

Chemiluminescent Western blotting technical guide and protocols

TR0067.1

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Introduction

Western blotting is a powerful and commonly used tool to identify and quantify a specific protein in a complex mixture. As originally conceived by Towbin *et al.*, the technique enables indirect detection of protein samples immobilized on a nitrocellulose or PVDF membrane. In a conventional Western blot, protein samples are first resolved by SDS-PAGE and then electrophoretically transferred to the membrane. Following a blocking step, the membrane is probed with a primary antibody (poly- or monoclonal) that was raised against the antigen in question. After a subsequent washing step, the membrane is incubated with an enzyme-conjugated secondary antibody that is reactive toward the primary. The activity of the enzyme, such as alkaline phosphatase (AP) and horseradish peroxidase (HRP), is necessary for signal generation. Finally, the membrane is washed again and incubated with an appropriate enzyme substrate, producing a recordable signal.

This guide describes the general principles and factors involved in Western blotting using chemiluminescent substrates. Relevant Thermo Scientific® Pierce Protein Research Products are referenced throughout, but the strategies and methods discussed are applicable to most enhanced chemiluminescent (ECL) assays for membrane-based protein detection.

Types and Factors of Chemiluminescent Western Blotting

The most popular Western blotting substrates are luminol-based and produce a chemiluminescent signal. Chemiluminescence is a chemical reaction that produces energy released in the form of light. In the presence of horseradish peroxidase (HRP) and a peroxide buffer, luminol oxidizes and forms an excited state product that emits light as it decays to the ground state. Light emission occurs only during the enzyme-substrate reaction and, therefore, once the substrate in proximity to the enzyme is exhausted, signal output ceases. In contrast, colorimetric substrates, such as DAB, produce precipitate that remains visible on the membrane even after the reaction has terminated. Several varieties (see Related Products section at the end of this guide) of Pierce® ECL and Thermo Scientific SuperSignal® Chemiluminescent Substrates for HRP are available that provide for different levels of sensitivity for chemiluminescent Western blotting.

A. Signal Capture

Although Western blotting is a powerful application that has become commonplace, attempting to capture that elusive chemiluminescent signal can be frustrating. Because a Western blot is composed of a series of linked techniques that require skill to perform, failure to capture signal can be caused by many factors. With so many variables (Table 1), troubleshooting a problem blot can be equated to that proverbial needle in a haystack. The classical protocol is often ineffective in detecting a particular sample. For example, the primary antibody may not recognize the immobilized antigen in its denatured state. Although the protein can be kept in its native state by using non-denaturing conditions, this makes determination of target molecular weight more challenging. Further, exceptionally large or hydrophobic proteins often preclude effective membrane transfer. Towbin's original protocol is therefore often modified to ensure target detection. Such modifications may involve using different reagents for indirect detection or a labeled primary probe for direct detection. Sometimes transfer is bypassed altogether and detection is accomplished in-gel.

B. Signal Intensity and Duration

When all Western blotting factors are optimal, a chemiluminescent signal can last for 6-24 hours, depending on the specific substrate used. How much light is generated and for how long depends on the specific substrate being used and the enzyme-to-substrate ratio present in the system. Although the amount of substrate on a blot is relatively constant, the amount of enzyme present depends on how much was added and other factors (Table 1). Too much enzyme conjugate applied to a Western blot system is the single greatest cause of signal variability, dark background, short signal duration and low sensitivity.

A signal emission curve that decays slowly (Figure 1) is desirable as it demonstrates that each component of the system has been optimized and allows reproducible results. A signal that decays too quickly can cause variability, low sensitivity and lack of signal documentation. A long-lasting signal minimizes variability with transfer efficiency, different manufacturer lots of substrate and other factors.

Although HRP continues its activity for as long as substrate is available, a predictable statistical probability exists for luminol to turn over HRP and render it inactive. Free radical production during the oxidation reaction can bind to HRP in such a way

Table 1. Factors that affect Western blotting results.

