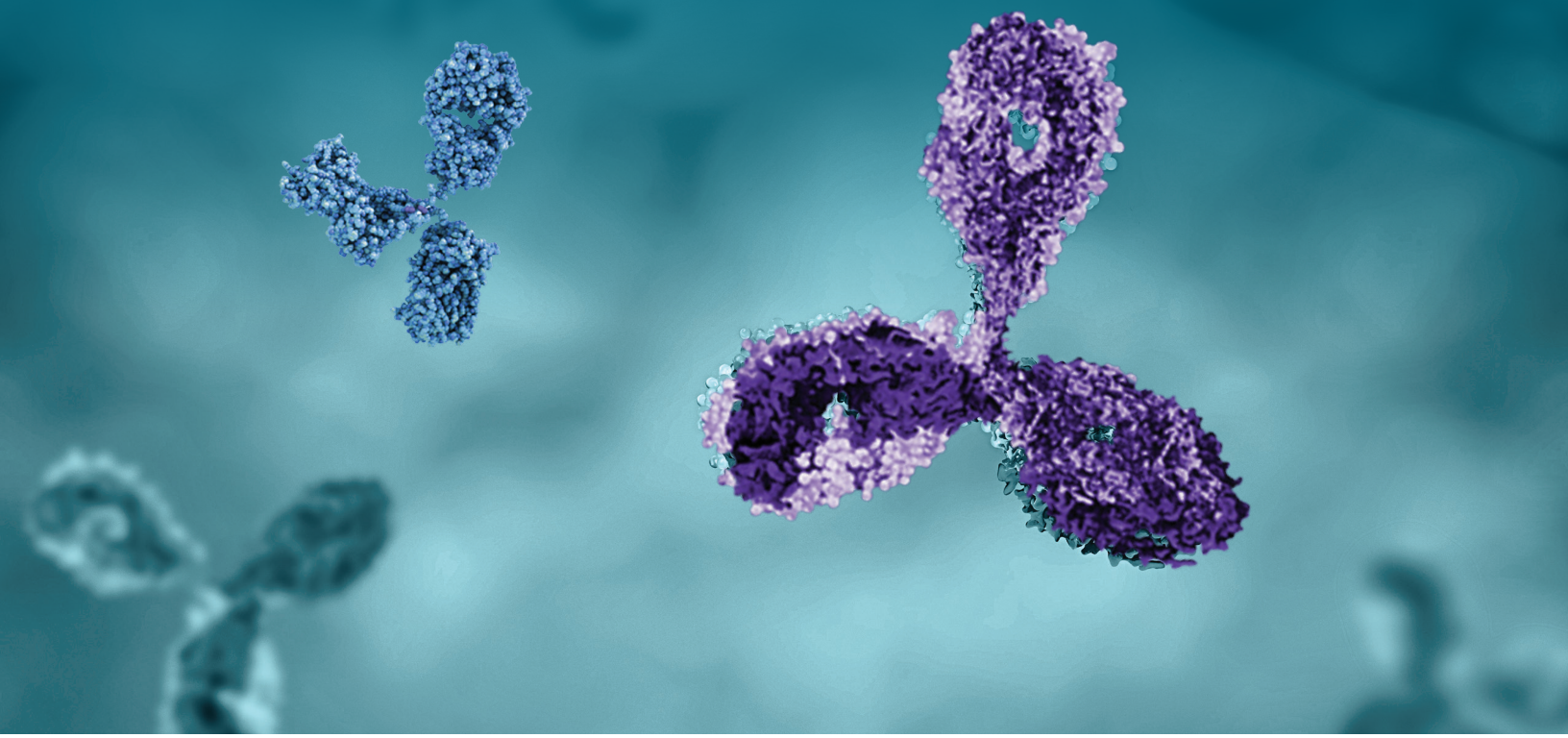
thermo scientific

HRP chemiluminescent substrates—see super signal like never before

Detect your protein, no matter how scarce

ThermoFisher SCIENTIFIC

Chemiluminescence is the western blot detection method of choice in many laboratories, as it provides high sensitivity and flexible detection with film or a digital imaging system. There are many chemiluminescent substrate choices available. The appropriate substrate selection depends on the detection level (sensitivity) required, the target protein's abundance, and the amount of sample available. The Thermo Scientific™ SuperSignal™ family of chemiluminescent substrates covers a broad range of sensitivity and enables you to obtain consistent and reproducible results you can count on.



