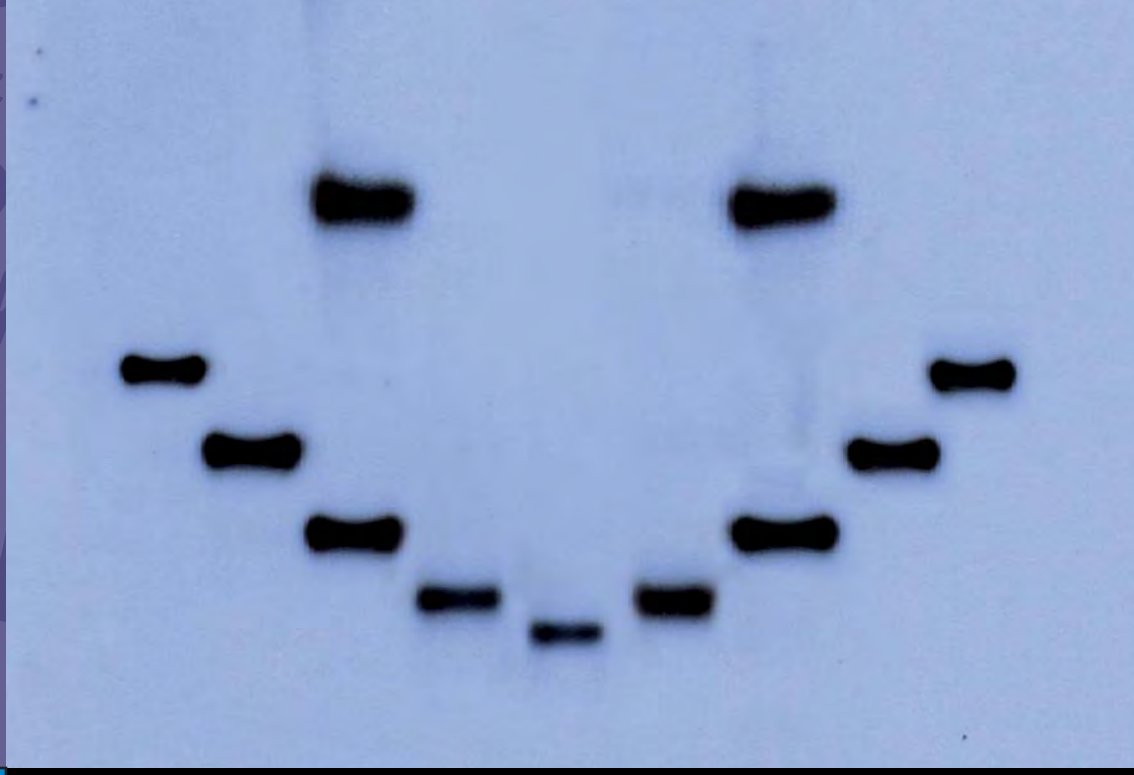
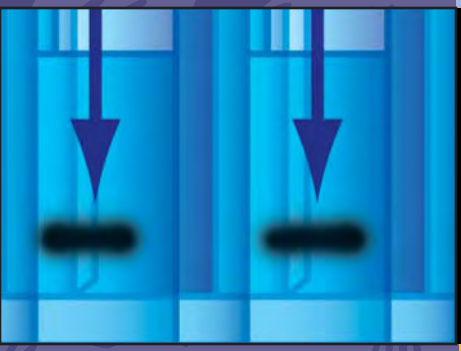




Western Blotting Handbook and Troubleshooting Guide

Featuring Thermo Scientific SuperSignal® Substrates and Pierce® Western Blotting Accessories

Part of Thermo Fisher Scientific

Thermo SCIENTIFIC

Table of Contents

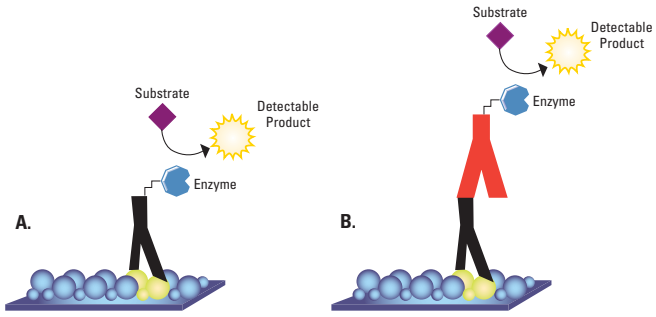
Introduction

The term "blotting" refers to the transfer of biological samples from a gel to a membrane and their subsequent detection on the surface of the membrane. Western blotting (also called immunoblotting because an antibody is used to specifically detect its antigen) was introduced by Towbin, *et al.* in 1979 and is now a routine technique for protein analysis. The specificity of the antibody-antigen interaction enables a target protein to be identified in the midst of a complex protein mixture. Western blotting can produce qualitative and semiquantitative data about that protein.

The first step in a Western blotting procedure is to separate the macromolecules using gel electrophoresis. After electrophoresis, the separated molecules are transferred or blotted onto a second matrix, generally a nitrocellulose or polyvinylidene difluoride (PVDF) membrane. Next, the membrane is blocked to prevent any nonspecific binding of antibodies to the surface of the membrane. The transferred protein is complexed with an enzyme-labeled antibody as a probe. An appropriate substrate is then added to the enzyme and together they produce a detectable product such as a chromogenic precipitate on the membrane for colorimetric detection. The most sensitive detection methods use a chemiluminescent substrate that, when combined with the enzyme, produces light as a byproduct. The light output can be captured using film, a CCD camera or a phosphoimager that is designed for chemiluminescent detection. Whatever substrate is used, the intensity of the signal should correlate with the abundance of the antigen on the blotting membrane.

Detailed procedures for detection of a Western blot vary widely. One common variation involves direct vs. indirect detection (Figure 1). With the direct detection method, the primary antibody that is used to detect an antigen on the blot is labeled with an enzyme or fluorescent dye. This detection method is not widely used as most researchers prefer the indirect detection method for a variety of reasons (Table 1).

In the indirect detection method, a primary antibody is added first to bind to the antigen. This is followed by a labeled secondary antibody that is directed against the primary antibody. Labels include biotin, fluorescent probes such as fluorescein or rhodamine, and enzyme conjugates such as horseradish peroxidase or alkaline phosphatase. The indirect method offers many advantages over the direct method (Table 2).



1A. Direct Detection **1B. Indirect Detection**

Figure 1A. In the direct detection method, **labeled primary antibody** binds to antigen on the membrane and reacts with substrate, creating a detectable signal. **1B**. In the indirect detection method, **unlabeled primary antibody** binds to the antigen. Then, a **labeled secondary antibody** binds to the primary antibody and reacts with the substrate.

Table 1. Direct detection method.

Advantages	Disadvantages
• It is a quick methodology because only one antibody is used	• Immunoreactivity of the primary antibody may be reduced as a result of labeling
• Cross-reactivity of secondary antibody is eliminated	• Labeling a primary antibody for each target protein is time-consuming and expensive
• Double probing is easily achieved using different labels on primary antibodies from the same host	• There is no flexibility in choice of primary antibody label from one experiment to another
	• Minimal signal amplification

Table 2. Indirect detection method.

Advantages	Disadvantages
• Sensitivity is increased because each primary antibody contains several epito bound by the labeled secondary antibody, which amplifies the signal	• Cross-reactivity may occur with the secondary antibody, resulting in nonspecific binding
• A wide variety of labeled secondary antibodies are available commercially	• An extra incubation step is required in the procedure
• Because many primary antibodies can be made in one species and the same labeled secondary antibody can be used for detection, it is versatile	
• Immunoreactivity of the primary antibody is maintained because it is not labeled	
• Different detection markers can be used with the same primary antibody	

Western Blotting is Easy with Thermo Scientific

STEP 1

SDS-PAGE
Separate protein sample by electrophoresis.

- Pierce SDS-PAGE Sample Prep Kit (Product # 89888)
- Precise™ Protein Gels (many available, see our website)
- Tris-Hepes-SDS Running Buffer (Product # 28398)
- Lane Marker Reducing Sample Buffer (5X) (Product # 39000)
- Lane Marker Non-Reducing Sample Buffer (5X) (Product # 39001)
- Pierce Blue Prestained Protein Molecular Weight Marker (Product #s 26681 and 26685)
- Pierce Chemiluminescent Prestained Peroxidase-labeled Protein Molecular Weight Marker (Product # 26651)
- Pierce Prestained 3-Color Protein Molecular Weight Marker (Product # 26691)

NEW!
- DyLight Dual-Labeled Fluorescent Marker (Product # 22859 and 26665)

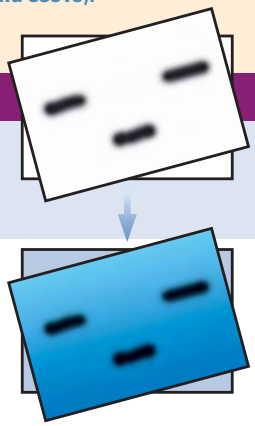
STEP 2

Electro-Transfer
Transfer proteins to membrane.

- Pierce Reversible Protein Stain Kit for Nitrocellulose Membranes (Product # 24580) and for PVDF Membranes (Product # 24585)
- Tris-Glycine Transfer Buffer (Product # 28380)
- Pierce Western Blot Signal Enhancer (Product # 21050)
- Pierce Antibody Extender NC (Product # 32110 and 32105)
- Nitrocellulose Membrane, 0.2 µm (Product #s 77012, 88013 and 88024)
- Nitrocellulose Membrane, 0.45 µm (Product #s 77010, 77011, 88014 and 88025)
- PVDF Membrane, 0.45 µm (Product #s 88585 and 88518)
- Low-fluorescence PVDF Membrane, 0.2 µm (Product # 22860)

For detection of proteins that cannot be efficiently transferred to a membrane, Thermo Scientific Pierce In-Gel Detection Technology† allows positive identification of proteins directly in a gel (Product #s 33500, 33505, 33510 and 33515).

STEP 3



Blocking
Block nonspecific sites.

