INSTRUCTIONS

SuperSignal® West Pico Chemiluminescent Substrate Kits

34081 34082 34083 34084 0683.10

Number	Description
34081	**SuperSignal West Pico Complete Mouse IgG Detection Kit** **Kit Contents:*** **Luminol/Enhancer Solution,** 250mL; **Stable Peroxide Solution,** 250mL **Peroxidase Conjugated Goat anti-Mouse IgG (H + L),** 2mL **SuperBlock® Blocking Buffer,** 1L **BupH™ Tris Buffered Saline Packs,** 4 each
34082	**SuperSignal West Pico Mouse IgG Detection Kit** **Kit Contents:*** **Luminol/Enhancer Solution,** 250mL; **Stable Peroxide Solution,** 250mL **Peroxidase Conjugated Goat anti-Mouse IgG (H + L),** 2mL
34083	**SuperSignal West Pico Rabbit IgG Detection Kit** **Kit Contents:*** **Luminol/Enhancer Solution,** 250mL; **Stable Peroxide Solution,** 250mL **Peroxidase Conjugated Goat anti-Rabbit IgG (H + L),** 2mL
34084	**SuperSignal West Pico Complete Rabbit IgG Detection Kit** **Kit Contents:*** **Luminol/Enhancer Solution,** 250mL; **Stable Peroxide Solution,** 250mL **Peroxidase Conjugated Goat anti-Rabbit IgG (H + L),** 2mL **StartingBlock™ (TBS) Blocking Buffer,** 1L **BupH Tris Buffered Saline Packs,** 4 each

Storage: Upon receipt store substrate components at room temperature. Refer to specification sheets or package labels of other kit components for specific storage information. Products are shipped at ambient temperature.

*Each kit contains sufficient volume of SuperSignal West Pico Substrate for 5000cm^2 of membrane.

IMPORTANT NOTE: Thermo Scientific 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.

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

Primary Antibody Dilution Range from a 1 mg/ml stock	Secondary Antibody Dilution Range from a 1 mg/ml stock
1:1000-1:5000 or 0.2-1.0µg/mL	1:20,000-1:100,000 or 10-50ng/mL

Table of Contents

Introduction

These kits include SuperSignal West Pico Substrate that is a highly sensitive enhanced chemiluminescent substrate for detecting horseradish peroxidase (HRP) on immunoblots. The kits also contain reagents for the specific detection of biotinylated antibodies, rabbit IgG or mouse IgG. The substrate's 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 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 system components including sample amount, antibody concentrations and the membrane and blocking reagents. SuperSignal West Pico Substrate is extremely sensitive and requires much less sample and antibodies than most commercially available substrates.
- The required antibody concentrations are more dilute than those used with precipitating colorimetric HRP systems. To optimize the appropriate concentrations, perform a systematic dot blot analysis.
- Empirical testing is essential to determine the optimal blocking buffer for each system. Using the proper blocking buffer can increase sensitivity and prevent nonspecific signal caused by cross-reactivity between the antibody and the blocking reagent. When switching from one substrate to another, a diminished signal or increased background may result if the blocking buffer is 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 reduce background.
- 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.
- 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 Thermo Scientific 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, as sodium azide is an inhibitor of HRP.

Procedure Summary

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

1. Dilute primary antibody to 0.2-1.0µg/mL or 1:1000-1:5000 dilution from a 1mg/mL stock.*
2. Dilute secondary antibody to 10-50ng/mL or 1:20,000-1:100,000 dilution from a 1mg/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 2.