Factor	Variable Characteristic
Target Antigen	conformation, stability, available epitope(s)
Polyacrylamide Gel	manufacturer, percent polyacrylamide, age, lot
Membrane	manufacturer, type, lot
Primary Antibody	specificity, titer, affinity, incubation time and temperature
HRP Conjugate	enzyme activation level and activity, source animal, concentration
Blocking Buffer	type, concentration, cross-reactivity
Washes	buffer, volume, duration, frequency
Substrate	sensitivity, manufacturer lot, age
Detection Method	film age, imaging instrument manufacturer, exposure time

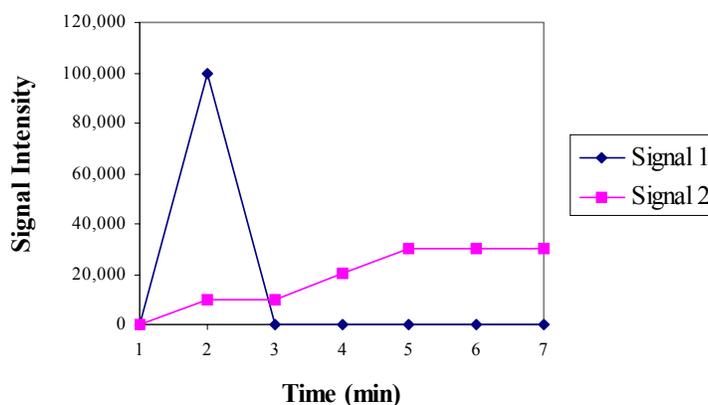


Figure 1. Example signal emission curves. When there is too much enzyme present in a Western blot system, signal output peaks soon after substrate application and rapidly exhausts the substrate (Signal 1). In an optimal system, the signal emission peaks approximately 5 minutes after applying the substrate and plateaus for several hours (Signal 2).

that the enzyme can no longer interact with the substrate. An abundance of HRP in the system in turn produces an abundance of free radicals that increases the probability of HRP inactivation. Free radicals also can damage the antigen, antibodies and the membrane, prohibiting re-probing effectiveness.

C. Direct and Indirect Methods

Direct detection uses a labeled primary antibody. Because incubation with a secondary antibody is eliminated, this strategy is performed in less time than a classical Western blot. Additionally, background signal from secondary antibody cross-reactivity is eliminated. Direct detection also enables probing for multiple targets simultaneously. Labeling a primary antibody, however, sometimes has an adverse effect on its immunoreactivity, and even in the best of circumstances, a labeled primary antibody cannot provide signal amplification. Consequently, the direct method is generally less sensitive than indirect detection and is best used only when the target is relatively abundant. One option is biotinylating the primary antibody, which is an indirect method that both amplifies the signal and eliminates the secondary antibody. Labeling with biotinylation reagents typically results in more than one biotin moiety per antibody molecule. Each biotin moiety is capable of interacting with an enzyme-conjugated avidin, streptavidin or Thermo Scientific NeutrAvidin™ Protein. These multiple enzymes catalyze the conversion of appropriate substrate to amplify the signal. Essentially, the avidin conjugate replaces a secondary antibody and its appropriate molar concentration is the same as if a secondary antibody were used.

D. Far-Western Methods

Occasionally, an antibody to a specific antigen is unsuitable for Western blot analysis or simply unavailable. Blotting is still possible if a binding partner to the target protein is available for use as a probe. This type of application is referred to as a far-Western blot and is routinely used for the discovery or confirmation of a protein:protein interaction. Variations on this theme are myriad and involve all the previously mentioned strategies. As with primary antibodies, labeled binding partners are used, which are frequently labeled in an *in vitro* translation reaction with ³⁵S. Biotinylation of the probe and detecting with an avidin or avidin-like conjugate is another possibility and has the added effect of amplifying the signal. Care must be taken to ensure the probe is not over-labeled lest its ability to interact with the target be compromised. Additionally, a recombinant probe can be expressed in bacteria with a tag, such as GST, HA, c-Myc or FLAG, in which detection occurs via a labeled antibody to the particular tag. As with other blotting applications, the far-Western method is effective for membrane or in-gel detection.