NEW!
- Protein-free Blocking Buffer (Product #s 37570, 37571, 37572 and 37573)
- StartingBlock™ Blocking Buffer in PBS (Product # 37538) and in TBS (Product # 37542)
- StartingBlock T20 Blocking Buffer (Contains 0.05% Tween®-20) in PBS (Product # 37539) or TBS (Product # 37543)
- SuperBlock® Buffer in PBS (Product # 37515) and in TBS (Product # 37535)
- SuperBlock T20 Blocking Buffer (Contains 0.05% Tween-20) in PBS (Product # 37516) or TBS (Product # 37536)
- SuperBlock Blocking Buffer – Blotting in PBS (Product # 37517) and in TBS (Product # 37537)
- Casein in PBS (Product # 37528) and in TBS (Product # 37532)
- BSA in PBS (Product # 37525) and in TBS (Product # 37520)
- SEA BLOCK Buffer (Product # 37527)
- BLOTTO in TBS (Product # 37530)

STEP 4A

Formulate Wash Buffers
Choose a buffer.

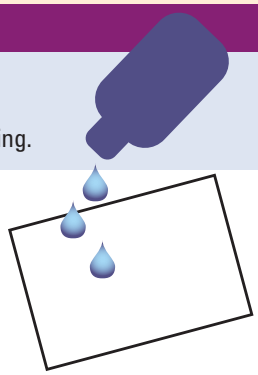
- Phosphate Buffered Saline (PBS, Product #s 28372 and 28348)
- Tris Buffered Saline (TBS, Product #s 28376, 28379 and 28358)
- Modified Dulbecco's PBS (Product #s 28374 and 28344)
- Carbonate-Bicarbonate Buffer Packs (Product # 28382)
- MES Buffered Saline (Product # 28390)
- BupH™ Borate Buffer Packs (Product #s 28384 and 28341)
- BupH Citrate-Carbonate Buffer Pack (Product # 28388)

For more information, or to download product instructions, visit www.thermo.com/pierce

Products

STEP 4B

Formulate Wash Buffers
Add detergent to blocking/wash buffers to reduce nonspecific binding.



Skip this step if you use StartingBlock T20 Blocking Buffer in PBS (Product # 37539) or TBS (Product # 37543) or SuperBlock T20 Blocking Buffer in PBS (Product # 37516) or TBS (Product # 37536). These buffers already contain Tween-20 Detergent at optimized concentrations.

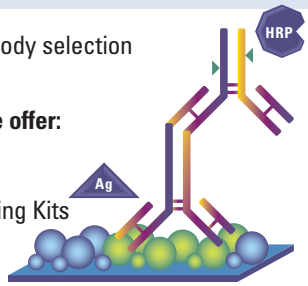
Surfact-Amps® Detergents containing:

- Tween-20 (Product # 28320) and Tween-80 (Product # 28328)
- Triton® X-100 (Product # 28314) and Triton X-114 (Product # 28332)
- Nonidet P-40 (Product # 28324)
- Brij®-35 (Product # 28316) and Brij-58 (Product # 28336)

For convenience and economy, we also offer complete Western blotting kits that include chemiluminescent substrates, enzyme-conjugated antibodies, blocking buffers and standard buffers.

STEP 5

Primary and Secondary Detection Reagents
Incubate the membrane with antibody.



For a complete list, visit the antibody selection guide on our website.

For direct detection methods we offer:

- Monoclonal Antibodies
- Fluorescent Probes and Labeling Kits
- Enzyme Labeling Kits

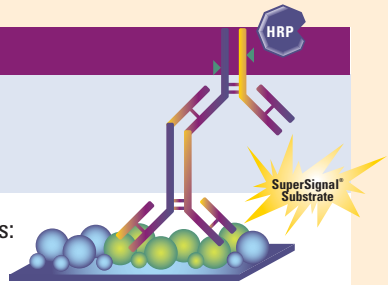
For indirect detection methods we offer:

- Biotinylation Kits
- Protein A, Protein G and Protein L labeled with fluorescein, rhodamine, HRP, AP or biotin
- Avidin, Streptavidin and NeutrAvidin Biotin-Binding Protein labeled with fluorescein, rhodamine, HRP or AP
- Secondary antibodies labeled with fluorescein, rhodamine, HRP, AP or biotin

NEW!
- DyLight Secondary Antibody and Streptavidin Conjugates [Photostable and inexpensive alternatives to CyDye™ Fluors (GE) and Alexa Fluor® Dye (Invitrogen)].

STEP 6

Enzyme Substrates
Add the detection reagent.



Chemiluminescent Substrates:

- Pierce ECL Substrate (Product #s 32106, 32209 and 32109)
- SuperSignal West Pico Chemiluminescent Substrate (Product #s 34077 and 34080); also available in an economical 1-L package (Product # 34078)
- SuperSignal West Femto Maximum Sensitivity Substrate (Product #s 34096 and 34095)
- SuperSignal West Dura Extended Duration Substrate (Product #s 34076 and 34075)
- Lumi-Phos WB Substrate (Product # 34150)

Colorimetric Substrates:

- Pierce Chloronaphthol (Product # 34012)
- TMB-Blotting (Product # 34018)
- NBT/BCIP (Product # 34042)
- Metal Enhanced DAB (Product # 34065)

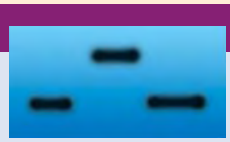
STEP 7

Film
Expose the membrane to X-ray film.

- CL-XPosure Film 5 x 7" sheets (Product #s 34090 and 34092); 8 x 10" sheets (Product #s 34091 and 34093); 9.5 x 11.8" sheets (Product # 34097); 18 x 24 cm sheets (Product # 34089) and 35 x 43 cm sheets (Product # 34099)
- Pierce Background Eliminator Kit (Product # 21065)

STEP 8

Stripping Buffer
Reprobe the blot if necessary.



- Restore Western Blot Stripping Buffer (Product # 21059)

NEW!
- Restore PLUS Western Blotting Stripping Buffer (Product #s 46428 and 46430) for High-Affinity Antibodies
- IgG Elution Buffer (Product #s 21004 and 21009)

†See patent information on inside back cover.

Transfer Protein to a Membrane

After electrophoresis, the protein must be transferred from the gel to a membrane. There are a variety of methods that have been used for this process, including diffusion transfer, capillary transfer, heat-accelerated convectional transfer, vacuum blotting transfer and electroelution. The transfer method that is used most commonly for proteins is electroelution or electrophoretic transfer because of its speed and transfer efficiency. This method uses the electrophoretic mobility of proteins and involves placing a protein-containing polyacrylamide gel in direct contact with a piece of nitrocellulose or other suitable protein-binding support and "sandwiching" this between two electrodes submerged in a conducting solution (Figure 1). When an electric field is applied, the proteins move out of the gel and onto the surface of the membrane where the proteins become tightly attached. The resulting membrane is a copy of the protein pattern that was in the polyacrylamide gel.

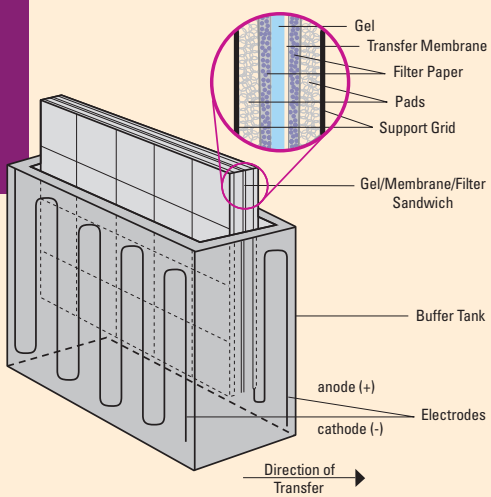


Figure 1. Electrophoretic transfer.

Transfer efficiency can vary dramatically among proteins, based upon the ability of a protein to migrate out of the gel and its propensity to bind to the membrane under a particular set of conditions. The efficiency of transfer depends on factors such as the composition of the gel, whether there is complete contact of the gel with the membrane, the position of the electrodes, the transfer time, size and composition of proteins, field strength, and the presence of detergents. Optimal transfer of proteins is generally obtained in low-ionic strength buffers and with low electrical current.

We offer a wide selection of the most commonly used membranes for Western blotting, including nitrocellulose and polyvinylidene difluoride (PVDF).

At this stage, before proceeding with the Western blot, it is often desirable to stain all proteins on the membrane with a reversible stain to check the transfer efficiency. Although the gel can be stained to determine if protein left the gel, this does not ensure efficient binding of protein on the membrane. Ponceau S stain is the most widely used reagent for staining proteins on a membrane. However, it has limited sensitivity, does not photograph well and fades with time. Pierce Reversible Stain is a superior alternative for staining protein on nitrocellulose (Product # 24580) or PVDF (Product # 24585) membranes. Pierce Reversible Stain detects low nanogram levels of protein, is easily photographed, does not fade with time and takes less than 30 minutes to stain, photograph and erase.

Featured Product: Thermo Scientific Transfer Buffers

BupH Tris-Glycine and Tris Buffered Saline

Great for Western blots!

BupH Tris-Glycine Buffer Packs

Each pack yields 500 ml of 25 mM Tris and 192 mM glycine at a pH of approximately 8 when dissolved in 400 ml deionized water and 100 ml of methanol (20 L total).

BupH Tris Buffered Saline Packs

Each pack yields 500 ml of 25 mM Tris, 0.15 M NaCl, pH 7.2 when dissolved in 500 ml deionized water (10 pack makes 5 L total; 40 pack makes 20 L total).

Ordering Information

Product #	Description	Pkg. Size
28380	BupH Tris-Glycine Buffer Packs	40 pack
28376	BupH Tris Buffered Saline Packs	40 pack
28379	BupH Tris Buffered Saline Packs	10 pack

Complementary Products: Transfer Membranes

Nitrocellulose Membranes

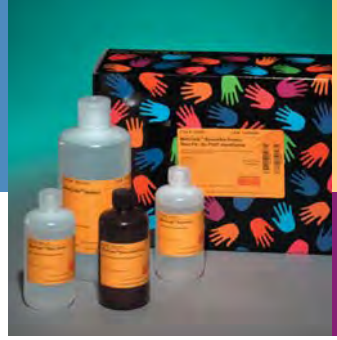
Product #	Description	Pkg. Size
88013	Nitrocellulose Membrane, 0.2 µm 7.9 cm x 10.5 cm	15/pkg.
88018	Nitrocellulose Membrane, 0.45 µm 33 cm x 3 m	1 roll
88014	Nitrocellulose Membrane, 0.45 µm 7.9 cm x 10.5 cm *Minimum 87 sheets when cut to 7.9 cm x 10.5 cm; minimum 52 sheets when cut to 11.5 cm x 12.5 cm.*	15/pkg.
88024	Nitrocellulose Membrane, 0.2 µm 8 cm x 8 cm	15/pkg.
77012	Nitrocellulose Membrane, 0.2 µm 8 cm x 12 cm	25/pkg.
88025	Nitrocellulose Membrane, 0.45 µm 8 cm x 8 cm	15/pkg.
77010	Nitrocellulose Membrane, 0.45 µm 8 cm x 12 cm	25/pkg.

