INSTRUCTIONS

PIERCE
3747 N. Meridian Road
P.O. Box 117
Rockford, IL 61105

SuperSignal® West Pico Chemiluminescent Substrate

34077 34078 34079 34080

0636.3

Number	Description
34079	**SuperSignal® West Pico Chemiluminescent Substrate,** sufficient for 5 mini-blots or 400 cm^2 of membrane **Kit Contents:** **SuperSignal® West Pico Luminol/Enhancer Solution,** 25 ml **SuperSignal® West Pico Stable Peroxide Solution,** 25 ml
34077	**SuperSignal® West Pico Chemiluminescent Substrate,** sufficient for 10 mini-blots or 800 cm^2 of membrane **Kit Contents:** **SuperSignal® West Pico Luminol/Enhancer Solution,** 2 × 25 ml **SuperSignal® West Pico Stable Peroxide Solution,** 2 × 25 ml
34080	**SuperSignal® West Pico Chemiluminescent Substrate,** sufficient for 50 mini-blots or 4,000 cm^2 of membrane **Kit Contents:** **SuperSignal® West Pico Luminol/Enhancer Solution,** 250 ml **SuperSignal® West Pico Stable Peroxide Solution,** 250 ml
34078	**SuperSignal® West Pico Chemiluminescent Substrate,** sufficient for 100 mini-blots or 8,000 cm^2 of membrane **Kit Contents:** **SuperSignal® West Pico Luminol/Enhancer Solution,** 500 ml **SuperSignal® West Pico Stable Peroxide Solution,** 500 ml

Storage: Upon receipt store reagents at room temperature.

Table of Contents

IMPORTANT NOTE: SuperSignal® West Pico Chemiluminescent Substrate is a high-sensitivity substrate that is more sensitive than most chemiluminescent products including ECL™, LumiGLO®, Renaissance® and Western Lightning™ Substrates. For optimal performance of SuperSignal® Substrate, antibodies must be more dilute than those used with these other substrates. **If you have been using one of the substrates listed above or another "entry-level" chemiluminescent substrate, dilute both primary and secondary antibodies at least 5-fold more.** For example: If you have been using the primary antibody at 1:100 dilution with ECL™ Substrate, then use a ~1:500 dilution with SuperSignal® West Pico Substrate. Recommended dilution ranges are listed in Table 1.

Table 1. Recommended antibody dilutions to use with SuperSignal® West Pico Chemiluminescent Substrate.

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

Introduction

SuperSignal® West Pico Chemiluminescent Substrate is a highly sensitive enhanced substrate for detecting horseradish peroxidase (HRP) on immunoblots. This substrate's extremely intense signal output enables detection of picogram amounts of antigen. The sensitivity, intensity and duration of the signal allow for easy detection of HRP using photographic or other imaging methods. Blots can also be repeatedly exposed to film to obtain optimal results or stripped of the immunodetection reagents and reprobed.

Important Product Information

- For best results, it is ESSENTIAL to optimize all components of the system including sample amount, primary and secondary antibody concentration, and the choice of membrane and blocking reagents. Because the substrate is extremely sensitive, SuperSignal® West Pico Substrate requires the use of much less sample and primary and secondary antibodies than most commercially available substrates, usually by a factor of at least 10-20.
- The antibody concentrations required will be much more dilute than those used with precipitating colorimetric HRP systems. To optimize the appropriate concentrations, perform a systematic dot blot analysis.
- Because no blocking reagent is optimal for all systems, empirical testing is essential to determine the appropriate blocking buffer for each Western blot system. Determining the proper blocking buffer can help increase sensitivity and prevent nonspecific signal caused by cross-reactivity between the antibody and the blocking reagent. Furthermore, when switching from one substrate to another, a diminished signal or increased background sometimes results because the blocking buffer was not optimal for the new system.
- Avoid using milk as a blocking reagent when using avidin/biotin systems because milk contains variable amounts of endogenous biotin.
- Use a sufficient volume of wash buffer, blocking buffer, antibody solution and Substrate Working Solution to cover blot and ensure that it never becomes dry. Large blocking and wash buffer volumes may result in reduced nonspecific signal.
- For optimal results, use a shaking platform during incubation steps.
- Add Tween®-20 (final concentration of 0.05%) to the blocking buffer and when preparing all antibody dilutions to reduce nonspecific signal. Use only high-quality products such as Surfact-Amps®-20 (Product No. 28320), which is a purified detergent packaged in ampules and guaranteed to be low in peroxides and other contaminants.
- Do not use sodium azide as a preservative for buffers. Sodium azide is an inhibitor of HRP and could interfere with this system.
- Do not handle membrane with bare hands. Always wear gloves or use clean forceps.
- All equipment must be clean and free of foreign material. Metallic devices (e.g., scissors) must have no visible signs of rust. Rust may cause speckling and/or high background.
- The Substrate Working Solution is stable for 8 hours at room temperature. Exposure to the sun or any other intense light can harm the Working Solution. For best results keep the Working Solution in an amber bottle and avoid prolonged exposure to any intense light. Short-term exposure to typical laboratory lighting will not harm the Working Solution.
- Pierce offers a variety of protein transfer membranes, blocking buffers, primary antibodies, enzyme-labeled secondary antibodies, buffers and detergents. Please consult the Pierce web site or catalog for product and ordering information.