Optimization

Each Western blot system must be optimized to obtain consistently good results. Many factors influence the intensity and longevity of a signal, and each of these factors can be optimized. Included below are discussions on several factors. The protocol section at the end of this guide includes specific instructions and suggestions about optimization procedures.

A. Blotting Membrane

HRP-luminol interactions and subsequent signal generation are likely unaffected by membrane composition. Nitrocellulose and PVDF membranes, however, do differ in their protein-binding properties. Generally, nitrocellulose binds proteins better, often produces crisper bands and sometimes results in greater sensitivity. PVDF is more hydrophobic, is difficult to wet and sometimes results in more background signal; however, it has high tensile strength and excellent handling characteristics. For best results, empirically determine which membrane type, manufacturer and lot is optimal for each Western blotting system. Once a specific membrane has proven effective in a system, it may be beneficial to use the same lot throughout the course of the study.

B. Target Protein

Transfer efficiency can vary dramatically among proteins. Proteins differ in their ability to migrate from the gel and their propensity to bind to the membrane using a particular set of conditions. Transfer efficiency depends on factors such as gel composition, the gel-membrane contact, position of the electrodes, transfer duration, protein size and composition, field strength, and the presence of detergents. Optimal transfer of most proteins is obtained in low-ionic strength buffers and with low electrical current. Transfer efficiency can be evaluated by staining the membrane with an immunoblot-compatible or reversible stain.

C. Blocking Buffer

Many different blocking reagents are available for Western blotting. Because no blocking reagent is appropriate for all systems, empirical testing is essential. An optimal blocking buffer maximizes the signal-to-noise ratio and does not react with the system's antibodies or target. For example, using 5% nonfat milk as a blocking reagent when using avidin/biotin systems results in high background because milk contains variable amounts of endogenous biotin, which binds the avidin. When switching substrates, antibodies or the target, a diminished signal or increased background can result simply because the blocking buffer was not optimal for the new system.

Some systems may benefit from adding a surfactant, such as Tween[®]-20, to the blocking solution. Surfactants can minimize background by preventing the blocking reagent from nonspecifically binding to the target. Adding too much detergent, however, can prevent adequate blocking. Typically, a final concentration of 0.05% detergent is used; however, for best results, determine if detergents enhance a specific system and at what concentration it is optimal. Always use a high-quality detergent that is low in contaminants.

D. Antibodies

Not only is the affinity of the primary antibody for the antigen important, primary and secondary antibody concentrations have a profound affect on signal emission. Too much HRP captured on the blot may be a result of either primary and secondary antibody concentrations or both. Minimal primary antibody is advantageous, as it promotes target-specific binding and low background.

If a blot failed to generate an adequate signal, removing all detection reagents from the blot and re-probing with either a different primary antibody or different concentrations of antibodies often conserves valuable sample and time; however, insufficient stripping can leave active HRP on the blot that will produce a signal. Applying substrate on the stripped blot and subsequent detection indicates if active HRP remains on the blot. Also, if there is an abundance of inactive HRP molecules not removed by stripping, it may inhibit the primary antibody from binding to the target. Stripping and re-probing blots is an effective method to gain information about a specific system, but it is not a definitive way to determine the optimal system parameters.

E. Detection Method

Traditionally, film has been used to detect a Western blot chemiluminescent signal. Film requires no expensive equipment and provides excellent sensitivity. Unfortunately each piece of film can be used only once and must be developed before one can know whether or not the exposure time was appropriate. Trial and error (i.e., using several sheets of film) is often required. The dynamic range of film is quite large, and even overexposed film retains accurate signal-to-noise ratio. Because of this, overexposed film can be “rescued” using reagents such as Thermo Scientific Pierce Background Eliminator for Film (Figure 2).