Polyvinylidene Difluoride (PVDF) Membranes

Product #	Description	Pkg. Size
22860	Low-Fluorescence PVDF Transfer Membrane, 0.2 µm 7 cm x 8.4 cm	10/pkg.
88585	PVDF Transfer Membrane, 0.45 µm 10 cm x 10 cm	10 sheets
88518	PVDF Transfer Membrane, 0.45 µm 26.5 cm x 3.75 m	1 roll

Western Blotting Filter Paper

Product #	Description	Pkg. Size
88600	Western Blotting Filter Paper	100 sheets



Featured Product: Thermo Scientific Pierce Reversible Protein Stain for Nitrocellulose and PVDF Membranes

A great alternative to Ponceau S stain.

For years the red Ponceau S has been the best option for staining before Western blotting, despite its major shortcomings. Pierce Reversible Protein Stains decrease staining time, increase staining sensitivity and enhance the immunoreactivity of antigens in subsequent Western blotting (Figures 2-4). Try these reversible protein stains for nitrocellulose and PVDF membranes and you will never use Ponceau S again.

Highlights:

- Sensitive, general protein stain that binds tightly to proteins
- Stain is protein-specific, avoiding interference from other biomolecules
- From stain to destain in minutes
- Turquoise bands are easily photographed
- Stained bands do not fade with time
- Enhances Western blot detection
- All components are room temperature-stable

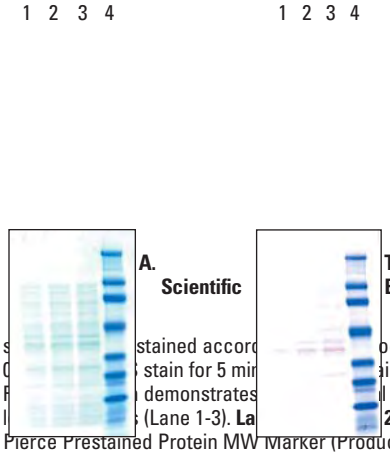
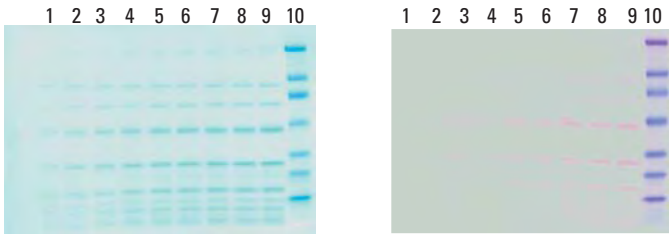


Figure 2. Thermo Scientific Pierce Reversible Protein Stain and Ponceau S Stain: A comparison of GST lysate staining on nitrocellulose. Increasing amounts of GST lysate protein were applied onto two 4-20% Tris-glycine SDS-polyacrylamide gels and electroblotted. **Blot A** was treated with Pierce Reversible Stain for 30 s stained accord ocol. **Blot B** was stained with stain for 5 mi ained. The blot stained with Pierce demonstrates l detection of bands. GST lysate (Lane 1-3). **La 2**. 10 µl, **Lane 3**. 15 µl and **Lane 4**. Pierce Prestained Protein MW Marker (Product # 26681), 10 µl.



A. Thermo Scientific Pierce Reversible Stain

B. Ponceau S Stain

Figure 3. Comparison of Thermo Scientific Pierce Reversible Protein Stain with Ponceau S stain on PVDF membrane. Pierce Unstained Protein MW Markers (Product # 26671) were serially diluted and applied to two 4-20% Tris-glycine SDS-polyacrylamide gels. **Lanes 1-9**. Both gels were electroblotted to PVDF membrane. **Blot A** was stained with Pierce Reversible Stain for 1 minute and destained according to the protocol. **Blot B** was stained with 0.1% Ponceau S stain in 5% acetic acid for 5 minutes and destained according to the published protocol. **Lane 10.** Pierce Prestained MW Marker (Product # 26681).

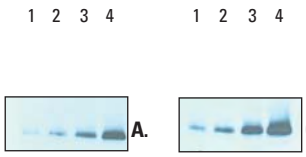


Figure 4. Immunoblot analysis of GST by chemiluminescent detection after Thermo Scientific Pierce Reversible Staining, destaining and stain reversal. Different amounts of purified GST protein were applied to two 10% Tris-glycine SDS-polyacrylamide gels and electroblotted to nitrocellulose membranes. The control membrane (**Panel A**) was not treated. **Panel B** was subjected to the staining, detaining and stain-erasing protocol of the Pierce Kit. Both membranes were probed with anti-GST incubated with goat anti-rabbit IgG-HRP conjugate and detected using SuperSignal West Dura Substrate (Product # 34075). **Lane 1**. 125 pg, **Lane 2**. 250 pg, **Lane 3**. 500 pg and **Lane 4**. 1 ng.

Ordering Information

Product #	Description	Pkg. Size
24580	**Pierce Reversible Protein Stain Kit for Nitrocellulose Membranes** *Sufficient material to stain protein and reverse the stain from 10 (8 cm x 8 cm) nitrocellulose membranes.* Includes: Pierce Reversible Stain *A broad-spectrum stain for proteins transferred to nitrocellulose membranes.* Pierce Destain* *Enhances protein band detection by eliminating background stain.* Pierce Stain Eraser* *Reverses protein band staining on demand.*	Kit 250 ml 1,000 ml 500 ml
24585	**Pierce Reversible Protein Stain Kit for Polyvinylidene Difluoride Membrane** *Sufficient material to stain protein and reverse the stain from 10 (8 cm x 8 cm) PVDF membranes.* Includes: Pierce Sensitizer *PVDF membrane pre-treatment agent.* Pierce Reversible Stain *A broad-spectrum stain for proteins transferred to PVDF membrane.* Pierce Destain* *Enhances protein band detection by eliminating background stain.* Pierce Stain Eraser* *Reverses protein band staining on demand.*	Kit 250 ml 250 ml 1,000 ml 500 ml

**Reagent-grade methanol (required, but not supplied) supplements the Destain and Stain Eraser formulations.*

Table 1. Comparison of Thermo Scientific Pierce Reversible Protein Stain with Ponceau S Stain.

Ponceau S Reversible Stain	Thermo Scientific Pierce Reversible Protein Stain
• Weak-binding, low-sensitivity general protein stain	• Tight-binding, higher sensitivity general protein stain
• Detection limit: 250 ng	• Detection limit: 25-50 ng
• Red bands are difficult to photograph	• Turquoise blue bands are photographed easily
• Stained protein bands fade within hours	• Turquoise bands do not fade over time, but they can be reversed
• Typical staining time: 5 minutes	• Typical staining time: 60 seconds
	• Background eliminated quickly with low pH wash

Transfer Protein to a Membrane

Another method of verifying target protein transfer is to check the transfer of the molecular weight (MW) markers. We offer a wide variety of MW markers, including unstained markers, prestained markers and peroxidase-conjugated markers.

Featured Product: Thermo Scientific Pierce Blue Prestained Molecular Weight Markers

Room temperature-stable markers are ready when you are.

1. Open the plastic pouch and remove the Pierce Prestained Protein Molecular Weight Marker Mix, which is packaged with a desiccant in a moisture-resistant, resealable pouch.

2. Load 10 µl of DI water into a pipette tip, puncture the foil over a single tube and dissolve the Pierce Prestained Markers.

3. Dispense 5-10 µl of the marker into a sample well of the gel. Each tube can be used for one or two gel lanes.

4. Return the Pierce Prestained Marker Mix to its pouch and reseal. The markers are stable at room temperature and can be kept on your bench-top ready for your next gel.

References

Foubert, T.R., *et al.* (2001). *J. Biol. Chem.* **276**, 38852-38861.
Prozialeck, W.C., *et al.* (2002). *Infect. Immun.* **70**, 2605-2613.

Featured Product: Thermo Scientific Pierce 3-Color Prestained Markers

Fresh marker every time, with reference bands, too.

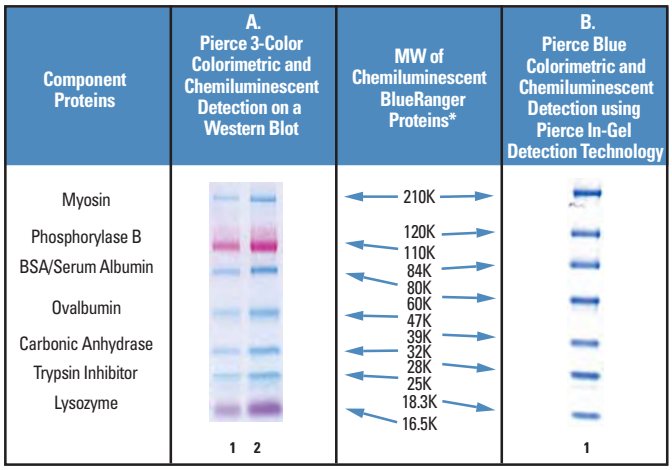


Figure 5. Thermo Scientific Pierce Prestained Marker Protein molecular weights.* Each tube of the Pierce Marker consists of a stabilized and lyophilyzed formulation of seven proteins, ranging from 16.5K to 210K. Each protein in the mixture is proportioned to yield uniform band intensities. Two specially modified bands (one red, one violet) serve as references for the order of the marker proteins.
**These are representative molecular weight values. The covalently bound dye and enzyme alter the apparent molecular weight (MW) of the component proteins relative to their unstained counterparts. Lot-specific MW values are provided with each package.*

Ordering Information

Product #	Description	Pkg. Size
26681	Pierce Blue Prestained Protein Molecular Weight Marker Mix *Sufficient material for loading 48-96 gel lanes.*	1 x 48 microtube plate
26685	Pierce Blue Prestained Protein Molecular Weight Marker Mix *Sufficient material for loading 240-480 gel lanes.*	5 x 48 microtube plates
26691	Pierce 3-Color Prestained Protein Molecular Weight Marker Mix *Sufficient material for loading 48-96 gel lanes in a 6 x 8 microtube-plate format.*	1 x 48 microtube plate

Featured Product: Thermo Scientific DyLight Fluorescent and Infrared MW Markers

One- or two-color fluorescent detection with one protein MW marker.

