

HRP chemiluminescent substrates— our signals are loud and clear

Detect your protein, no matter how scarce



Thermo Scientific™ horseradish peroxidase (HRP) chemiluminescent substrates are capable of detection sensitivities in the low-femtogram level with minimal background interference. Take advantage of the longer signal duration and broader detection levels to obtain high-quality western blot results time after time.

Our chemiluminescent substrates enable you to obtain fast, reproducible results you can count on, attested to by over 7,000 references in major research publications over the past three years.*

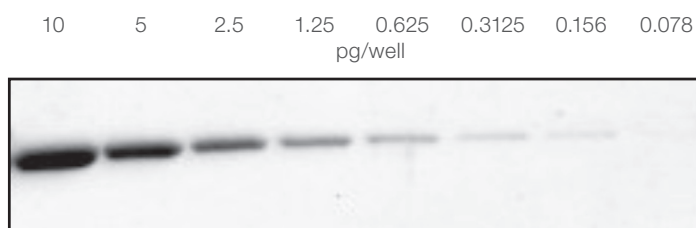


Figure 1. Low-picogram detection with SuperSignal West Pico PLUS Substrate. TurboGFP containing a 6x-His tag was diluted in reducing sample buffer for electrophoresis. Lane 1 contained 10 pg of the purified protein with serial dilutions prepared 1:1 and applied at 10 μ L/well. After electrophoresis, proteins were transferred to a Thermo Scientific™ Nitrocellulose Membrane (Cat. No. PI88018), using the Thermo Scientific™ Pierce™ Power Blotter (Cat. No. PI22834) and Pierce™ 1-Step Transfer Buffer (Cat. No. PI84731). The membrane was blocked with Thermo Scientific™ SuperBlock™ T20 (TBS) Blocking Buffer (Cat. No. PI37536). The membrane was then incubated with Invitrogen™ 6x-His Epitope Tag Antibody (Cat. No. MA1-21315) at 1 μ g/mL, followed by incubation with Invitrogen™ Goat anti-mouse IgG (H+L) Secondary Antibody, HRP conjugate (Cat. No. PI32430) at 100 ng/mL. SuperSignal West Pico PLUS Substrate (Cat. No. PI34577) was used for detection. A 30-second exposure was acquired on the Thermo Scientific™ myECL™ Imager (Cat. No. PI62236).

Highlights:

- **Excellent sensitivity**—five substrates, providing picogram-to-femtogram sensitivity
- **Strong light emission**—longer signal duration, allowing for multiple exposures
- **Long shelf life**—stable for at least one year
- **Antibody savings**—optimized to work with more dilute primary and secondary antibodies
- **Flexibility**—signal can be detected with X-ray film and charge-coupled device (CCD) imaging

Introducing the newest member of our western blot substrate family

Thermo Scientific™ SuperSignal™ West Pico PLUS Chemiluminescent Substrate is an enhanced chemiluminescent (ECL) substrate designed for low-picogram to high-femtogram detection levels.

SuperSignal West Pico PLUS Chemiluminescent Substrate offers better sensitivity (Figure 1), longer duration, and brighter intensity than our original Thermo Scientific™ SuperSignal™ West Pico substrate. The intensity of the light emission combined with exceptional duration allows acquisition of multiple exposures to effortlessly obtain publication-quality blot images.

Our newest chemiluminescent substrate is compatible with different membranes and a wide range of antibody dilutions, making it an ideal choice for most western blotting applications (Figure 2).

Benefits you can expect from SuperSignal West Pico PLUS Chemiluminescent Substrate:

- **Picogram-to-femtogram sensitivity**—detect low-picogram to high-femtogram amounts of target protein on nitrocellulose or PVDF membrane
- **Enhanced stability**—stable signal duration over the critical 4-hour time period with signal light output up to 24 hours (Figure 3)
- **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 dilution from a 1 mg/mL stock solution
- **Reagent stability**—8-hr working solution stability; 1-year kit stability at room temperature

One size does not fit all when it comes to western blotting

Sensitivity requirements for western blotting may vary depending on the abundance of the target protein, the sample availability, and the quality of the antibodies. No single substrate performs best for all western blot systems and meets all sensitivity requirements. For example, our SuperSignal West Pico PLUS substrate is recommended for detecting a new protein of interest when western blotting conditions are not yet optimized. The long-lasting signal combined with low-picogram sensitivity provided by this substrate makes it the prime choice for detecting new target proteins.