Materials Required

- Completed Western blot membrane: Use any suitable protocol to separate proteins by electrophoresis and transfer them to a nitrocellulose membrane. PVDF membranes may 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 5mL of 10% Tween-20 Detergent (Surfact-Amps® 20, Product No. 28320) to 1000mL of Dilution Buffer (the final concentration of Tween-20 Detergent will be 0.05%.).
- Blocking Reagent: Add 0.5mL of 10% Tween-20 Detergent to 100mL of a blocking buffer such as Thermo Scientific SuperBlock (PBS) Blocking Buffer (Product No. 37515), SuperBlock (TBS) Blocking Buffer (Product No. 37535), StartingBlock (TBS) Blocking Buffer (Product No. 37542, or Blocker BLOTTO in TBS (Product No. 37530). Choose a blocking buffer with the same base component as the Dilution Buffer.
- Primary Antibody: (See Important Note on page 2.) Prepare stock solution in Dilution Buffer. Use the Blocking Reagent to make all working dilutions of this antibody stock. Prepare a working dilution at **0.2-1.0µg/mL** (i.e., 1:1000-1:5000 from a 1mg/mL stock). The optimal dilution to use depends on the specific primary antibody and the amount of antigen on the membrane.
- HRP-conjugate: (See Important Note on page 2.) Refer to the HRP-conjugate product insert for reconstitution information. Prepare stock solution in Dilution Buffer. Use Blocking Reagent to make all working dilutions of this conjugate stock. Prepare a working dilution at **10-50ng/mL** (i.e., 1:20,000-1:100,000 from a 1mg/mL stock). The optimal dilution to use varies depending on the specific 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.

Detailed Western Blotting Procedure

For optimal results, use a shaking platform during incubation steps. Also, do not handle membrane with bare hands; always wear gloves or use clean forceps.

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.

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

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

   Note: Membrane MUST be thoroughly washed after incubation with the HRP-conjugate.
6. Prepare Working Solution by mixing equal parts of the Stable Peroxide Solution and the Luminol/Enhancer Solution. Use 0.1mL of Working Solution per cm^2 of membrane. The Working Solution is stable for 8 hours at room temperature.

   Note: 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.
7. Incubate blot with Working Solution for 5 minutes.
8. 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.
9. 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.
10. Carefully place a piece of film on top of the membrane. A recommended first exposure time is 60 seconds. Vary exposure time to achieve optimal results. Enhanced or pre-flashed autoradiographic film is not necessary.

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

    Note: If the signal is too intense, reduce exposure time or optimize the system by decreasing the antigen and/or antibody concentrations. Alternatively, use Thermo Scientific Pierce 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 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.
11. Develop film using appropriate developing solution and fixative. Blot may be stripped and reprobed if necessary. For optimal results, use Thermo Scientific Restore Western Blot Stripping Buffer (Product No. 21059).

Additional Information

Please visit the Thermo Scientific website for additional information relating to this product including the following items:

- Tech Tip #24: Optimize antigen and antibody concentrations for Western blots
- Tech Tip #21: Convert to SuperSignal West Pico Substrate from ECL Substrate

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 depleted the substrate and 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 Pierce® Background Eliminator (Product No. 21065)
	Antigen or antibody is too concentrated	Dilute antigen or antibody working solution
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 0.2µm filter or centrifuge and use supernatant
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 1-2mL 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.

Related Products

34090 **CL-XPosure™ Film,** 5" × 7" sheets, 100 sheets/pkg

34075 **SuperSignal West Dura Substrate,** 100mL

34095 **SuperSignal West Femto Maximum Sensitivity Substrate,** 100mL

32106 **Pierce ECL Western Blotting Substrate,** 500mL

21059 **Restore™ Western Blot Stripping Buffer,** 500mL

21065 **Pierce Background Eliminator Kit,** for eliminating background from X-ray film

37515 **SuperBlock (PBS) Blocking Buffer,** 1L

37535	**SuperBlock (TBS) Blocking Buffer,** 1L
37530	**Blocker™ BLOTTO (TBS),** 1L
32110	**Antibody Extender Solution NC,** 500mL, allows three times less primary antibody while maintaining signal intensity on nitrocellulose membrane
88018	**Nitrocellulose Membrane, 0.45µm, 33cm × 3m,** 1 roll
77010	**Nitrocellulose Membrane, 0.45µm, 8 × 12cm,** 25/pkg
88025	**Nitrocellulose Membrane, 0.45µm, 8 × 8cm,** 15/pkg
88600	**Western Blotting Filter Paper, 8cm × 10.5cm,** 100 sheets
24580	**Pierce Reversible Protein Stain Kit for Nitrocellulose Membranes**
24585	**Pierce Reversible Protein Stain Kit for PVDF Membranes**

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:**306-8.

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.

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non infringement. Buyer's exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).

There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

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