Charged-coupled device (CCD) cameras are becoming common. These imagers and their accompanying analytical software are capable of adjusting the background signal and performing densitometry. The imager has advantages over film-based detection. The large dynamic range and the high degree of exposure control allow for the best possible image documentation without issues of high background or high signal intensity obscuring data. Additionally, optimization of exposure time avoids band signal saturation and allows observation of minor variations in density. In contrast, film has a small dynamic range, and band signal can quickly reach saturation. When signal intensity is high, film’s low dynamic range, propensity to reach saturation quickly and exposure control limitations often result in over-exposed images.

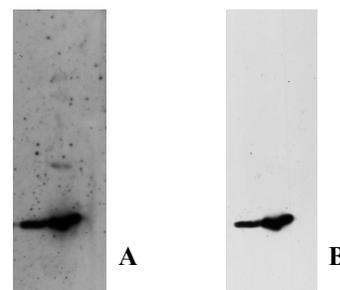


Figure 2. Recombinant human TNF α was separated by SDS-PAGE using a 4-20% gel and transferred to a nitrocellulose membrane. After blocking, the blot was probed with mouse anti-human TNF α and goat anti-mouse-HRP antibodies and detected with SuperSignal West Dura Substrate. The blot was exposed to film for 30 seconds, resulting in considerable background speckling (A). The film was then treated with Pierce Background Eliminator for 2 minutes to remove speckling and band overexposure (B).

Common Occurrences and Explanations

A. No Signal

An initial exposure that fails to capture the signal indicates a Western blotting system that requires optimization. Frequently, lack of signal is caused by too much HRP in the system. It may seem counterintuitive to use less enzyme conjugate when a signal cannot be detected; however, for successful signal documentation, the correct balance of enzyme and substrate must be present. Substrate oxidation by the enzyme is irreversible and, therefore, once the substrate is oxidized, it can no longer interact with the enzyme to generate light. Because enzyme activity persists, the substrate is the limiting factor and once exhausted, signal output ceases. Rarely, lack of signal is caused by an insufficient amount of active enzyme present. Too much or not enough enzyme can be caused by any of the factors involved in the Western blot system.

To produce a signal that can be captured, adjust the system’s parameters. For reproducible results, prepare a new gel and apply less sample or titrate the antibodies. When optimizing antibody concentrations, image the blot twice: once immediately

after substrate incubation and a second time at an interval after incubating the substrate (e.g., 1 hour). The second detection provides information about the optimal enzyme concentration and helps optimize parameters.

Also, if the initial exposure did not capture a signal, a second incubation in substrate may yield a signal if enough HRP was inactivated while some remains active. Stripping all detection reagents from the blot and re-probing can save valuable sample while optimizing parameters. Perform an additional incubation in the substrate and strip the blot only to recover some information about the system. If blot-to-blot consistency is desired, the same conditions must be used and the same procedure must be followed each time the experiment is performed.

B. Signal Fades Quickly

When a particular system produces a signal that fades quickly, the Western blotting system requires optimization, as described in the above section. The good news is that a signal was obtained, indicating that you are getting close to the optimal blotting conditions.

Sometimes a particular system produces a signal that fades more quickly than usual although all parameters are the same. This type of result is minimized in a fully optimized system. A successful but suboptimal system is subject to the slight variations inherent to the method, such as transfer efficiency and changes in sample and antibody activity during storage and handling.

C. New Bottle of Substrate does not Produce a Signal

Occasionally, a signal cannot be captured when the only variable that has changed in a particular system is the bottle or lot of substrate used. Typically this result is caused by a Western blot system that has not been fully optimized. Western blotting substrates are inherently variable. Many manufacturers simply control for a specific sensitivity, and it is possible that the new substrate is more sensitive than the previously used lot. In a fully optimized blotting system, substrate sensitivity variations, as well as other variables, are minor or unnoticeable.