DyLight Fluorescent and Infrared Protein Molecular Weight Markers are optimized for direct visualization of marker proteins after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Each protein† in the mixture fluoresces at two wavelengths in the near infrared region of the spectrum to enable one- or two-color detection with the LI-COR Odyssey® (Infrared Markers only) or common CCD instruments. The markers are compatible with Western blotting and can be detected by virtually any in-gel staining method. The DyLight Fluorescent Protein Molecular Weight Markers consist of nine proteins with MW in the range of 6K to 200K (Figure 6).

Highlights:

- Excitation/emission maxima – 557/570 and 652/673 or 682/715 and 770/794
- Easily multiplexed – two excitation and emission maxima enable one- or two-color fluorescent detection
- Saves time – no awkward marking or overlay procedures
- Fluorescent and colorimetric – detect in-gel or on-membrane
- Instrument-compatible – spectra are compatible with LI-COR Odyssey (infrared markers only) and CCD instruments
- Photostable – capture multiple images with no decrease in fluorescent intensity

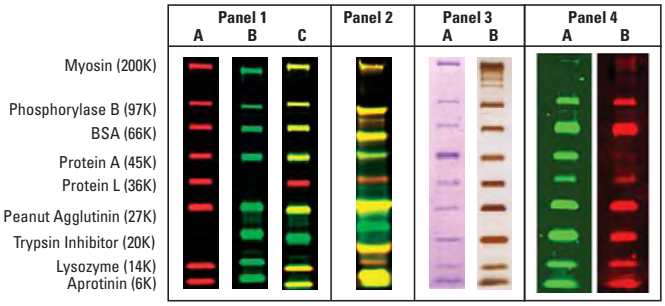


Figure 6. Detection methods for the DyLight Fluorescent and Infrared Markers.

Panel 1. Direct in-gel fluorescent detection. Marker proteins (10 µl) were separated in 4-20% Tris-glycine gels and detected with the LI-COR Odyssey Infrared Imaging System using intensity level 5 with the **A.** 680/720 nm excitation/emission setting, **B.** 780/820 nm excitation/emission setting and **C.** combined image.

Panel 2. Fluorescent detection on membranes. Proteins were separated in 4-20% Precise Protein Gels and transferred to low-fluorescence PVDF membrane. The membrane was blocked overnight in SEA BLOCK Blocking Buffer and imaged with the LI-COR Odyssey System.

Panel 3. Colorimetric in-gel detection. Marker proteins (10 µl) were separated in 4-20% Tris-glycine gels and stained with **A.** Imperial Protein Stain and **B.** the Pierce Silver Stain Kit II.

Panel 4. Fluorescent Western blot detection. Marker proteins (5 µl) were separated in 4-20% Tris-glycine gels and transferred to **A.** nitrocellulose or **B.** PVDF membrane. Blots were imaged with the Typhoon® 9410 at 500V PMT using the **A.** Cy3 Fluor and **B.** Cy5 Fluor laser settings.

Note: Proteins in the marker mix produce uniform fluorescent intensities in SDS-PAGE applications; however, variations in protein-transfer efficiency affect intensity. For example, high MW proteins, such as myosin (200K), typically transfer less efficiently than low MW proteins.

Ordering Information

Product #	Description	Pkg. Size
26665	DyLight Fluorescent Protein Molecular Weight Markers *Sufficient material for loading 50 gel lanes.*	250 µl
22859	DyLight Infrared Protein Molecular Weight Markers *Sufficient material for loading 50 gel lanes.*	250 µl

† Patent pending on Dual-labeled Fluorescent Molecular Weight Marker Technology.

Featured Product: Thermo Scientific Pierce Chemiluminescent Molecular Weight Markers

New protein MW standard looks and acts like a typical pre-stained marker for SDS-PAGE and can also "light up" after transfer or in-gel.

The Pierce Chemiluminescent Marker consists of seven proteins spanning the molecular weight range from 18K to 220K. Each marker component is covalently linked to a blue dye and chemically modified to impart peroxidase capability. Unlike any other chemiluminescent detection-compatible marker for Western blot applications, Pierce Chemiluminescent Marker does not need an HRP-antibody conjugate to yield a chemiluminescent signal.

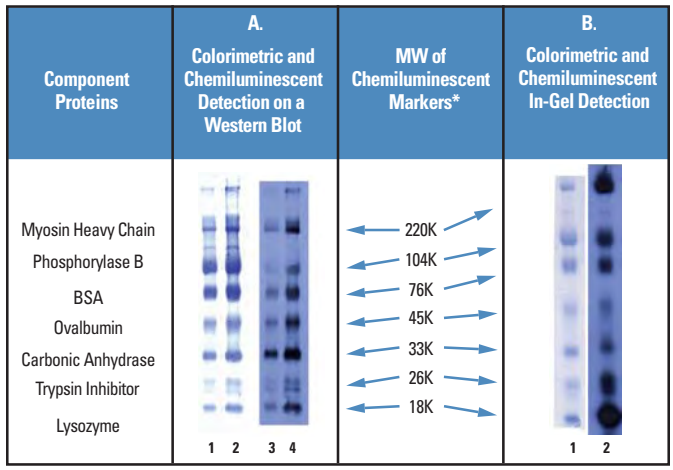


Figure 7. On-membrane and in-gel detection using the Thermo Scientific Pierce Chemiluminescent Molecular Weight Markers.

**These are representative MW values. The covalently bound dye and enzyme alter the apparent MW of the component proteins relative to their unstained counterparts. Lot-specific MW values are provided with each package.*

Highlights:

- Colorimetric and chemiluminescent – detect on-membrane or in-gel
- Visual detection in-gel – already prestained; does not require staining to detect in-gel
- Self-contained peroxidase activity, does not require an HRP-antibody conjugate for chemiluminescence
- Compatible with streptavidin-HRP conjugates
- Room temperature stable
- Convenient packaging – single dose in 48-well microtube plate

Key consideration when using the Pierce Chemiluminescent Marker

The peroxidase activity associated with the Pierce Chemiluminescent Marker is enzymatic. Avoid denaturing or deactivating conditions to preserve activity. Heating the gel during electrophoresis, pH extremes, denaturing agents, strong reducing agents, oxidizing agents and chelating agents will attenuate or quench peroxidase activity.

Ordering Information

Product #	Description	Pkg. Size
26651	Pierce Chemiluminescent Prestained Peroxidase-Labeled Protein Molecular Weight Marker Mix	1 x 48 microtube plate

Transfer Protein to a Membrane

Featured Product: Thermo Scientific Pierce Antibody Extender NC

Reduce the amount of primary antibody used in a Western blot by three- to 100-fold.

A simple 10-minute, post-transfer treatment of the target protein on nitrocellulose can reduce the amount of primary antibody used by three-, 10-, 25- and even 100-fold, while maintaining equivalent signal compared to an untreated control.

Our Antibody Extender NC Promise

Proper use of Pierce Antibody Extender NC will retain post-transfer detection of your target protein on nitrocellulose membrane when using **at least three times less primary antibody** than you are currently using. If you do not experience a minimum of three-fold reduction in primary antibody requirement with an equivalent or better performance on nitrocellulose membrane, we will refund the cost of the reagent.

Highlights:

- **Achieves equivalent signal while using less antibody** – uses three- to 100-fold less primary antibody [average Primary Antibody Reduction Factor (PAR) is 28.2-fold]
- **Inexpensive** – costs approximately US$5 to treat an 8 x 10 cm blot
- **Conserves antibody, regardless of detection system** – works with colorimetric, chemiluminescent, HRP and AP systems
- **Simple and ready to use** – fast 10-minute protocol

Ordering Information

Product #	Description	Pkg. Size
32110	Pierce Antibody Extender Solution NC *Sufficient reagent for up to 20 nitrocellulose membranes (1,600 cm²).*	500 ml
32105	Pierce Antibody Extender Solution NC Trial Pack *Sufficient reagent to treat two nitrocellulose membranes (160 cm²).*	50 ml

How much will you save?

Primary Antibody Cost: US$230 Primary Antibody Volume: 200 µg † Minimum Savings: US$211.60

Primary Antibody Reduction Factor (PAR Factor)	Primary antibody cost/blot vs. PAR Factor	Primary antibody cost per 20 blots – AES treated vs. untreated	Primary antibody cost savings – AES treated vs. untreated	Savings (including cost of Pierce Antibody Extender NC)
0 (Untreated)	$23	$460	$0	$0
3X	$7.67	$153.40	$306.60	$207.60
12X	$1.91	$38.20	$421.80	$322.80
25X	$0.92	$18.40	$441.60	$342.60

† ***Assumptions:*** *(1) Analysis based on 20 blots using an 8 cm x 10 cm nitrocellulose membrane. (2) Pierce Antibody Extender NC, 500 ml, treats 20 blots. (3) Primary antibody cost based on US$1.15 per µg. (4) 1:500 primary antibody dilution from a 1 mg/ml stock = 2 µg/ml with an ECL Substrate. (5) 10 ml of primary antibody solution used per blot. (6) 20 µg of primary antibody used per untreated blot.*

Featured Products: Thermo Scientific Pierce Antibody Extender NC vs. Pierce Western Blot Signal Enhancer

Which one should you use?

Pierce Antibody Extender NC and Pierce Western Blot Signal Enhancer are mutually exclusive; i.e., you cannot extend your antibody and increase signal at the same time, so you can use only one of these products.

Use Pierce Antibody Extender NC when

- You want a costly primary antibody to last as long as possible
- You have plenty of target, but the detection antibody is available in limited amount

Use Pierce Western Blot Signal Enhancer when

- You have a low abundance of target protein (antigen), but adequate primary antibodies with which to detect it
- You want to obtain a stronger signal under the conditions you typically use to detect your target protein

Increase the Sensitivity of a Western Blot

Featured Product: Thermo Scientific Pierce Western Blot Signal Enhancer

It's like having an intensifying screen in a bottle.

There are many ways to increase the sensitivity of a Western blot. Some methods are as simple as switching substrates or blocking buffers, while others are more time-consuming such as optimizing antibody titer or checking for proper protein transfer. Those solutions are detailed in the troubleshooting section of this handbook.

One of the more certain and easiest ways to increase the sensitivity of any Western blot is to use Pierce Western Blot Signal Enhancer.

Pierce Western Blot Signal Enhancer does for enzyme-/substrate-based blotting what intensifying screens do for radioactive blotting – it increases the signal up to 10-fold (or one order of magnitude) in only 15 minutes (Figures 1-2).