Use Thermo Scientific™ SuperSignal™ West Dura Extended Duration Substrate for sensitive detection of low-abundance proteins and 24-hour stable signal output, and use Thermo Scientific™ SuperSignal™ West Femo Maximum Sensitivity Substrate for the highest level of sensitivity to the low-femtogram range of detection.

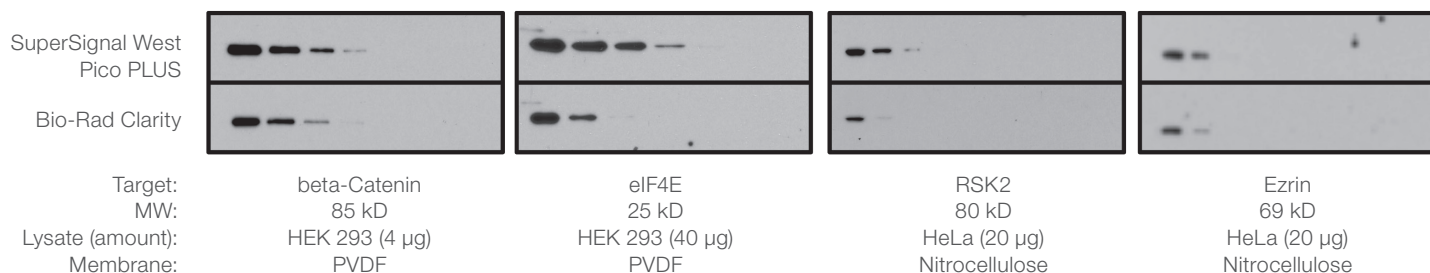


Figure 2. SuperSignal West Pico PLUS substrate outperforms Bio-Rad™ Clarity™ substrate. Detection of the indicated targets was performed using 2-fold serial dilutions of HEK 293 or HeLa cell lysates, starting with the amount indicated in parentheses. Following separation by SDS-PAGE, proteins were transferred to either Thermo Scientific™ PVDF (Cat. No. PI88518) or nitrocellulose (Cat. No. PI88018) membranes using the Pierce Power Blotter (Cat. No. PI22834) and 1-Step Transfer Buffer (Cat. No. PI84731). The membranes were blocked with 5% nonfat dry milk dissolved in Thermo Scientific™ Pierce™ 20X TBS Tween™ 20 Buffer (Cat. No. PI28360), and incubated with Invitrogen™ antibodies against beta-Catenin (Cat. No. MA1-300; 1:5,000), eIF4E (Cat. No. MA1-089; 1:1,000), RSK2 (Cat. No. MA5-15920; 1:1,000), or Ezrin (Cat. No. MA5-13862; 1:1,000), followed by incubation with Goat anti-Mouse IgG (H+L) Secondary Antibody, HRP Conjugate (Cat. No. PI31430) at a concentration of 20 ng/mL. Chemiluminescent detection and substrate comparison was performed following a 5-minute incubation with either SuperSignal West Pico PLUS or Bio-Rad Clarity substrate. Signal was captured using X-ray film.

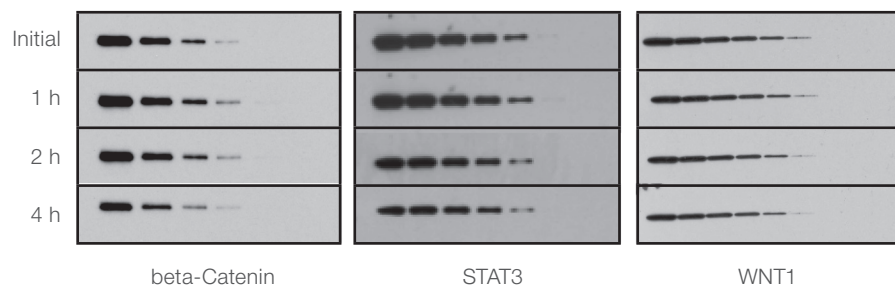


Figure 3. Robust signal for minimum of 4 hours. Detection of the indicated targets was performed using 2-fold serial dilutions of HEK 293 or HeLa cell lysates, starting at 4 µg/well or 20 µg/well, respectively. Following separation by SDS-PAGE, proteins were transferred to either PVDF (Cat. No. PI88518) or nitrocellulose (Cat. No. PI88018) membranes using the Pierce Power Blotter (Cat. No. PI22834) and 1-Step Transfer Buffer (Cat. No. PI84731). The membranes were blocked with 5% nonfat dry milk dissolved in Pierce 20X TBS Tween 20 Buffer (Cat. No. PI28360), and incubated with Invitrogen antibodies against beta-Catenin (Cat. No. MA1-300), STAT3 (Cat. No. MA1-13042), or WNT1 (Cat. No. MA5-15544), followed by incubation with Goat anti-Mouse IgG (H+L) Secondary Antibody, HRP Conjugate (Cat. No. PI31430) at a concentration of 20 ng/mL. Chemiluminescent detection was performed following a 5-minute incubation with SuperSignal West Pico PLUS substrate. Signal was captured on X-ray film at the indicated time points after addition of substrate.