Introducing the SuperSignal West Atto Ultimate Sensitivity Substrate—a new standard for high-sensitivity chemiluminescent HRP substrates

The Thermo Scientific™ SuperSignal™ West Atto Ultimate Sensitivity Substrate is the newest member of our SuperSignal family. It is an ultrasensitive enhanced chemiluminescent (ECL) substrate that enables protein detection down to the high-attogram level by western blot analysis using horseradish peroxidase (HRP). It provides the greatest sensitivity and better signal-to-noise ratios than other commonly used HRP substrates. For detection of very low-abundance targets or for precious samples that require maximum sensitivity, SuperSignal West Atto substrate is the ideal choice.

Choose the right SuperSignal substrate for your needs.

	SuperSignal West Pico PLUS	SuperSignal West Dura	SuperSignal West Femto	SuperSignal West Atto
				
Detection level	Low-picogram to high-femtogram	Mid-femtogram	Mid- to low-femtogram	Low-femtogram to high-attogram
Signal duration (hours)	Up to 24 hours	Up to 24 hours	Up to 8 hours	Up to 6 hours
Antibody dilution	1°: 1:1K–5K 2°: 1:10K–1:100K	1°: 1:1K–50K 2°: 1:50K–1:250K	1°: 1:5K–100K 2°: 1:100K–1:500K	1°: 1:1K–1:5K 2°: 1:100K–1:250K
Advantages	Superior sensitivity and intensity and longer duration than other entry-level ECL substrates	Best for use with imaging equipment; long-lasting signal duration	Good sensitivity with optimized conditions	Most sensitive substrate for HRP detection with high signal-to-noise ratio
Select when:	Routine western blots; working with new protein target when western blotting conditions are not yet optimized	Maximum signal duration is needed	Target is low in abundance/sample is precious; system is optimized	Target is very low in abundance/sample is precious; requires maximum sensitivity with less optimization

p23 detection in HeLa lysates (lane 1: 10 µg total protein; lanes 2–9: serially diluted 1:1) was performed using SuperSignal HRP chemiluminescent substrates. The blots were developed using an anti-p23 monoclonal antibody (Cat. No. MA3-414), followed by an HRP-conjugated goat anti–mouse IgG secondary antibody (Cat. No. 31430). Images were captured using an Invitrogen™ iBright™ Imaging System.

Why choose SuperSignal West Atto substrate?

- **Superior sensitivity and signal-to-noise ratio**—exhibits superior intensity and sensitivity to detect proteins that were previously undetectable using other high-performance ECL substrates on the market (Figure 1)
- **Use less and see more**—low-femtogram to high-attogram ultrasensitivity enables strong signal with very dilute samples (Figure 2); allows users to save more sample to expend in other applications, making it an ideal choice when working with precious or dilute samples
- **Excellent signal duration**—up to 6 hours of usable light output when conditions are optimized
- **Stable**—working solution is stable for 48 hours; kit is stable at 2–8°C for 12 months