The Pierce Western Blot Signal Enhancer membrane treatment is a simple, 15-minute procedure (Figure 3) that can be added to your current Western blotting protocol. The result is an increase in the intensity of target protein bands on the Western blot or detection of target proteins at a level that could not previously be detected. Some protein targets have resulted in a 10-fold increase in band intensity after treatment with the Western Blot Signal Enhancer compared to the typical detection protocol without treatment.

Highlights:

Enhances chemiluminescent, fluorescent and colorimetric detection up to 10-fold

- Treatment with Western Blot Signal Enhancer can boost the band intensity from three- to 10-fold, regardless of which substrate is used

Enhances detection of targets transferred to either nitrocellulose or PVDF, independent of membrane pore size

- Works with the most commonly used Western blotting membranes
- Signal intensity has been increased with targets such as mouse IL-6, p53, NF-κB, BRCA1 and EGF

Room temperature-stable, ready-to-use reagents

- No thawing, formulating or diluting necessary

15-minute protocol

- Optimized to save time and improve detection capability of your specific analyte

Ordering Information

Product #	Description	Pkg. Size	U.S. Price
21050	Pierce Western Blot Signal Enhancer*	Kit	$ 104
	Sufficient reagent for ten 10 cm x 10 cm blots.		
	Includes: Enhancer Reagent 1	250 ml	
	Enhancer Reagent 2	250 ml	

** Signal enhancement of proteins on PVDF membrane has been shown to be variable from no significant enhancement for some proteins, to several-fold enhancement for others.*

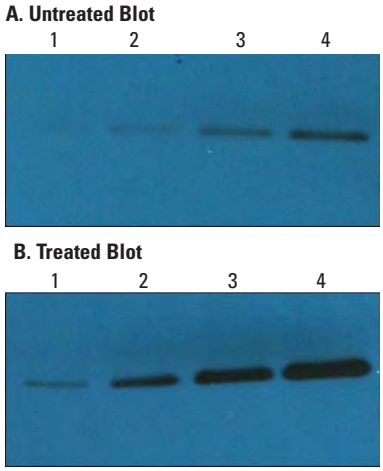


Figure 1. Enhanced chemiluminescent detection of identical serial dilutions of IL-6 Panel A: before and Panel B: after treatment with Thermo Scientific Pierce Western Blot Signal Enhancer. Lane 1. 250 pg, **Lane 2.** 500 pg, **Lane 3.** 1,000 pg and **Lane 4.** 2,000 pg.

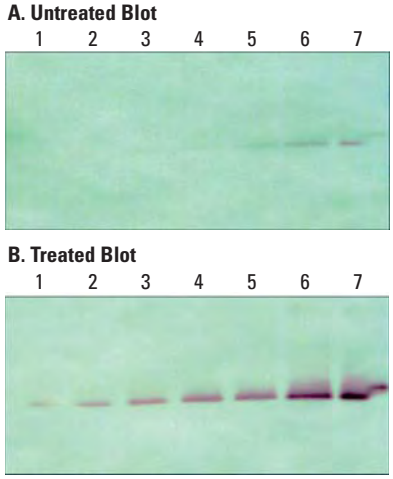


Figure 2. Enhanced chromogenic detection of identical serial dilutions of IL-6 Panel A: before and Panel B: after treatment with Thermo Scientific Pierce Western Blot Signal Enhancer. Lane 1. 100 pg, **Lane 2.** 200 pg, **Lane 3.** 300 pg, **Lane 4.** 400 pg, **Lane 5.** 500 pg, **Lane 6.** 1,000 pg and **Lane 7**. 5,000 pg.

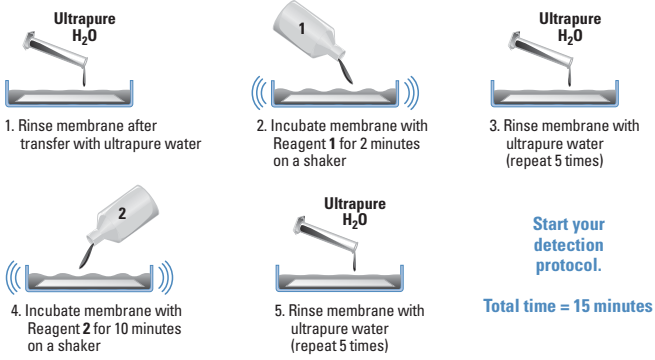


Figure 3. Thermo Scientific Pierce Western Blot Signal Enhancer Protocol performed after transfer and before blocking.

Block Nonspecific Binding Sites

In a Western blot, it is important to block the unreacted sites on the membrane to reduce the amount of nonspecific binding of proteins during subsequent steps in the assay. A variety of blocking buffers ranging from milk or normal serum to highly purified proteins have been used to block unreacted sites on a membrane. The blocking buffer should improve the sensitivity of the assay by reducing background interference. Individual blocking buffers are not compatible with every system. For this reason, a variety of blockers in both Tris-buffered saline (TBS) and phosphate-buffered saline (PBS) are available. The proper choice of blocker for a given blot depends on the antigen and on the type of enzyme conjugate to be used. For example, with applications using an alkaline phosphatase conjugate, a blocking buffer in TBS should be selected because PBS interferes with alkaline phosphatase. The ideal blocking buffer will bind to all potential sites of nonspecific interaction, eliminating background without altering or obscuring the epitope for antibody binding.

For true optimization of the blocking step for a particular immunoassay, empirical testing is essential. Many factors can influence nonspecific binding, including various protein:protein interactions unique to a given set of immunoassay reagents. The most important parameter when selecting a blocker is the signal-to-noise ratio, which is measured as the signal obtained with a sample containing the target analyte as compared to that obtained with a sample without the target analyte. Using inadequate amounts of blocker will result in excessive background noise and a reduced signal-to-noise ratio. Using excessive concentrations of blocker may mask antibody:antigen interactions or inhibit the marker enzyme, again causing a reduction of the signal-to-noise ratio. When developing any new immunoassay, it is important to test several different blockers for the highest signal-to-noise ratio in the assay. No single blocking agent is ideal for every occasion because each antibody-antigen pair has unique characteristics. If a blocking buffer that does not cross-react with your system cannot be found, Pierce In-Gel Protein Detection is an alternate choice. This method specifically detects proteins within the gel and requires no blocking (see page 58 for more information).

We offer a complete line of blocking buffers for Western blotting, including BLOTTO, Casein, BSA, SEA BLOCK and the exclusive SuperBlock and StartingBlock Blocking Buffers.

Which blocking buffer is most likely to cause a high background?

Nonfat Dry Milk Ingredients:

β-lactoglobulin, α-lactalglobulin, antibodies, serum albumin, three or more different caseins, enzymes, hormones, growth factors, nutrient transporters, disease-resistance factors, entire leukocytes, other proteins, lactose, glucose, galactose, amino sugars, sugar phosphates, neutral and acid oligonucleotides, nucleotide sugars, monosaturated fatty acids, polyunsaturated fatty acids, saturated fatty acids, A, B-6, B-12, D, E, H (biotin), folate, niacin, pantothenic acid, riboflavin, thiamin, calcium, iron, magnesium, phosphorous, potassium, sodium, zinc, copper, manganese, and selenium

Thermo Scientific SuperBlock and StartingBlock Blocking Buffer Ingredients:

A single protein, PBS or TBS buffer, and a Kathon® Preservative

Blocking Buffer Optimization

The most appropriate blocking buffer for Western blotting use is often system-dependent. Determining the proper blocking buffer can help to increase the system's signal-to-noise ratio. Occasionally, when switching from one substrate to another, the blocking buffer that you are using will lead to diminished signal or increased background. Empirically testing various blocking buffers with your system can help achieve the best possible results. Avoid using milk as a blocking reagent for blots that rely on the avidin/biotin system because milk contains variable amounts of biotin. Although SuperBlock Blocking Buffer (Product # 37515) often gives excellent results, we recommend testing several blocking reagents for their suitability in a particular system. There is no blocking reagent that will be the optimal reagent for all systems.

Various proteins were analyzed by Western blotting to determine the optimal blocking condition for nonspecific sites (Figure 1). The resulting blots were analyzed for signal-to-noise and compared. The results indicate that there is no single blocking reagent that is optimal for all systems.

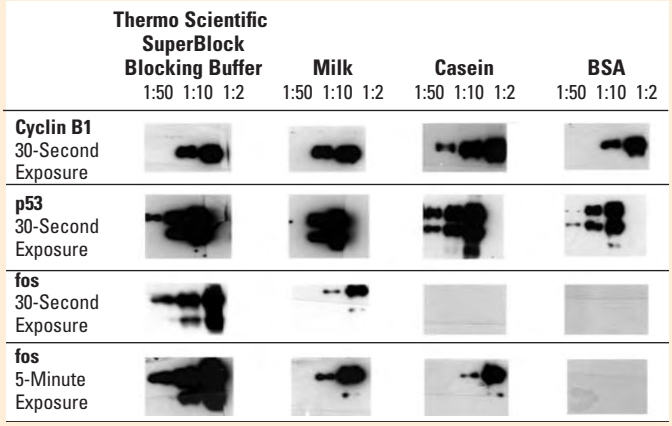


Figure 1. Blocking buffer optimization. Recombinant human cyclin B1, wild-type p53 and mouse fos baculovirus lysates were diluted in Lane Marker Reducing Sample Buffer (1:50, 1:10 or 1:2) and separated electrophoretically on a 12% SDS-polyacrylamide gel. The proteins were transferred to nitrocellulose membrane and cut into strips. The membrane strips were blocked for 1 hour at RT with shaking in Blocker Casein in TBS, 1% BSA in TBS, SuperBlock Blocking Buffer in TBS or 5% nonfat milk in TBS. Tween-20 (0.05%) was added to all blocking buffers. The membranes were then incubated with the appropriate primary antibody at 0.5 µg/ml prepared in the different blocking solutions for one hour at RT with shaking. Each membrane strip was washed with TBS followed by a one-hour incubation in HRP-conjugated goat anti-mouse antibody prepared in the different blocking buffers at 25 ng/ml. The membranes were washed with TBS. A working solution of SuperSignal West Pico Chemiluminescent Substrate was prepared and added to each membrane for 5 minutes. The membranes were placed in sheet protectors and exposed to film for 30 seconds and 5 minutes as indicated. The film was developed per the manufacturer's instructions.

