INSTRUCTIONS

SuperSignal West Pico Chemiluminescent Substrate

34077 34078 34079 34080 34087 0636.10

Number	Description
34079	**SuperSignal West Pico Chemiluminescent Substrate,** sufficient for 500cm^2 of membrane **Kit Contents:** **SuperSignal West Pico Luminol/Enhancer Solution,** 25mL **SuperSignal West Pico Stable Peroxide Solution,** 25mL
34077	**SuperSignal West Pico Chemiluminescent Substrate,** sufficient for 1000cm^2 of membrane **Kit Contents:** **SuperSignal West Pico Luminol/Enhancer Solution,** 50mL **SuperSignal West Pico Stable Peroxide Solution,** 50mL
34087	**SuperSignal West Pico Chemiluminescent Substrate,** sufficient for 2000cm^2 of membrane **Kit Contents:** **SuperSignal West Pico Luminol/Enhancer Solution,** 100mL **SuperSignal West Pico Stable Peroxide Solution,** 100mL
34080	**SuperSignal West Pico Chemiluminescent Substrate,** sufficient for 5000cm^2 of membrane **Kit Contents:** **SuperSignal West Pico Luminol/Enhancer Solution,** 250mL **SuperSignal West Pico Stable Peroxide Solution,** 250mL
34078	**SuperSignal West Pico Chemiluminescent Substrate,** sufficient for 10,000cm^2 of membrane **Kit Contents:** **SuperSignal West Pico Luminol/Enhancer Solution,** 500mL **SuperSignal West Pico Stable Peroxide Solution,** 500mL
	Storage: Upon receipt store reagents at room temperature. Products are shipped at ambient temperature.

Introduction

The Thermo Scientific™ SuperSignal™ West Pico Chemiluminescent Substrate is a sensitive, luminol-based enhanced chemiluminescent substrate for detecting horseradish peroxidase (HRP) on immunoblots. SuperSignal West Pico Substrate enables picogram detection of antigen by oxidizing luminol in the presence of HRP and peroxide. This reaction produces a prolonged chemiluminescence which can be visualized on X-ray film or an imaging system. Optimal signal intensity and duration can be attained with appropriate primary and secondary antibody dilutions (see Table 1).

Table 1. Antibody dilution ranges to use with SuperSignal West Pico Chemiluminescent Substrate.

Primary Antibody Dilution Range from a 1mg/mL stock	Secondary Antibody Dilution Range from a 1mg/mL stock
1:1,000-1:5,000 or 0.2-1.0µg/mL	1:20,000-1:100,000 or 10-50ng/mL

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Important Product Information

- Western blot results require optimizing the process components and steps, including sample amount, gel type, transfer method, membrane type, blocking reagent, wash buffer, primary antibody concentration, secondary antibody concentration and incubation times.
- Use a sufficient volume of all solutions to ensure membrane never becomes dry.
- For optimal results, use a shaking or rocking platform during incubation steps.
- Do not use sodium azide as a preservative for buffers, as it inhibits HRP.
- Always wear gloves or use clean, plastic forceps. Metallic devices (e.g., scissors) must have no visible signs of rust, which may cause speckling and/or high background.
- The substrate Working Solution is stable for 24 hours at room temperature. Exposure to the sun or any other intense light can harm the Working Solution. Short-term exposure to laboratory lighting will not harm the Working Solution.

Additional Materials Required

- **Western blot membrane**: Use any suitable protocol to separate proteins by electrophoresis and transfer them to a membrane.
- **X-ray film or imaging system** (e.g., Thermo Scientific™ MYECL™ Imager, Product No. 62236)
- **Rotary or rocking platform shaker**: For agitation of membrane during incubations.

Procedure

Note: Consult Tech Tip #67: Chemiluminescent Western blotting technical guide and protocols or our SuperSignal Substrate Western Blotting Handbook for a detailed Western Blotting protocol.

Note: Western blot results require optimizing the process components and steps. See Important Product Information.

1. Incubate the blot with 0.01-0.2µg/mL primary antibody for one hour to overnight.
2. Sufficiently wash the blot with appropriate buffer.
3. Incubate the blot with 2-10ng/mL secondary antibody for approximately 30-60 minutes.
4. Prepare Working Solution by mixing equal parts of the Stable Peroxide Solution and the Luminol/Enhancer Solution. Use 0.1mL Working Solution per cm^2 of membrane. The Working Solution is stable for 24 hours at room temperature.
5. Incubate the blot in Working Solution for 5 minutes.
6. Remove the blot from Working Solution and drain excess reagent.
7. Place the blot in clear plastic wrap or sheet protector and remove bubbles.
8. Expose the blot to X-ray film or imaging system.

Image Acquisition with MYECL Imager

1. Place blot on the black Chemiluminescence Exposure Screen.
2. Place the exposure screen on top of the transilluminator and close the imager door.
3. Select the **Chemi** tab and then the **Interactive Chemi** button. The imager acquires a 15-second exposure.
4. The imager calculates and displays the exposure time with maximum dynamic range and minimum pixel saturation.
5. Use the exposure time slider bar and updated image preview to obtain the desired exposure time. Drag and release the slider bar to update the image preview. Alternatively, select the **Time Display/Input** button to enter a custom exposure time that updates the image preview and slider bar location.
6. Select the **Acquire** button to capture an image with the indicated exposure time.
7. Alternatively, select a pre-set or custom exposure time in the **Chemi** tab to acquire an image.