D. Brown or Yellow Bands on the Membrane

HRP becomes brown when it is oxidized and inactive. Within a given amount of enzyme conjugate, there always exists a portion that is oxidized. In an optimized system, the amount of oxidized HRP is miniscule and cannot be visualized on the blot. The appearance of yellow or brown bands indicates the presence of a large amount of HRP and, therefore, the oxidized and inactive portion is visible. A blotting system that results in yellow bands requires optimization using much less enzyme conjugate. Additionally, too much HRP in a localized area produces an excess of free radicals, in the presence of substrate. These free radicals can inactivate HRP and damage antibodies, target and the membrane, prohibiting effective re-probing.

E. Bands or Entire Blot Glowing in the Darkroom

If a pattern of bands or the entire blot is glowing after incubation in the substrate, then there is too much HRP present in the system. This occurrence indicates that further dilution of the secondary HRP-conjugated antibody is required and possibly the primary antibody as well. Too much enzyme can be caused by many of the factors involved in the Western blotting system. If the entire blot is glowing, optimization of blocking and washing also may be necessary.

F. Ghost/Hollow Bands

Protein bands that appear as a halo with no signal in the middle of the band or an entire band that appears white in a dark background are typically referred to as ghost bands. This occurrence indicates depletion of substrate in the white area. The most common causes for ghost-band effects are applying too much target protein to the gel and using concentrations of secondary antibody antibodies that are too high.

G. High Background

High background signal is the result of either insufficient blocking, antibodies cross-reacting with the blocking buffer or the use of too much enzyme conjugate. Researchers sometimes believe that a particular substrate causes background or can increase background. The substrate in itself cannot cause any signal without the enzyme being present. When using a substrate with greater sensitivity than what was previously used, high background often results if the parameters were not altered to compensate for the substrate's sensitivity. Using optimal concentrations of antibodies promotes target-specific binding and low background.

General Protocol for Blotting and Stripping using Chemiluminescent Substrates

This section contains in-depth protocols for Western blotting, stripping membranes, and optimizing and troubleshooting. While this guide does not describe every eventuality and aspect of Western blotting it nevertheless serves as an accurate overview of the uses, limits and potential of this powerful technique.

Thermo Scientific Pierce Protein Research Products include a wide selection of pre-cast gels, electrophoresis buffers, blocking and wash buffers, primary and secondary antibodies, chemiluminescent substrates, film and other essential reagents for Western blotting. See the abbreviated list of Related Products on page 9 or visit the web site for more information.

A. Western Blotting Protocol

1. Separate the proteins in the sample by gel electrophoresis.
2. Prepare the transfer buffer: Use Tris-glycine transfer buffer dissolved in 400 ml of ultrapure water plus 100 ml methanol (25 mM Tris, 192 mM glycine, pH 8.0, 20% methanol). Use and store the transfer buffer at 4°C.
3. Construct a gel “sandwich” (Figure 3) for wet transfer. For semi-dry transfer, prepare the sandwich in the same order between the anode and cathode.
4. Transfer proteins from the gel to a membrane. For wet transfer using a mini-transfer apparatus designed for a 8 × 10 cm gel, transfer at 40 V for 90 minutes keeping the buffer temperature at 4°C. For semi-dry transfer use 15 V for 90 minutes.
5. Remove the membrane and block nonspecific binding sites with a blocking buffer for 20-60 minutes at room temperature (RT) with shaking.
6. Incubate the blot with the primary antibody solution (see Table 2) containing 10% blocking solution with rocking for 1 hour. If desired, incubate the blot overnight at 2-8°C.