Blocking Buffers Application Chart

Product #	Blocking Buffer	ELISA	Western Blot	Dot Blot	Immuno-histo-chemistry	DNA/RNA Hybridiza-tions	
37538	StartingBlock (PBS) Blocking Buffer	✔	✔	✔	✔		$135
37542	StartingBlock (TBS) Blocking Buffer	✔	✔	✔	✔		
37539	StartingBlock T20 (PBS) Blocking Buffer	✔	✔	✔	✔		$152
37543	StartingBlock T20 (TBS) Blocking Buffer	✔	✔	✔	✔		
37515	SuperBlock Blocking Buffer in PBS	✔	✔	✔	✔	✔	$132
37535	SuperBlock Blocking Buffer in TBS	✔	✔	✔	✔	✔	
37517	SuperBlock Blocking Buffer – Blotting in PBS		✔	✔	✔		$124
37537	SuperBlock Blocking Buffer – Blotting in TBS		✔	✔	✔		
37516	SuperBlock T20 PBS Blocking Buffer	✔	✔	✔	✔	✔	$146
37536	SuperBlock T20 TBS Blocking Buffer	✔	✔	✔	✔	✔	
37527	SEA BLOCK Blocking Buffer	✔	✔	✔			$134
37520	Blocker BSA in TBS	✔	✔	✔	✔	✔	
37525	Blocker BSA in PBS	✔	✔	✔	✔	✔	$112
37532	Blocker Casein in TBS	✔	✔	✔	✔	✔	
37528	Blocker Casein in PBS	✔	✔	✔	✔	✔	$ 92
37530	Blocker BLOTTO in TBS	✔	✔	✔	✔	✔	
37570	Protein-Free (TBS) Blocking Buffer	✔	✔	✔	✔		$129
37571	Protein-Free T20 (TBS) Blocking Buffer	✔	✔	✔	✔		
37572	Protein-Free (PBS) Blocking Buffer	✔	✔	✔	✔		$129
37573	Protein-Free T20 (PBS) Blocking Buffer	✔	✔	✔	✔		

Blocking Buffer Optimization

Thermo Scientific Protein-Free Blocking Buffers

Eliminate or minimize cross-reactivity to reduce background and increase signal.

Traditional blocking buffers contain proteins that can cross-react with a system, resulting in high background and reduced signal (Figures 2-3). Protein-Free Blocking Buffers eliminate or minimize cross-reactivity associated with protein-based blocking buffers in ELISA, Western blotting, arrays and other immunodetection applications.

Highlights:

- **Protein-free** – eliminate or minimize cross-reactivity associated with protein-based blocking buffers
- **Compatible with multiple detection systems** – can be used in Western blots, ELISA or arrays; does not interfere with avidin-biotin systems
- **High signal-to-background** – for optimal sensitivity
- **1X formulation** – ready-to-use
- **Available with 0.05% Tween-20 Detergent already added** – saves time and money

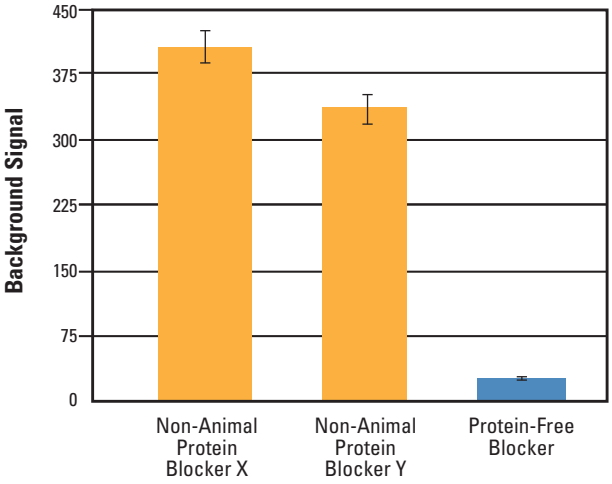


Figure 2. Thermo Scientific Protein-Free Blocking Buffers exhibit less background than other blocking buffers in Thermo Scientific SearchLight® Multiplex Arrays. SearchLight Arrays were created by spotting up to 12 cytokine capture antibodies per well. The plates were blocked with the indicated blocking buffers and the background for each well determined. Error bars represent the standard deviation for triplicate microplate wells.

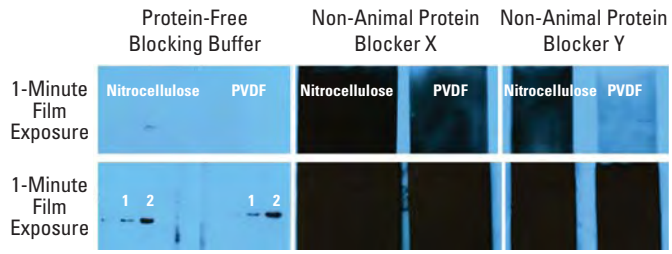


Figure 3. Thermo Scientific Protein-Free Blocking Buffer efficiently blocks Western blotting membranes. Jurkat apoptotic lysate (**Lane 1**. 0.25 µg, **Lane 2**. 0.50 µg) was separated in 4-20% Tris-glycine gels and transferred to nitrocellulose or PVDF membranes. The membranes were blocked for 1 hour at RT with the indicated blocking buffer, probed with mouse anti-PARP (0.25 µg/ml) followed by goat anti-mouse HRP (4 ng/ml) and detected by SuperSignal West Dura Chemiluminescent Substrate.

Ordering Information

Product #	Description	Pkg. Size
37570	**Protein-Free (TBS) Blocking Buffer** *Proprietary formulation in Tris-buffered saline at pH 7.4 with Kathon Antimicrobial Agent.*	1 L
37571	**Protein-Free T20 (TBS) Blocking Buffer** *Proprietary formulation in Tris-buffered saline at pH 7.4 with 0.05% Tween-20 Detergent and Kathon Antimicrobial Agent.*	1 L
37572	**Protein-Free (PBS) Blocking Buffer** *Proprietary formulation in phosphate-buffered saline at pH 7.4 with Kathon Antimicrobial Agent.*	1 L
37573	**Protein-Free T20 (PBS) Blocking Buffer** *Proprietary formulation in phosphate-buffered saline at pH 7.4 with 0.05% Tween-20 Detergent and Kathon Antimicrobial Agent.*	1 L

Featured Product: **Thermo Scientific StartingBlock Blocking Buffer**

Simplify the selection of a blocker for Western blot and ELISA applications.

Although no blocking buffer is ideal for every system, you can improve the odds dramatically with StartingBlock Blocking Buffer because it is compatible with the widest variety of antibodies.

For example: StartingBlock Blocking Buffers are compatible with biotin-containing systems, while milk-based protein blockers interfere. StartingBlock Buffers rarely cross-react with rabbit antibodies, while many other blockers do. StartingBlock Blocking Buffers are also free of potentially interfering serum proteins.

StartingBlock Blocking Buffers offer a high level of performance – regardless of the system you choose for your Western blotting or ELISA application (Figure 4). They may be the only blockers you ever use.

Figure	4A	4B	4C	4D
Membrane Type	Nitrocellulose	PVDF	Nitrocellulose	PVDF
Film Exposure Time	30 minutes	30 minutes	24 hours*	24 hours*

**Full duration of SuperSignal West Dura Chemiluminescent Substrate light emission.*

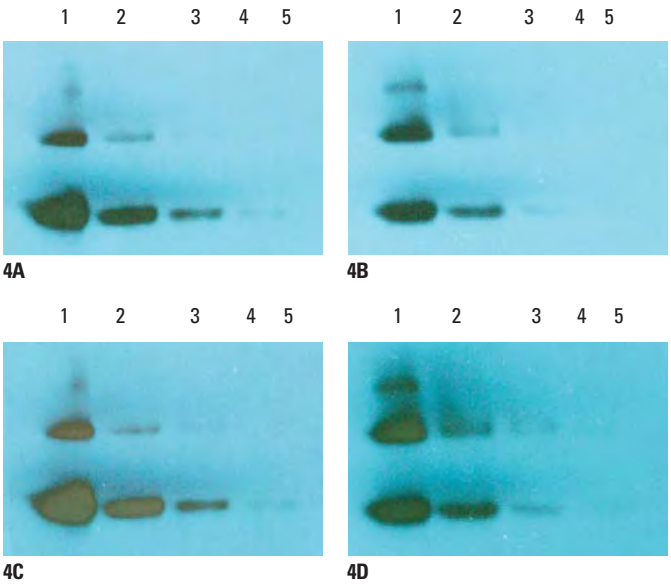


Figure 4A-D. Comparison of Thermo Scientific StartingBlock Blocking Buffer performance after stripping and reprobing. Nitrocellulose vs. PVDF when probed for the transferrin receptor (CD71).

Highlights:

Compatible with a wide range of detection systems
- Works in both Western and ELISA applications
- Rarely cross-reacts with rabbit antibodies
- Serum protein-free
- Biotin-free

Shorter blocking times
- Western blotting – 1-15 minutes
- ELISA – "no-wait" blocking capability

Strip and reprobe – no reblocking necessary
- Blots stay blocked with StartingBlock Blocker when our Restore Stripping Buffer (Product # 21059) is used, allowing reprobing of the same blot without re-blocking

Superior signal-to-noise ratios in ELISA applications
- Signal-to-noise ratios in the range of 10:1-20:1 have been realized with StartingBlock Blocking Buffer

Ordering Information

Product #	Description	Pkg. Size
37538	**StartingBlock (PBS) Blocking Buffer** *A protein-based blocker formulation in phosphate-buffered saline (pH 7.5) for use in Western blotting and ELISA applications.*	1 L
37542	**StartingBlock (TBS) Blocking Buffer** *A protein-based blocker formulation in Tris-buffered saline (pH 7.5) for use in Western blotting and ELISA applications.*	1 L

StartingBlock Blocking Buffers are also available with an optimized amount of Tween-20 Detergent to provide the lowest background.

Ordering Information

Product #	Description	Pkg. Size
37539	**StartingBlock T20 (PBS) Blocking Buffer** *A protein-based blocker formulation in phosphate-buffered saline at pH 7.5 with 0.05% Tween-20 Detergent and Kathon Antimicrobial Agent.*	1 L
37543	**StartingBlock T20 (TBS) Blocking Buffer** *A protein-based blocker formulation in Tris-buffered saline at pH 7.5 with 0.05% Tween-20 Detergent and Kathon Antimicrobial Agent.*	1 L

Featured Product: **Thermo Scientific SuperBlock Blocking Buffers**

Guaranteed to be biotin-free.

Our most popular blocking buffer, SuperBlock Blocking Buffer, now comes in both dry and liquid formats! Many researchers have discovered that SuperBlock Blocking Buffer is the only blocking buffer needed for all of their applications.

