over 3 orders of magnitude, ideal for quantitative Westerns. WesternBright Sirius has an extremely bright signal, making it the substrate of choice when detecting low-abundance proteins by either Western blotting or ELISA. Both substrates have extremely low background, for high signal to noise ratios and increased ability to detect low abundance bands. WesternBright Quantum also demonstrates no substrate depletion at high protein loads, expanding the linear dynamic range of detection relative to substrates that do experience substrate depletion. WesternBright Quantum and WesternBright Sirius also have stable, long-lasting signals, allowing blots to be imaged multiple times, or hours after substrate incubation.

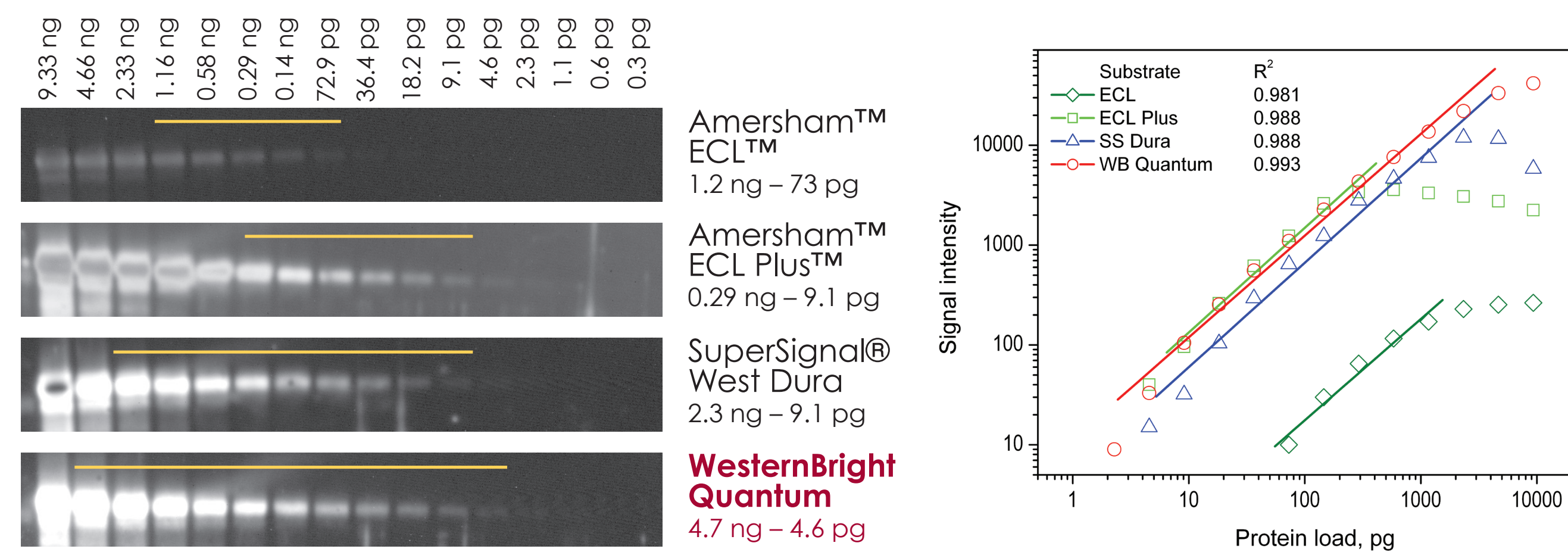


Long-lasting signal



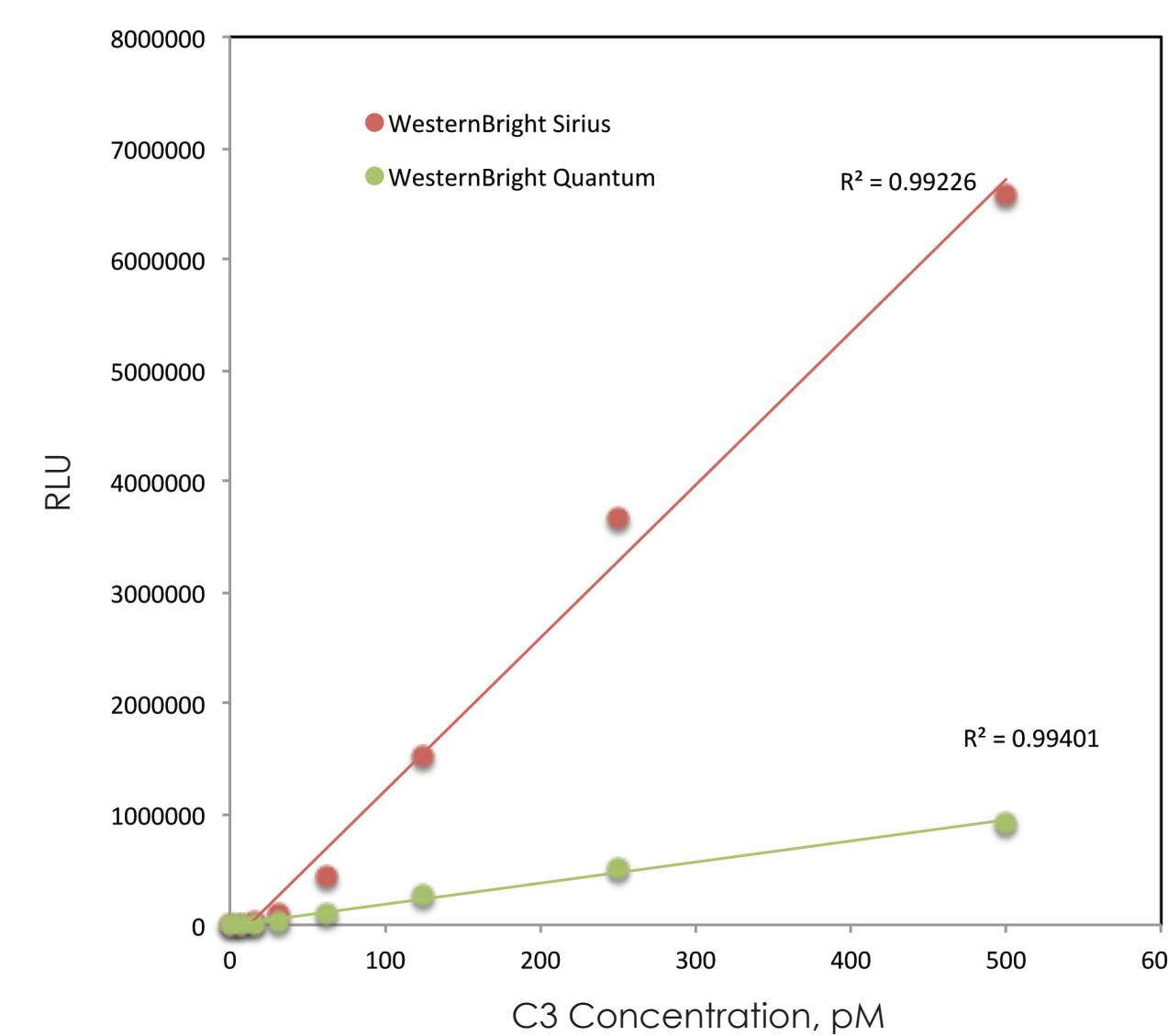
WesternBright Quantum produces the most stable chemiluminescent signal. Blots detected using WesternBright Quantum and three other chemiluminescent substrates, were re-imaged simultaneously at several times over a 10 hour period. The intensity of one band is plotted. One hour after substrate incubation, WesternBright Quantum retains 