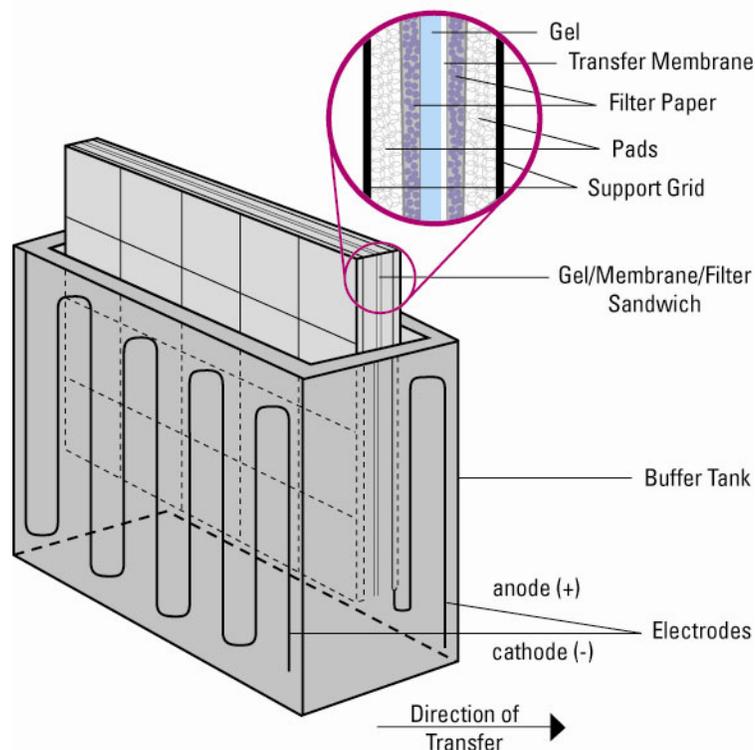


Figure 3. Electrophoretic transfer setup.

Table 2. Primary antibody concentrations to use with Thermo Scientific Chemiluminescent Substrates.

Substrate	Pierce ECL	West Pico	West Dura	West Femto
Primary Antibody Concentration	0.2-10 µg/ml	0.2-1 µg/ml	0.02-1 µg/ml	0.01-0.2 µg/ml

7. Wash the membrane three times for 5 minutes each with Tris-buffered saline (TBS), phosphate-buffered saline (PBS) or other physiological wash buffer containing 0.05% Tween-20. If using an enzyme-conjugated primary antibody, proceed to Step 10.
8. Incubate blot with the enzyme conjugate (see Table 3) containing 10% blocking solution for 1 hour with rocking at RT.

Table 3. Secondary antibody concentrations to use with Thermo Scientific Chemiluminescent Substrates.

Substrate	Pierce ECL	West Pico	West Dura	West Femto
Secondary Antibody Concentration	67-1,000 ng/ml	10-50 ng/ml	4-20 ng/ml	2-10 ng/ml

9. Wash the membrane five times for 5 minutes each in wash buffer to remove any nonbound conjugate. It is crucial to thoroughly wash the membrane after incubation with the enzyme conjugate.
10. Prepare the substrate. Use a sufficient volume to ensure that the blot is completely wetted with substrate and the blot does not become dry (0.1 ml/cm²).
11. Incubate the blot with substrate for 1 minute when using Pierce ECL or 5 minutes when using SuperSignal Substrates.
12. Remove the blot from the substrate and place it in a plastic membrane protector. A plastic sheet protector works well, although plastic wrap also may be used. Remove all air bubbles between the blot and the surface of the membrane protector.
13. Image the blot using film or a cooled CCD camera.