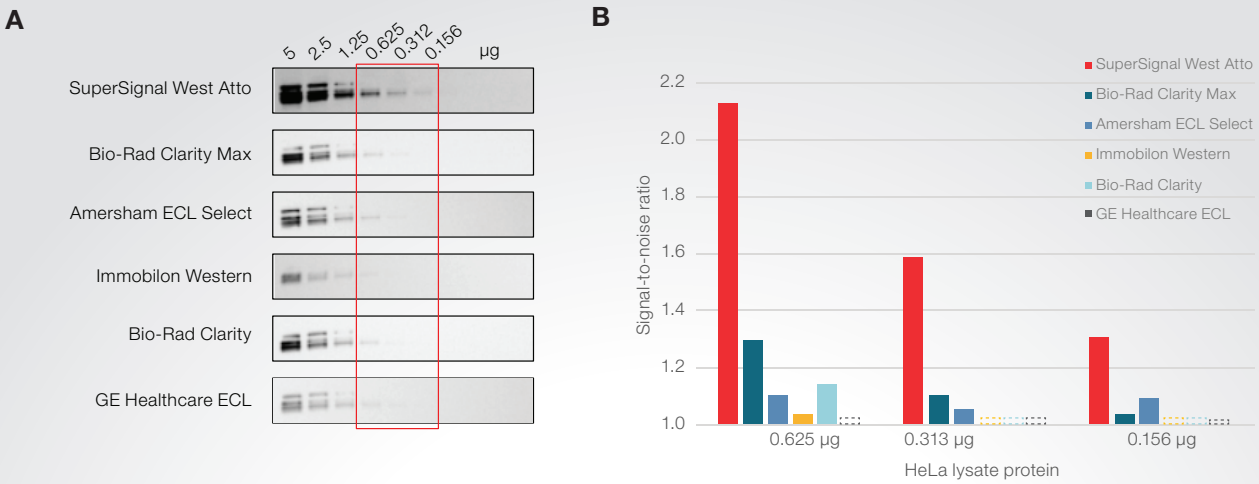


Figure 1. Comparison of SuperSignal West Atto substrate and other chemiluminescent substrates. Serial dilutions of HeLa lysate were prepared, separated by SDS-PAGE, and transferred to membranes for detection of AKT (pan). The proteins were detected using Invitrogen™ AKT Pan Monoclonal Antibody (J.314.4) (Cat. No. MA5-14916) followed by an Invitrogen™ HRP-conjugated goat anti-rabbit IgG secondary antibody (Cat. No. 32460) diluted per manufacturer's instructions. Blots were then incubated with the respective substrates per product instructions. **(A)** Images were captured at 2 min using an Invitrogen™ iBright™ imager. **(B)** SuperSignal West Atto substrate provided the best signal-to-noise ratios across all protein loads.

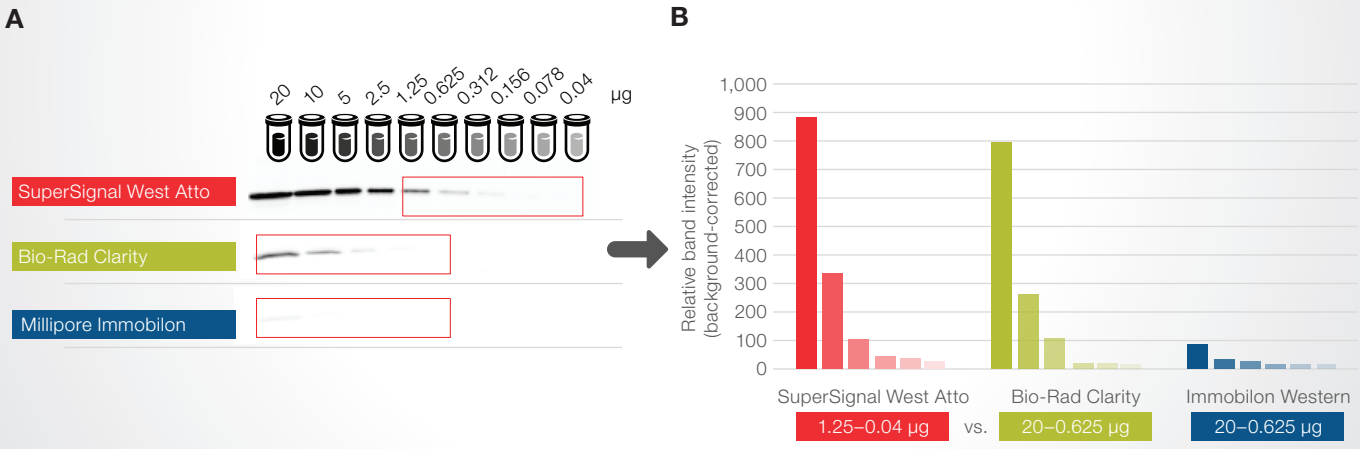


Figure 2. SuperSignal West Atto substrate enables detection of even low concentrations of target. Serial dilutions of HeLa lysate were prepared, separated by SDS-PAGE, and transferred to nitrocellulose membranes for detection of p23. The protein was detected using an Invitrogen™ mouse anti-p23 primary antibody (Cat. No. MA3-414) followed by an Invitrogen™ HRP-conjugated goat anti-mouse secondary antibody (Cat. No. 32430) diluted per manufacturer's instructions. Blots were then incubated with SuperSignal West Atto substrate (Cat. No. A38558), Clarity™ Western ECL Blotting Substrate (Bio-Rad Laboratories, Inc.), or Immobilon™ Western Chemiluminescent HRP Substrate (MilliporeSigma) per product instructions. All blots were simultaneously imaged using the Invitrogen™ iBright™ FL Imaging System **(A)**. Compared to the amounts of lysate required for detectable signal using the other substrates, only 1/16 of the amounts is needed for comparable signal intensities using the SuperSignal West Atto substrate **(B)**, conserving precious samples.