Procedure Summary

Note: Antigen and antibody amounts may require optimization. Recommended antibody dilutions must be used to consistently obtain positive results. For recommended dilution ranges please see Additional Materials Required section.

1. Dilute primary antibody to 0.2-1.0 µg/ml or 1:1,000-1:5,000 dilution from a 1 mg/ml stock.*
2. Dilute secondary antibody to 10-50 ng/ml or 1:20,000-1:100,000 dilution from a 1 mg/ml stock.*
3. Mix the two substrate components at a 1:1 ratio to prepare the substrate Working Solution.

   Note: Exposure to the sun or any other intense light can harm the Working Solution. For best results keep the Working Solution in an amber bottle and avoid prolonged exposure to any intense light. Short-term exposure to typical laboratory lighting will not harm the Working Solution.
4. Incubate blot 5 minutes in SuperSignal® West Substrate Working Solution.
5. Drain excess reagent. Cover blot with clear plastic wrap.
6. Expose blot to X-ray film.

*See Important Note on page 1.

Additional Materials Required

- Completed Western blot membrane: Use any suitable protocol to separate proteins by electrophoresis and transfer them to a nitrocellulose membrane. Other membrane types can be used; however, optimization may be required.[1]
- Dilution Buffer: Use either Tris Buffered Saline (TBS, Product No. 28376) or Phosphate Buffered Saline (PBS, Product No. 28374).
- Wash Buffer: Add 5 ml of 10% Tween®-20 (Surfact-Amps™ 20, Product No. 28320) to 1,000 ml Dilution Buffer. (The final concentration of Tween®-20 will be 0.05%.)
- Blocking Reagent: Add 0.5 ml of 10% Tween®-20 to 100 ml of a blocking buffer such as SuperBlock® (PBS) Blocking Buffer (Product No. 37515) or SuperBlock® (TBS) Blocking Buffer (Product No. 37535). Choose a blocking buffer with the same base component as the Dilution Buffer.
- Primary Antibody:* Choose an antibody that is specific to the target protein(s). Prepare a **1 mg/ml** stock solution of this antibody in Dilution Buffer. Use the Blocking Reagent to make all working dilutions of this antibody stock. Prepare a working dilution between **1:1,000** to **1:5,000** or **0.2-1 µg/ml**. The optimal dilution to use depends on the specific primary antibody and the amount of antigen on the membrane.
- HRP-conjugated Secondary Antibody:* Choose an HRP-conjugate that specifically binds to the primary antibody. Prepare a **1 mg/ml** stock solution in Dilution Buffer. Use the Blocking Reagent to make all working dilutions of this antibody stock. Prepare a working dilution between **1:20,000** to **1:100,000** or **10-50 ng/ml**. This range also applies when using either Streptavidin-HRP (Product No. 21124) or NeutrAvidin™-HRP (Product No. 31001). The optimal dilution to use will vary depending on the HRP conjugate and the amount of antigen on the membrane.
- Film cassette, developing and fixing reagents: For processing autoradiographic film.
- Rotary platform shaker: For agitation of membrane during incubations.

*See Important Note on page 1.

Detailed Western Blotting Procedure

1. Remove blot from the transfer apparatus and block nonspecific sites with Blocking Reagent for 20-60 minutes at room temperature (RT) with shaking. For best results, block for 1 hour at RT.

Please Note: It is critical to use the recommended antibody dilution indicated in the previous Additional Materials Required section

2. Remove the Blocking Reagent and add the appropriate primary antibody dilution. Incubate blot for 1 hour with shaking. If desired, blots may be incubated with primary antibody overnight at 2-8°C.
3. Wash membrane by suspending it in Wash Buffer and agitating for ≥5 minutes. Replace Wash Buffer at least 4-6 times. Increasing the wash buffer volume and/or the number of washes may help reduce background.

   Note: Briefly rinsing membrane in wash buffer before incubation will increase wash efficiency.

Please Note: It is critical to use the recommended HRP-conjugate dilution indicated in the previous Additional Materials Required section

7. Incubate blot with the appropriate HRP-conjugate dilution for 1 hour at RT with shaking.
8. Repeat Step 3 to remove non-bound HRP-conjugate.

   Note: Membrane MUST be thoroughly washed after incubation with the HRP-conjugate.
9. Prepare Working Solution by mixing equal parts of the Stable Peroxide Solution and the Luminol/Enhancer Solution. Use 0.125 ml Working Solution per cm^2 of membrane. The Working Solution is stable for 8 hours at room temperature.