Troubleshooting

Problem	Possible Cause	Solution
Reverse image on film (i.e., white bands with a black background)	Too much HRP in the system	Further dilute the HRP-conjugate (see guidelines in Table 1)
Membrane has brown or yellow bands		
Blot glows in the darkroom		
Signal duration is less than 8 hours		
Weak or no signal	Too much HRP in the system depleted the substrate and caused the signal to fade quickly	Further dilute the HRP-conjugate (see guidelines in Table 1)
	Insufficient quantities of antigen or antibody	Increase amount of antibody or antigen
		Use SuperSignal Western Blot Enhancer (Product No. 46640)
	Inefficient protein transfer	Optimize transfer
	Reduction of HRP or substrate activity	**See note below
High background	Too much HRP in the system	Further dilute the HRP-conjugate (see guidelines in Table 1)
	Inadequate blocking	Optimize blocking conditions
	Inappropriate blocking reagent	Try a different blocking reagent
	Inadequate washing	Increase length, number or volume of washes
	Film has been overexposed	Decrease exposure time or use Pierce Background Eliminator (Product No. 21065)
	Concentration of antigen or antibody is too high	Decrease amount of antigen or antibody
	Poor antibody specificity	Use SuperSignal Western Blot Enhancer (Product No. 46640)
Spots within the protein bands	Inefficient protein transfer	Optimize transfer procedure
	Unevenly hydrated membrane	Perform manufacturer's recommendations for hydrating membrane properly
	Bubble between the film and the membrane	Remove bubbles before exposing blot to film
Speckled background on film	Aggregate formation in the HRP-conjugate	Filter conjugate through a 0.2µm filter
Nonspecific bands	Too much HRP in the system	Further dilute the HRP-conjugate (see guidelines in Table 1)
	SDS caused nonspecific binding to protein bands	Do not use SDS during the Western blot procedure
	Poor antibody specificity	Use SuperSignal Western Blot Enhancer (Product No. 46640)
	Insufficient blocking	Increase blocking time or use different blocking reagent

**To test the activity of the system in the darkroom, prepare 1-2mL of the SuperSignal Substrate Working Solution in a clear test tube. With the lights turned off, add 1µL undiluted HRP-conjugate to the Working Solution. The solution should immediately emit a blue light that will fade over the next several minutes. If no light emission is evident, test another source of HRP to determine the root cause.

Additional Information

Please visit the website for additional information on this product including the following items:

- Tech Tip #23: Strip and reprobe Western blots
- Tech Tip #24: Optimize antigen and antibody Concentrations for Western blots
- Tech Tip #67: Chemiluminescent Western blotting technical guide and protocols
- Request a free copy of the 24-page SuperSignal Substrate Western Blotting Handbook, which contains a 10-page section on troubleshooting, detailed Western Blotting protocols and dozens of SuperSignal Substrate references

Related Products

62288	**Pierce G2 Fast Blotter,** 2-part unit
62236	**MYECL Imager,** 1 unit
32430	**Stabilized Goat Anti-Mouse IgG (H+L), Peroxidase Conjugated (10µg/mL),** 2mL
32460	**Stabilized Goat Anti-Rabbit IgG (H+L), Peroxidase Conjugated (10µg/mL),** 2mL
34075	**SuperSignal West Dura Extended Duration Substrate,** 100mL
34096	**SuperSignal West Femto Maximum Sensitivity Substrate,** 200mL
34090	**CL-XPosure™ Film,** 5 × 7in. sheets, 100 sheets/pkg
34091	**CL-XPosure Film,** 8 × 10in. sheets, 100 sheets/pkg
21059	**Restore Western Blot Stripping Buffer,** 500mL
46430	**Restore PLUS Western Blot Stripping Buffer,** 500mL
46640	**SuperSignal Western Blot Enhancer,** 500mL kit

General References

CRC Handbook of Immunoblotting of Proteins: Volume 1 Technical Description. Eds Ole J. Bjerrum, Ph.D., M.D. and Niels H.H. Heegaard, M.D. CRC Press, Inc.: Boca Raton, FL, 1988.

Kaufmann, S.H., *et al.* (1987). The erasable Western blot. *Anal. Biochem.* **161:**89-95.

Mattson, D.L. and Bellehumeur, T.G. (1996). Comparison of three chemiluminescent horseradish peroxidase substrates for immunoblotting. *Anal. Biochem.* **240:**306-308.

Walker, G.R., *et al.* (1995). SuperSignal™ CL-HRP: A new enhanced chemiluminescent substrate for the development of the horseradish peroxide label in Western blotting applications. *J. of NIH Research* **7:**76.

SuperSignal Technology is protected by U.S. Patent #6,432,662.

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.

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