SuperSignal West Pico PLUS Chemiluminescent Substrate—your ideal choice for routine western blotting applications

The Thermo Scientific™ SuperSignal™ West Pico PLUS Chemiluminescent Substrate is an ECL substrate designed for low-picogram to high-femtogram detection levels. It offers better sensitivity, longer duration, and brighter intensity than most commonly used entry-level ECL substrates (Figure 3). This substrate is flexible with a wide range of antibody dilutions, making it an ideal choice for routine western blotting applications (Figure 4). It is also recommended for detecting a new protein of interest when western blotting conditions are not yet optimized. The long-lasting signal combined with improved sensitivity helps you obtain publication-quality results the first time, without spending time repeating experiments.

Why choose SuperSignal West Pico PLUS substrate?

- **Exceptional robustness**—high performance even outside of the recommended antibody ranges, including the most commonly used 1:5,000 to 1:10,000 secondary antibody dilutions from a 1 mg/mL stock solution
- **Excellent sensitivity**—low-picogram to high-femtogram levels
- **Long signal duration**—no loss in signal sensitivity for up to 4 hours, with signal light output up to 24 hours
- **Stable**—working solution is stable for 8 hours; kit is stable at room temperature for 1 year

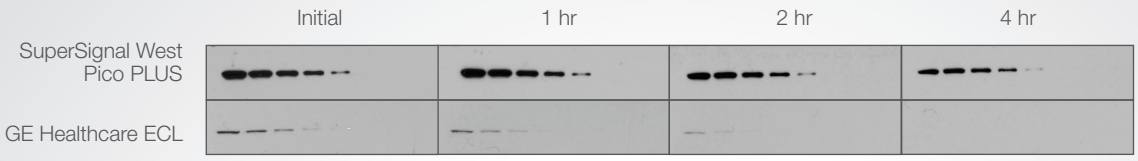


Figure 3. SuperSignal West Pico PLUS substrate provides better sensitivity, longer duration, and brighter intensity than another entry-level ECL-based product. Two-fold serial dilutions of HeLa lysate were prepared, starting at 20 µg/well. Following separation by SDS-PAGE, proteins were transferred to nitrocellulose membranes (Cat. No. 88018) using a semi-dry transfer method. The membranes were blocked with 5% nonfat dry milk dissolved in Thermo Scientific™ Pierce™ 20X TBS Tween™ 20 Buffer (Cat. No. 28360) and then incubated with an antibody against STAT3 (Cat. No. MA1-13042) followed by goat anti-mouse IgG (H+L) secondary antibody, HRP conjugate (Cat. No. 32430). Chemiluminescent detection was performed following a 5-minute incubation with the indicated substrates. Signal was captured on X-ray film at the indicated times after addition of substrate.

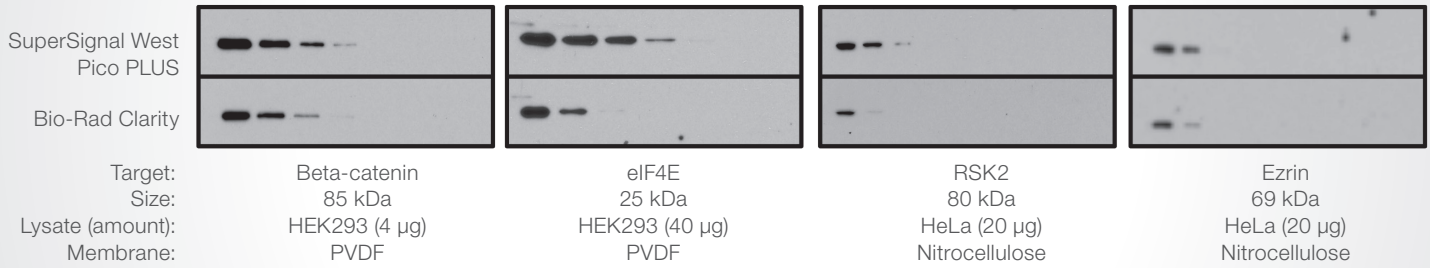
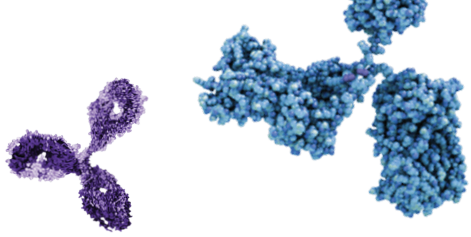