   Note: Exposure to the sun or any other intense light can harm the Working Solution. For best results keep the Working Solution in an amber bottle and avoid prolonged exposure to any intense light. Typical laboratory lighting will not harm the Working Solution.
10. Incubate blot with Working Solution for 5 minutes.
11. Remove blot from Working Solution and place it in a plastic membrane protector; a plastic sheet protector or plastic wrap may be used. Use an absorbent tissue to remove excess liquid and to carefully press out any bubbles from between the blot and surface of the membrane protector.
12. Place the protected membrane in a film cassette with the protein side facing up. Turn off all lights except those appropriate for film exposure (e.g., a red safelight).

    Note: Film must remain dry during exposure. For optimal results, perform the following precautions:
    - Make sure excess substrate is removed from the membrane and the membrane protector.
    - Use gloves during the entire film-handling process.
    - Never place a blot on developed film, as there may be chemicals on the film that will reduce signal.
13. Carefully place a piece of film on top of the membrane. A recommended first exposure time is 60 seconds. Exposure time may be varied to achieve optimal results. Enhanced or pre-flashed film is not necessary.

    Caution: Light emission is intense and any movement between the film and membrane can cause artifacts on the film.

    Note: The exposure time may be varied to achieve optimal results. If the signal is too intense, reduce exposure time or optimize the system by decreasing the antigen and/or antibody concentrations. Alternatively, use Erase-It™ Background Eliminator (Product No. 21065).

    Light emission is most intense during the first 5-30 minutes after substrate incubation. Light emission will continue for several hours, but will decrease with time. Longer exposure times may be necessary as the blot ages.

    If using a storage phosphor imaging device (e.g., Bio-Rad's Molecular Imager® System) or a CCD Camera (e.g., Alpha-Innotech Corporation's ChemiImager™ System), longer exposure times may be necessary.
14. Develop film using appropriate developing solution and fixative. Blot may be stripped and reprobed if necessary. For best results, use Restore™ Western Blot Stripping Buffer (Product No. 21059).

Troubleshooting

Problem	Possible Cause	Solution
Reverse image on film (i.e., white bands with a black background)	Too much HRP in the system	Dilute HRP-conjugate at least 10-fold
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 caused the signal to fade quickly	Dilute HRP-conjugate at least 10-fold
	Insufficient quantities of antigen or antibody	Increase amount of antibody or antigen
	Inefficient protein transfer	Optimize transfer
	Reduction of HRP or substrate activity	**See note below
High background	Too much HRP in the system	Dilute HRP-conjugate at least 10-fold
	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 Erase-it™ Background Eliminator (Product No. 21065)
	Concentration of antigen or antibody is too high	Decrease amount of antigen or antibody
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 all bubbles before exposing blot to film
Speckled background on film	Aggregate formation in the HRP-conjugate	Filter conjugate through a 0.2 µm filter
Nonspecific bands	Too much HRP in the system	Dilute HRP-conjugate at least 10-fold
	SDS caused nonspecific binding to protein bands	Do not use SDS during immunoassay procedure

**To test the activity of the system in the darkroom, prepare 1-2 ml of the SuperSignal® Substrate Working Solution in a clear test tube. With the lights turned off, add 1 µl undiluted HRP-conjugate to the Working Solution. The solution should immediately emit a blue light that will fade over the next several minutes.

Additional Information

Please visit the Pierce web site for additional information on this product including the following items:

- Tech Tip Protocol: Optimize antigen and antibody concentrations for Western blots
- Tech Tip Protocol: Determine source of nonspecific background signal in Western blots detected using SuperSignal® Chemiluminescent Substrates
- Tech Tip Protocol: Convert to SuperSignal West Pico Substrate from ECL Substrate
- HRP conjugates for use with SuperSignal® West Pico Substrate
- How to request your 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 Pierce Products

34090	**CL-XPosure™ Film,** 5" × 7" sheets, 100 sheets/pkg
34075	**SuperSignal® West Dura Extended Duration Substrate,** 100 ml
34095	**SuperSignal® West Femto Maximum Sensitivity Substrate,** 100 ml
21059	**Restore™ Western Blot Stripping Buffer,** 500 ml
21065	**Erase-It® Background Eliminator Kit,** for eliminating background from X-ray film
37515	**SuperBlock® (PBS) Blocking Buffer,** 1 L
37535	**SuperBlock® (TBS) Blocking Buffer,** 1 L
32110	**Antibody Extender Solution NC,** 500 ml, for using three times less primary antibody while maintaining signal intensity on nitrocellulose membrane
88018	**Nitrocellulose Membrane, 0.45 µm, 33 cm × 3 m,** 1 roll
77010	**Nitrocellulose Membrane, 0.45 µm, 8 × 12 cm,** 25/pkg.
88025	**Nitrocellulose Membrane, 0.45 µm, 8 × 8 cm,** 15/pkg.
88600	**Western Blotting Filter Paper, 8 cm x 10.5 cm,** 100 sheets

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.

The most current versions of all product instructions are available at *www.piercenet.com.* For a faxed copy, contact customer service (in the USA call 800-874-3723) or your local distributor.