70% of its initial signal strength, while the signal from competing substrates decays to 5% or less. Enjoy more flexibility in imaging blots, knowing the signal will not decay substantially over several hours. Also, long exposures can be conducted if needed to detect very low abundance bands.

High sensitivity, large dynamic range



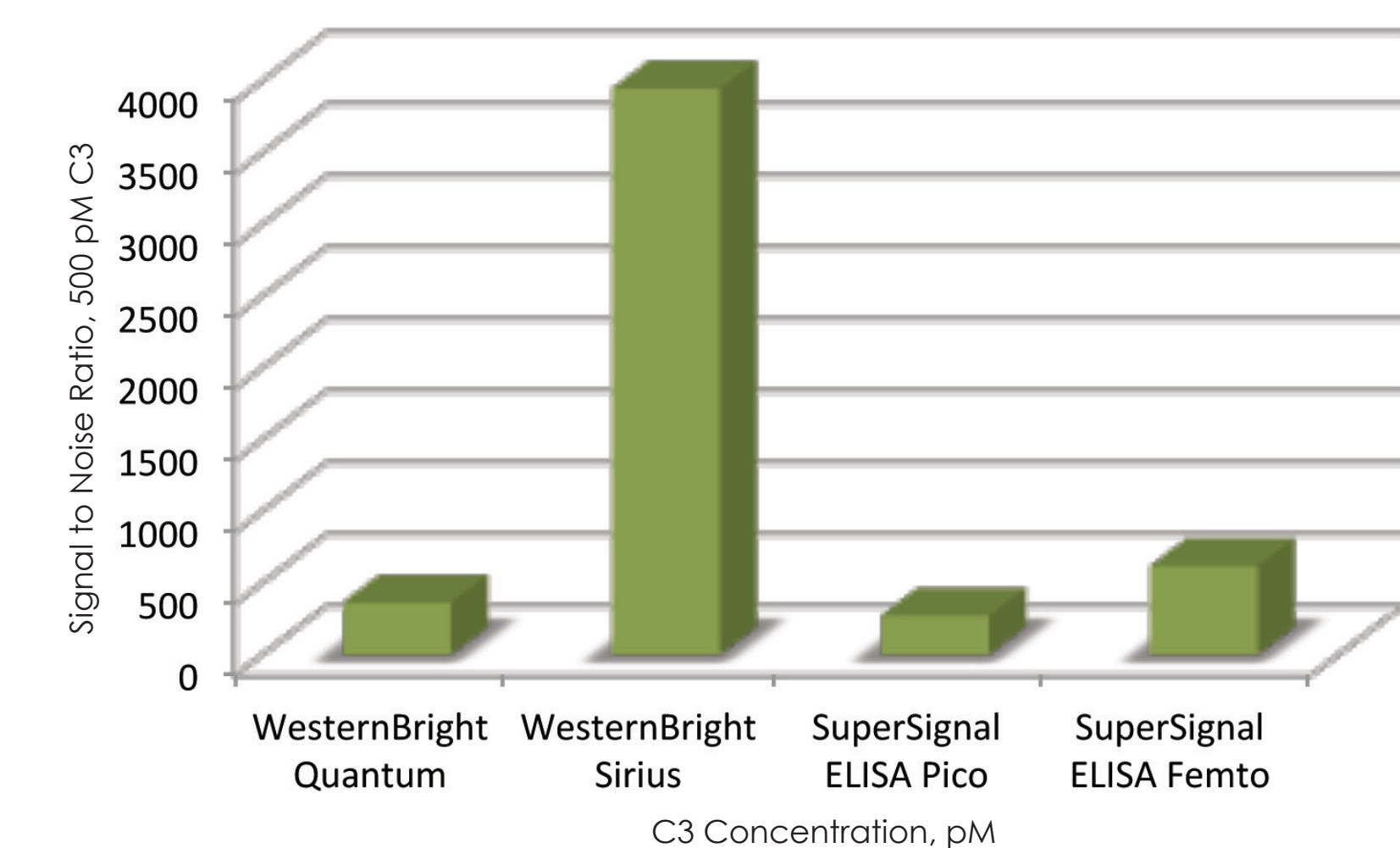
Linear dynamic range of WesternBright Quantum. Identical Western blots containing serial dilutions of transferrin were probed with a rabbit-anti-transferrin primary antibody, and a goat-anti-rabbit secondary antibody conjugated to horseradish peroxidase. The blots were incubated with chemiluminescent substrates as recommended by each manufacturer. All blots were simultaneously imaged for 2 minutes with a CCD imager. Display parameters were set identical across all images shown. Band intensities were plotted and a best fit linear regression conducted for each substrate. WesternBright Quantum shows the largest dynamic range out of all four substrates with the highest R² value. Bands that fall on the linear part of the curve for each substrate are indicated on the image.