B. Western Blot Stripping Protocol

1. Prepare the stripping buffer. Use one of the following suggested stripping buffers:
 - Thermo Scientific Restore[®] Western Blot Stripping Buffer (Pierce Product No. 21059)
 - Thermo Scientific Restore[®] Plus Western Blot Stripping Buffer (Pierce Product No. 46428)
 - 0.1 M glycine•HCl (pH 2.5-3.0)
 - 50 mM Tris•HCl (pH 7), 2% sodium dodecyl sulfate (SDS), 50 mM dithiothreitol (DTT)
2. Place the blot to be stripped in stripping buffer. Use a sufficient volume to ensure that the blot is completely wetted (approximately 20 ml for an 8 × 10 cm blot).
3. Incubate for 5-15 minutes at room temperature. Optimization of both incubation time and temperature is essential for best results. Some interactions require at least 15 minutes and may require incubation at 37°C. If using buffer C, incubate for 30 minutes at 70°C.
4. Remove the blot from the stripping buffer and wash using wash buffer (PBS/TBS or other physiological buffer containing 0.05% Tween-20).
5. To test for complete removal of the enzyme conjugate and primary antibody, perform the tests listed below. If signal is detected in either case, repeat steps 2-4, stripping for an additional 5-15 minutes or increasing the temperature to 37°C. Optimize stripping time and temperature to ensure complete removal of antibodies while preventing damage to the antigen.
 - To test for complete removal of the enzyme conjugate, incubate the membrane with substrate and image the blot. If no signal is detected after a 5-minute exposure to film, the enzyme conjugate has been successfully removed from the membrane.
 - To test for complete removal of the primary antibody, incubate the membrane with enzyme conjugate then wash with wash buffer. Incubate with substrate and image the blot. If no signal is detected after a 5-minute exposure to film, the primary antibody has been successfully removed from the membrane.
6. After determining that the membrane is properly stripped, commence the second probing experiment. Typically, a blot can be stripped and re-probed several times but may require longer exposure times or a more sensitive substrate. Subsequent re-probing may result in decreased signal if the antigen is labile. Analysis of the individual system is required.

Optimization Procedures for Chemiluminescent Western Blotting

A. Optimizing Antigen Concentration

1. Prepare different concentrations of the protein sample in SDS-PAGE sample buffer. Test a wide range of sample concentration, keeping in mind the detection limit of the substrate being used.
2. Apply an equal volume of each concentration on the gel and separate by electrophoresis. Transfer the samples to a membrane.
3. Block the membrane with a standard blocking reagent and probe with primary antibody followed by enzyme conjugate. If optimized dilutions have not yet been determined, use a mid-range value according to the sensitivity of the substrate.
4. Wash membrane and add the substrate. Image the blot as desired.

B. Optimizing Membrane Blocking

1. Separate the protein sample by electrophoresis and transfer to a membrane or dot protein samples onto the membrane as described in Section A notes.
2. Cut strips from the membrane according to the number of conditions being tested. The following combinations should be tested with each blocker:
 - Blocker + primary antibody + enzyme conjugate + substrate
 - Blocker + enzyme conjugate + substrate
 - Blocker + substrate
3. Add the strips to various blocking solutions, ensuring the strip is completely immersed in the solution. Incubate each strip for 1 hour at room temperature with shaking.
4. Add primary antibody and/or enzyme conjugate solutions containing 10% blocking agent to appropriate groups. If optimized dilutions have not yet been determined, use a mid-range value according to the sensitivity of the substrate.
5. Wash membrane and add the substrate. Image the blot as desired.

C. Optimizing the Primary Antibody Concentration

1. Separate the protein sample by electrophoresis and transfer to the membrane. Alternatively, dot the protein sample onto the membrane as described in the section for optimizing antigen concentration. Block the membrane using an appropriate blocking reagent. Cut strips from the membrane according to the number of primary antibody conditions being tested.
2. Prepare dilutions of primary antibody in wash buffer containing 1/10 volume of blocking agent and apply to the membrane strips. Incubate for 1 hour at room temperature.
3. Wash the strips and incubate with enzyme conjugate for 1 hour at room temperature. Wash again and develop signal using an appropriate substrate. Detect signal using film or a CCD camera.