Choose the Thermo Scientific™ substrate that best suits your western blot sensitivity needs from our range of high-quality products.

	Pierce ECL	Pierce ECL 2	SuperSignal West Pico PLUS	SuperSignal West Dura	SuperSignal West Femto
Advantage	Same signal, lower price than other entry-level ECL substrates	Same signal and lower price than competing ECL Plus substrates	Excellent sensitivity, intensity, and duration than other ECL substrates in its class	Best for use with imaging equipment	Most sensitive substrate for HRP detection
Detection level	Low to mid picogram	Low picogram	Low picogram to high femtogram	Mid femtogram	Low to mid femtogram
Signal duration	30 min–2 hr	5 hr	Up to 24 hr	24 hr	8 hr
Detection methods	X-ray film, CCD imager	X-ray film, CCD imager, fluorescence imager	X-ray film, CCD imager	X-ray film, CCD imager	X-ray film, CCD imager
Recommended primary and secondary antibody dilutions	1° 1:1K 2° 1:1K–1:15K	1° 1:1K 2° 1:25K–1:200K	1° 1:1K 2° 1:20K–1:100K	1° 1:5K 2° 1:50K–1:250K	1° 1:5K 2° 1:100K–1:500K
Select when...	Target is abundant, sample is abundant, and substrate is for everyday use	Target is less abundant, sample is limited, and for chemifluorescent detection	Target is less abundant, sample is limited, and you need more sensitivity than an entry-level ECL substrate	Target is less abundant, sample is limited, and for CCD image capture	Target is least abundant, sample is precious, and for maximum sensitivity
Value to you	Low cost; easy to switch from other entry-level ECL substrates	Best detection flexibility with chemifluorescent detection option	Best value; works for majority of western blots	Best signal duration	Best sensitivity

For data above: STAT3 detection in HeLa cell lysate (lane 1: 20 µg total protein; lanes 2–6: serially diluted 1:1) was performed using Thermo Scientific™ HRP chemiluminescent substrates. The blots were developed using Invitrogen™ anti-STAT3 Antibody (Cat. No. MA1-13042) and Goat anti-Mouse IgG Secondary Antibody, HRP conjugate (Cat. No. PI31430). Images were captured using the myECL Imager (Cat. No. PI62236).

HRP chemiluminescent substrates for western blotting

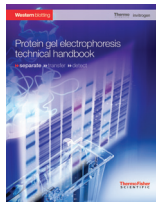


Ordering information

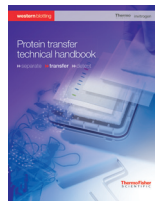
Description	Unit size	Cat. No.
Pierce ECL Substrate		
Pierce ECL Western Blotting Substrate	50 mL	PI32109
	250 mL	PI32209
	500 mL	PI32106
Pierce ECL 2 Substrate		
Pierce ECL Plus Western Blotting Substrate	25 mL	PI80197
	100 mL	PI80196
	3 x 100 mL	PI80196X3
SuperSignal West Pico PLUS Substrate		
SuperSignal West Pico PLUS Chemiluminescent Substrate	20 mL	PI34579
	200 mL	PI34577
	500 mL	PI34580
	1 L	PI34578

Description	Unit size	Cat. No.
SuperSignal West Dura Substrate		
SuperSignal West Dura Extended Duration Substrate	20 mL	PI37071
	100 mL	PIA34075
	200 mL	PI34076
SuperSignal West Femto Substrate		
SuperSignal West Femto Maximum Sensitivity Substrate	20 mL	PI34094
	100 mL	PI34095
	200 mL	PI34096
Related products		
SuperSignal Western Blot Enhancer	500 mL	PI46640
Restore PLUS Western Blot Stripping Buffer	500 mL	PI46430
CL-XPosure Film 5 x 7 in.	50 sheets	PI34088
SuperBlock (PBS) Blocking Buffer	1 L	PI37517
StartingBlock (PBS) Blocking Buffer	1 L	PI37538
MagicMark XP Western Protein Standard	250 µL	LC5602

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