Large linear dynamic range for ELISA



Linear Dynamic Range of WesternBright Quantum and WesternBright Sirius in ELISA. WesternBright Quantum or WesternBright Sirius were used as substrates to develop duplicate chemiluminescent ELISA experiments. Two-fold serial dilutions of human C3 protein were made in 1% BSA-PBST, spanning the range from 7.81 to 500 pM. 50 µl of each C3 protein solution was added per well, in triplicate, to EIA plates coated with purified anti-C3 antibody, and blocked with 1% BSA-PBST. C3 protein was detected with 50 µl per well of a 0.5 µg/ml solution of a monoclonal anti-huC3a antibody diluted in 1% BSA-PBST followed by a goat anti-mouse-HRP conjugate diluted 1:30,000 in 1% BSA-PBST. Both substrates result in a signal linear with respect to target protein concentration, with R² values approaching 1.

Highest ELISA signal / noise ratio



WesternBright Sirius provides the highest signal/noise ratio in chemiluminescent ELISA experiments. WesternBright Sirius, WesternBright Quantum, SuperSignal® ELISA Pico or SuperSignal ELISA Femto substrate (Thermo Fisher) were used as substrates to develop replicate chemiluminescent ELISA experiments. Two-fold serial dilutions of human C3 protein were made in 1% BSA-PBST, spanning the range from 7.81 to 500 pM. 50 µl of each C3 protein solution was added per well, in triplicate, to EIA plates coated with purified anti-C3 antibody, and blocked with 1% BSA-PBST. C3 protein was detected with 50 µl per well of a 0.5 µg/ml solution of a monoclonal anti-huC3a antibody diluted in 1% BSA-PBST followed by a goat anti-mouse-HRP conjugate diluted 1:30,000 in 1% BSA-PBST. WesternBright Sirius produces a signal to noise ratio over 6-times greater than that of SuperSignal ELISA Femto.

Advantages of WesternBright Quantum

- **Quantitative** – WesternBright Quantum has a broad linear range, exceeding 3 orders of magnitude
- **High sensitivity** – Detect attomoles of protein per band
- **Low background** – WesternBright Quantum provides extremely high signal to noise, a distinct advantage when detecting low abundance proteins
- **No substrate depletion** – With WesternBright Quantum, substrate depletion (observed as inverse bands with other substrates) is not a problem at high protein loads. This extends the dynamic range of the substrate
- **Long-lasting signal** – One hour after substrate incubation, 70% of the WesternBright Quantum signal remains. With this signal stability, blots can be imaged multiple times without concern for loss of information

Advantages of WesternBright Sirius

- **Highest sensitivity** – 2-4 times more sensitive than WesternBright Quantum in chemiluminescent Western blotting
- **Highest signal to noise** – Extremely low background in ELISA experiments for higher signal to noise ratios
- **Flexibility** – Image chemiluminescence or chemifluorescence

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