D. Optimizing Membrane Washing

1. Use a wash buffer such as PBS or TBS or other physiological buffer containing 0.05% Tween-20.
2. Wash the membrane by agitating at least three times for 5 minutes each after primary antibody incubation, and at least five times for 5 minutes each after incubating with enzyme conjugate.
3. If nonspecific background appears upon final detection, use larger volumes of wash buffer or increase the number and time of each wash. If no improvement occurs, the problem lies with another variable.

E. Optimizing Enzyme Conjugate Concentration

When determining the optimal concentration for a new Western blotting system, a simple experiment often saves much frustration with signal variability.

1. Apply the same amount of target in three (or more) wells of the gel.

2. Separate the protein sample by electrophoresis and transfer to the membrane. Block the nonspecific binding sites and probe with primary antibody.
3. After washing, cut the blot into strips containing the target.
4. Probe each strip with a different enzyme conjugate concentration. For example, for SuperSignal West Pico Substrate use 1:40 K, 1:60 K and 1:80 K dilutions (from a 1 mg/ml stock). Incubate strips for 1 hour at RT with rocking.
5. Wash the strips and add the substrate. After substrate incubation, image the strips.
6. Wait 1-2 hours and image the strips a second time.
7. Evaluate the results. For example, if the second exposure results in a signal for the 1:80 K dilution and the other two signals are faint, then the 1:80 K dilution is closest to the optimum. But if the 1:40K dilution has a strong signal and the other two dilutions are faint, then the more concentrated dilution is closer to the optimum.

F. Optimizing the Detection Method

1. Separate the protein sample by electrophoresis and transfer to membrane or dot the protein sample onto the membrane as described in “Optimization of Antigen Concentration” section.
2. Block nonspecific binding sites and probe with primary antibody and enzyme conjugate containing 1/10 volume of blocking agent.
3. If antibody concentrations have not been optimized, choose a mid-range value. Wash the membrane after each incubation.
4. Cut strips from the membrane according to the number of substrate exposure conditions being tested. Prepare working solution of substrate to be tested.
5. Incubate the membrane strips with the substrate for a time period consistent with the manufacturer’s instructions.
6. Remove the strips from the substrate using forceps, and gently tap edge onto a paper towel to remove excess substrate.
7. Place the strips in a plastic cover and image blot for varying lengths of time. Select a time with clear signal and low background.

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Thermo Scientific Products for Western Blotting

Please visit the web site for a complete list of Western blotting reagents and kits. The following list represents only a small fraction of related Pierce Protein Research Products.

32209	Pierce ECL Western Blotting Substrate , 250 ml
34080	SuperSignal West Pico Chemiluminescent Substrate for HRP , 100 ml
34075	SuperSignal West Dura Chemiluminescent Substrate for HRP , 100 ml
34095	SuperSignal West Femto Maximum Sensitivity Chemiluminescent Substrate for HRP , 100 ml
34090	CL-Xposure[®] Film , 5 × 7" (13 × 18 cm), 100 sheets/pkg.
21065	Pierce Background Eliminator for Film , Kit
21059	Restore Western Blot Stripping Buffer , 500 ml
46428	Restore Plus Western Blot Stripping Buffer , 500 ml
37570	Protein-Free (TBS) Blocking Buffer , 1 L
37539	StartingBlock[®] T20 (PBS) Blocking Buffer , 1 L
37516	SuperBlock[®] T20 (PBS) Blocking Buffer , 1 L
37525	Blocker[®] BSA in PBS (10X) , 1 L
28380	Tris-Glycine Transfer Buffer Packs , 40 packs, each makes 500 ml
77010	Nitrocellulose Membrane , 0.45 µm, 8 × 12 cm, 25 sheets/pkg.
88585	PVDF Transfer Membrane , 0.45 µm, 10 × 10 cm, 10 sheets/pkg.
32430	Stabilized Goat Anti-Mouse IgG (H + L), Peroxidase Conjugated , 2 ml
32460	Stabilized Goat Anti-Rabbit IgG (H + L), Peroxidase Conjugated , 2 ml

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

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

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