Figure 4. SuperSignal West Pico PLUS substrate outperforms Bio-Rad Clarity substrate. Two-fold serial dilutions of HEK293 and HeLa lysates were prepared, starting with the amounts indicated in parentheses. Following separation by SDS-PAGE, proteins were transferred to either Thermo Scientific™ PVDF (Cat. No. 88518) or nitrocellulose (Cat. No. 88018) membranes using the Invitrogen™ Power Blotter (Cat. No. 22834) and Thermo Scientific™ Pierce™ 1-Step Transfer Buffer (Cat. No. 84731). The membranes were blocked with 5% nonfat dry milk dissolved in Pierce 20X TBS Tween 20 Buffer (Cat. No. 28360) and then incubated with Invitrogen™ antibodies against beta-catenin (Cat. MA1-300; 1:5,000), eIF4E (Cat. No. MA1-089; 1:1,000), RSK2 (Cat. No. MA515920; 1:1,000), or ezrin (Cat. No. MA513862; 1:1,000) followed by goat anti-mouse IgG (H+L) secondary antibody, HRP conjugate (Cat. No. 31430). Chemiluminescent detection and substrate comparison were performed following a 5-minute incubation with either SuperSignal West Pico PLUS or Bio-Rad Clarity substrate. Signal was captured on X-ray film.

thermo scientific



Ordering information

Description	Unit size	Cat. No.
SuperSignal West substrates		
SuperSignal West Pico PLUS Chemiluminescent Substrate	200 mL	34577
	500 mL	34580
	1 L	34578
SuperSignal West Dura Extended Duration Substrate	100 mL	34075
	200 mL	34076
SuperSignal West Femto Maximum Sensitivity Substrate	100 mL	34095
	200 mL	34096
SuperSignal West Atto Ultimate Sensitivity Substrate	100 mL	A38555
	200 mL	A38556
SuperSignal West substrate combo kits		
SuperSignal Western Blot Substrate Bundle—Pico PLUS (500 mL) + Trial size Femto (20 mL)	Combo kit	A43840
SuperSignal Western Blot Substrate Bundle—Femto (200 mL) + Trial size Pico PLUS (20 mL)	Combo kit	A43841
SuperSignal Western Blot Substrate Bundle—Pico PLUS (500 mL) + Trial size Atto (20 mL)	Combo kit	A45915
SuperSignal Western Blot Substrate Bundle—Femto (100 mL) + Trial size Atto (20 mL)	Combo kit	A45916
SuperSignal Western Blot Substrate Bundle—Atto (100 mL) + Trial size Pico PLUS (20 mL)	Combo kit	A45917
SuperSignal Western Blot Substrate Trial Pack—Pico PLUS (20 mL) and Atto (20 mL)	Combo kit	A45918
SuperSignal West substrates (trial size)		
SuperSignal West Pico PLUS Chemiluminescent Substrate	20 mL	34579
SuperSignal West Dura Extended Duration Substrate	20 mL	37071
SuperSignal West Femto Maximum Sensitivity Substrate	20 mL	34094
SuperSignal West Atto Ultimate Sensitivity Substrate	20 mL	A38554
Pierce ECL substrates		
Pierce ECL Western Blotting Substrate	50 mL	32109
	250 mL	32209
	500 mL	32106
Pierce ECL Plus Western Blotting Substrate	25 mL	32134
	100 mL	32132
	3 x 100 mL	32132X3
Invitrogen HRP-conjugated secondary antibodies (0.01 mg/mL)		
Goat Anti-Mouse IgG (H+L) Secondary Antibody, HRP	2 mL	32430
Goat Anti-Rabbit IgG (H+L) Secondary Antibody, HRP	2 mL	32460

To find out more or request a sample, go to
thermofisher.com/supersignal

ThermoFisher SCIENTIFIC