Highlights:
- Fast blocking – blocks ELISA plates in two minutes or membranes in five to 10 minutes
- Non-serum protein solution yields a high signal-to-noise ratio
- Plates blocked with SuperBlock Blocking Buffer can be stored dry for up to 12 months
- Liquid formulations available in PBS or TBS

Blocking Buffer Optimization

SuperBlock Dry Blend (TBS) Blocking Buffer

Delivers the ultimate in space-saving convenience.

Highlights:

- Delivers even more economy and stability
- Each pouch reconstitutes to form 200 ml of SuperBlock Blocking Buffer in TBS
- Room-temperature storage; small packaging takes up minimal shelf space

References

Ikeda, K., *et al.* (2003). *J. Biol. Chem.* **278**, 7725-7734.
Leclerc, G.J. and Barredo, J.C. (2001). *Clin. Cancer Res.* **7**, 942-951.
Subbarayan, V., *et al.* (2001). *Cancer Res.* **61**, 2720-276.
Walters, R.W., *et al.* (2002). *Cell* **100**, 789-799.

Ordering Information

Product #	Description	Pkg. Size
37515	SuperBlock (PBS) Blocking Buffer	1 L
37516	SuperBlock T20 (PBS) Blocking Buffer *(Contains 0.05% Tween-20 Detergent)*	1 L
37535	SuperBlock (TBS) Blocking Buffer	1 L
37536	SuperBlock T20 (TBS) Blocking Buffer *(Contains 0.05% Tween-20 Detergent)*	1 L
37517	SuperBlock (PBS) Blocking Buffer – Blotting*	1 L
37537	SuperBlock (TBS) Blocking Buffer – Blotting*	1 L
37545	SuperBlock (TBS) Blocking Buffer Dry Blend Blocking Buffer *Each pouch yields 200 ml when reconstituted.*	5 pouches

** Formulated for precipitating enzyme substrates. Added ingredient to keep precipitate from flaking. Not recommended for chemiluminescent substrates.*

SEA BLOCK Blocking Buffer

No mammalian proteins, reducing the risk of nonspecific interaction.

Highlights:

- Made from steelhead salmon serum
- Functions as a universal blocker
- Offers reduced background
- Can be diluted up to 1:10 with buffer

References

Hypolite, J.A., *et al.* (2001). *Am. J. Physiol. Cell Physiol.* **280**, C254-264.
Wang, L., *et al.* (2002). *J. Clin. Invest.* **110**, 1175-1184.

Ordering Information

Product #	Description	Pkg. Size
37527	SEA BLOCK Blocking Buffer	500 ml

Blocker™ Casein

Ready-to-use solution (1% w/v) of Hammersten Grade casein for blocking nonspecific sites.

Highlights:

- Preformulated for ease of use
- Use when skim milk produces high background
- Thimerosal-free formulation

Ordering Information

Product #	Description	Pkg. Size
37532	Blocker Casein in TBS *1% (w/v) Casein Hammersten Grade in TBS; Contains Kathon Antimicrobial Reagent as preservative, pH 7.4.*	1 L
37528	Blocker Casein in PBS *1% (w/v) Casein Hammersten Grade in PBS; Contains Kathon Antimicrobial Reagent as preservative, pH 7.4.*	1 L

Blocker BLOTTO

Ready-to-use blocking buffer made of nonfat dry milk.

Highlights:

- Preformulated for ease of use
- Available in TBS Buffer
- Anti-foaming agent added
- Merthiolate-free formulation

Ordering Information

Product #	Description	Pkg. Size
37530	Blocker BLOTTO in TBS *5% (w/v) nonfat powdered milk in TBS, 0.01% Anti-foam A; contains Kathon Antimicrobial Reagent as preservative, pH 7.4.*	1 L

Blocker BSA

For all blocking applications.

Highlights:

- 10% solutions of high-quality bovine serum albumin
- Concentrated formulation saves storage space
- No powder to dissolve; ready-to-dilute liquid concentrate

Ordering Information

Product #	Description	Pkg. Size
37525	Blocker BSA in PBS (10X)	200 ml
37520	Blocker BSA in TBS (10X)	125 ml

Surfact-Amps 20 Purified Detergent Solution

Specially purified form of Tween-20 Detergent.

Highlights:

- Guaranteed < 1 milliequivalent of peroxides and carbonyl in a 10% solution
- Enhances signal-to-background ratio

Ordering Information

Product #	Description	Pkg. Size
28320	Surfact-Amps 20 Purified Detergent Solution	6 x 10 ml

Wash the Membrane

Like other immunoassay procedures, Western blotting consists of a series of incubations with different immunochemical reagents separated by wash steps. Washing steps are necessary to remove unbound reagents and reduce background, thereby increasing the signal-to-noise ratio. Insufficient washing produces high background, while excessive washing may result in decreased sensitivity caused by elution of the antibody and/or antigen from the blot. As with other steps in performing a Western blot, a variety of buffers may be used. Occasionally, washing is performed in a physiological buffer such as Tris-buffered saline (TBS) or phosphate-buffered saline (PBS) without any additives. More commonly, a detergent such as 0.05% Tween-20 Detergent (Product # 28320) is added to the buffer to help remove nonspecifically bound material. Another common technique is to use a dilute solution of the blocking buffer along with some added detergent to help minimize background. For best results, use high-purity detergents, such as Surfact-Amps Detergents for Western blotting.

Thermo Scientific BupH Dry Buffers

The most advanced, versatile, time-saving buffer products available.

The ultimate in convenience

1. Reach for the sealed foil pack stored conveniently on the bench top.
2. Open, pour into beaker and add water.
3. The fresh buffer is ready to use in practical amounts so there's no waste.

The ultimate in versatility

1. Routine buffers are designed for use in Western blotting, dialysis, crosslinking, ELISAs, immunohistochemistry, protein plate-coating, biotinylation and other applications.
2. Using one buffer source maintains consistency and minimizes variables.

The ultimate in integrity

1. BupH Buffers are protected from contamination and are fresh every time.
2. Perform applications with confidence in quality buffers.
3. "Test-assured" with our commitment to quality management standards.

The ultimate in time savings

1. Making routine buffers is no longer time-consuming.
2. No component measurement, pH adjustment, quality validation, preparation tracking or refrigeration hassles.
3. Move forward with your research by eliminating re-tests from buffer problems.

BupH Phosphate Buffered Saline Packs (PBS)

Great wash buffer for Western blots!

Each pack yields 500 ml of 0.1 M phosphate, 0.15 M sodium chloride, pH 7.0 when dissolved in 500 ml deionized water (20 L total).

Ordering Information

Product #	Description	Pkg. Size
28372	BupH Phosphate Buffered Saline Packs	40 pack

BupH Tris Buffered Saline (TBS)

Great wash buffer for Western blots!

Each pack yields 500 ml of 25 mM Tris, 0.15 M sodium chloride, pH 7.2 when dissolved in 500 ml deionized water (10 pack makes 5 L total; 40 pack makes 20 L total).

Ordering Information

Product #	Description	Pkg. Size
28380	BupH Tris-Glycine Buffer Packs	40 pack
28376	BupH Tris Buffered Saline Packs	40 pack
28379	BupH Tris Buffered Saline Packs	10 pack

Surfact-Amps 20 Purified Detergent Solution

Specially purified form of Tween-20 Detergent.

Highlights:

- Can be added to PBS or TBS wash buffers to improve performance
- Guaranteed < 1 milliequivalent of peroxides and carbonyl in a 10% solution
- Enhances signal-to-background ratio

Ordering Information

Product #	Description	Pkg. Size
28320	Surfact-Amps 20	6 x 10 ml

Primary and Secondary Antibodies

The choice of a primary antibody for a Western blot will depend on the antigen to be detected and what antibodies are available to that antigen. A huge number of primary antibodies are available commercially and can be identified quickly by searching sites such as www.antibodyresource.com or www.linscottsdirectory.com. Alternatively, a primary antibody may be made to recognize the antigen of interest. Both polyclonal and monoclonal antibodies work well for Western blotting. Polyclonal antibodies are less expensive and less time-consuming to produce and they often have a high affinity for the antigen. Monoclonal antibodies are valued for their specificity, purity and consistency that result in lower background. Crude antibody preparations such as serum or ascites fluid are sometimes used for Western blotting, but the impurities present may increase background. To obtain antibodies with the greatest specificity, they can be affinity-purified using the immobilized antigen. For more information on affinity purification, request your FREE Affinity Purification Handbook from our website or contact a customer service representative at 800-874-3723 or 815-968-0747. Outside the United States, contact your local distributor.

A wide variety of labeled secondary antibodies can be used for Western blot detection. The choice of secondary antibody depends upon the species of animal in which the primary antibody was raised (the host species). For example, if the primary antibody is a mouse monoclonal antibody, the secondary antibody must be an anti-mouse antibody obtained from a host other than the mouse. The host species of the secondary antibody often will not affect the experiment. However, secondary antibodies are available from many host species and, if a secondary antibody causes high background in a particular assay, another host species may be chosen. Another option to reduce background is to use a secondary antibody that has been pre-adsorbed to serum proteins from other species. This pre-adsorption process removes antibodies that have the potential to cross-react with serum proteins, including antibodies, from those species. To expedite the process of choosing the appropriate secondary antibody, visit the Secondary Antibody Selection Guide on our website.

Antibody solutions for Western blotting are typically diluted from 1/100 to 1/500,000 beginning from a 1 mg/ml stock solution. The optimal dilution of a given antibody with a particular detection system must be determined experimentally. More sensitive detection systems require less antibody, which can result in substantial savings on antibody costs and allow a limited supply of antibody to be used for many experiments. It also produces a side benefit of reduced background because the limited amount of antibody is specific for the target with the highest affinity. Antibody dilutions are typically made in the wash buffer containing a blocking agent. The presence of a small amount of blocking agent and detergent in the antibody diluent often helps to minimize background.

We offer a wide variety of labeled secondary antibodies for use in Western blotting. The labels include biotin, fluorescein, rhodamine, DyLight Dyes, horseradish peroxidase and alkaline phosphatase. For the complete list of labeled secondary antibodies please refer to pages 19-21.

The choice of secondary antibody also depends upon the type of label that is desired. Many different labels can be conjugated to antibodies. Radioisotopes were used extensively in the past, but they are expensive, have a short shelf-life, offer no improvement in signal-to-noise ratio and require special handling. Alternative labels are biotin, fluorophores and enzymes. The use of fluorophores requires fewer steps and special equipment to view the fluorescence. Also, a photograph must be taken for a permanent record of the results. Enzymatic labels are used most commonly and consistently produce excellent results.

Alkaline phosphatase (AP) and horseradish peroxidase (HRP or POD) are the two enzymes that are used extensively. An array of chromogenic, fluorogenic and chemiluminescent substrates is available for use with either enzyme. For a detailed comparison of these two enzymes, see Table 1.

AP, a 140 kDa protein that is generally isolated from calf intestine, catalyzes the hydrolysis of phosphate groups from a substrate molecule, resulting in a colored or fluorescent product or the release of light as a byproduct. AP has optimal enzymatic activity at a basic pH (pH 8-10) and can be inhibited by cyanides, arsenate, inorganic phosphate and divalent cation chelators, such as EDTA. As a label for Western blotting, AP offers a distinct advantage over other enzymes. Because its reaction rate remains linear, detection sensitivity can be improved by simply allowing a reaction to proceed for longer.

HRP is a 40 kDa protein that catalyzes the oxidation of substrates by hydrogen peroxide, resulting in a colored or fluorescent product or the release of light as a byproduct. HRP functions optimally at a near-neutral pH and can be inhibited by cyanides, sulfides and azides. Antibody-HRP conjugates are superior to antibody-AP conjugates with respect to the specific activities of both the enzyme and antibody. In addition, its high turnover rate, good stability, low cost and wide availability of substrates make HRP the enzyme of choice for most applications.

Table 1. Comparison of AP and HRP enzymes.

	Alkaline Phosphatase	Horseradish Peroxidase
Size	140 kDa	40 kDa
Price	Relatively Expensive	Relatively Inexpensive
Stability (Storage)	Unstable at < 0°C	Stable at < 0°C
Number of Substrates	Few	Many
Kinetics	Slower	Rapid
pH optimum	8-10	5-7

Affinity-Purified Secondary Antibodies

Affinity-purified antibodies are available unconjugated or conjugated with biotin, alkaline phosphatase, horseradish peroxidase, fluorescein, rhodamine and DyLight Dyes. $F(ab')_2$ fragments of antibodies to immunoglobulins are also available in unconjugated or conjugated forms. These $F(ab')_2$ fragments of antibodies are especially useful in assays in which binding between the Fc portions of antibodies and Fc receptor-bearing cells must be eliminated.

Polyclonal antibodies are purified by immunoaffinity chromatography to eliminate nonspecific antibodies, resulting in high sensitivity and specificity and low background. The purification process involves an elution procedure, yielding antibodies with high avidity. These antibodies exhibit maximal binding to antigens and minimal cross-reactivity to other molecules. Conjugated antibodies are affinity-purified before the conjugation process.

Selected Pierce Antibodies have been further purified to minimize cross-reactivities to other species' serum proteins and is indicated by "min x Species Sr Prot." The key to abbreviations for the individual species is shown in Table 2.

Table 2. Key to abbreviations for individual species.

Bv = Bovine	Gu = Guinea Pig	Hs = Horse	Rt = Rat
Ch = Chicken	Ha = Hamster	Ms = Mouse	Sh = Sheep
Gt = Goat	Hn = Human	Rb = Rabbit	Sw = Swine

Pierce Polyclonal Conjugated Antibodies contain bovine serum albumin as a stabilizer. Table 3 lists the typical conjugate working dilutions for ELISAs, immunoblotting and immunohistochemical techniques.

Table 3. Typical dilution ranges for Thermo Scientific Pierce Polyclonal Conjugated Antibodies.

Conjugate	ELISA	Immunoblotting	Immunohistochemistry
AP	1:5,000-1:50,000	1:2,500-1:25,000	1:500-1:5,000
Peroxidase	1:5,000-1:200,000 (for SuperSignal ELISA Products)	1:25,000-1:500,000 (for SuperSignal West Products)	1:500-1:5,000
Fluorescein	—	—	1:50-1:200
Rhodamine	—	—	1:50-1:200
DyLight Dyes	1:100-1:500	1:10,000-1:75,000	1:1,000-1:5000

Storing Enzyme Conjugates

We provide a variety of reagents to help preserve enzyme conjugate activity. Typically, conjugates are aliquoted in 50-100 µl increments using purified ethylene glycol (Product # 29810) as a preservative for -20°C storage. Conjugates can maintain activity for up to two years. An alternative to aliquoting is to use Pierce Peroxidase Conjugate Stabilizer (Product # 31503), diluting the conjugate 1:1 in the stabilizer and storing at -20°C for up to one year as a stock solution. Pierce Peroxidase Stabilizer/Diluent (Product #s 37548 and 37552) allow peroxidase conjugates to be reconstituted and stored at 4°C as a 1:1,000 or a 1:100,000 dilution.

Conjugate Stabilizers

Ordering Information

Product #	Description	Pkg. Size
37548	Pierce Peroxidase Conjugate Stabilizer/Diluent (SD)	200 ml
37552	Pierce Peroxidase Conjugate Stabilizer/Diluent (SD)	1 L
31503	Pierce Peroxidase Conjugate Stabilizer	25 ml
29810	Ethylene Glycol (50% aqueous solution)	200 ml

Using Antibodies: A Laboratory Manual

Few technical manuals have become standards in bioresearch like *Antibodies: A Laboratory Manual* by Ed Harlow and David Lane, which has enjoyed that status for more than a decade.

The authors, however, have raised the standard with the publication of their book *Using Antibodies: A Laboratory Manual.* Harlow and Lane have completely revised their guide for using antibody reagents in the laboratory. Chapters have been entirely rewritten, reorganized and updated to provide background, context and step-by-step instructions for techniques ranging from choosing the right antibody and handling it correctly, to the proper methods for characterizing antigens in cells and solutions. They've also added new chapters on tagging proteins and epitope mapping.

Rather than presenting an array of solutions for working with antibodies and antigens, "Using Antibodies" identifies the best approach to specific problems. These recommendations include more detail in the protocols, extensive advice on avoiding and solving problems, information regarding proper controls, and thorough illustration of theory, methods and results. The book also includes a bonus – a set of portable protocols that include step-by-step instructions for the most frequently used and essential techniques. The protocols are printed on durable cards, enabling them to be used easily at the bench.

This helpful guide, along with high-quality Thermo Scientific Pierce Products, will help you purify, immobilize, label and store antibodies and perform common procedures such as immunoprecipitation, Western blotting and ELISA.

Ordering Information

Product #	Description	Pkg. Size
15051	Using Antibodies: A Laboratory Manual* Ed Harlow and David Lane Published by Cold Spring Harbor Laboratory Press, 1999. 495 pages; wire spiral-bound hardcover with nine separate portable protocols	1 book

** Sorry, books are nonreturnable.*

Primary and Secondary Antibodies

Featured Product: **Thermo Scientific DyLight 488, 549, 649, 680 and 800 Conjugates**

Bright new alternatives to Alexa Fluor, CyDye and LI-COR Fluorescent Dyes.

Thermo Scientific DyLight Dyes have absorption spectra ranging from 493 nm to 770 nm (Table 4) and match the principal output wavelengths of common fluorescence instrumentation. They exhibit higher fluorescence intensity and photostability than Alexa Fluor, CyDye and LI-COR Dyes in many applications and remain highly fluorescent over a broad pH range (pH 4-9). Additionally, DyLight Dye water solubility allows a high dye-to-protein ratio without precipitation during conjugation.

Highlights:

- Available conjugated to commonly used secondary antibodies, streptavidin and NeutrAvidin Protein; conjugated using a molar ratio (dye:protein) optimized to provide excellent fluorescent intensity
- Stable for 1 year at 4°C
- Antibody conjugates are affinity-purified to minimize cross-reactivity
- Superior photostability
- pH-insensitive (pH 4-9)
- High water solubility
- Compatible with common fluorescence instrumentation

Table 4. Spectral properties of Thermo Scientific DyLight Fluorescent Dyes.

Emission	Color	Thermo Scientific DyLight Dye	Ex/Em*	ε†	Spectrally Similar Dyes
Green		488	493/518	70,000	Alexa Fluor 488, fluorescein and FITC
Yellow		549	550/568	150,000	Alexa Fluor 546, Alexa Fluor 555, Cy3 and TRITC
Red		649	646/674	250,000	Alexa Fluor 647 and Cy5
Near Infrared		680	682/715	140,000	Alexa Fluor 680 and Cy5.5
		800	770/794	270,000	IRDye 800

*Excitation and emission maxima in nanometers
†Molar extinction coefficient (M^{-1} cm^{-1})

Ordering Information

Conjugates: *Package size for these items is 1 mg at 1 mg/ml.*

	Product #				
Description	DyLight 488 Dye	DyLight 549 Dye	DyLight 649 Dye	DyLight 680 Dye	DyLight 800 Dye
Goat Anti-Mouse IgG (H+L)	35502	35507	35515	35518	35521
Goat Anti-Rabbit IgG (H+L)	35552	35557	35565	35568	35571
Streptavidin	21832/	21837	21845	21848	21851
NeutrAvidin Protein	22832	22837	22845	22848	22853

Ordering Information

Specificity	Host	Product # / Pkg. Size Unconj.	Biotin-LC	Peroxidase	Alk. Phos.	Fluor Labeled
Anti-Chicken						
Chicken IgY (H+L)	Rabbit	**31104:** 2 mg $123	**31720:** 1.5 ml	**31401:** 1.5 ml $146		**31501:** Fluorescein, 1.5 mg $113
Anti-Goat						
Goat IgG (H+L) (min x HnMsRb Sr Prot)*	Mouse	**31107:** 1.5 mg $127	**31730:** 1 ml	**31400:** 1 ml $162		**31512:** Fluorescein, 1 mg $126 **31620:** Rhodamine, 1 mg $103 **31940:** Texas Red®, 1 mg $122
Goat IgG (H+L)	Rabbit	**31105:** 2 mg $115	**31732:** 1.5 mg	**31402:** 1.5 ml $136	**31300:** 1 ml	**31509:** Fluorescein, 1.5 mg $107 **31650:** Rhodamine, 1.5 mg $106 **31492:** Texas Red, 1.5 mg $102
Goat IgG [F(ab')$_2$]	Rabbit	**31153:** 2 mg $130	**31753:** 1.5 ml	**31403:** 1.5 ml $162	**31405:** 1 ml	**31553:** Fluorescein, 1.5 mg $125
Goat IgG (Fc)	Rabbit	**31133:** 2 mg $123	**31733:** 1.5 ml	**31433:** 1.5 ml $157	**31337:** 1 ml	**31533:** Fluorescein, 1.5 mg $122
Anti-Goat F(ab')$_2$ Fragment of Host Antibody						
Goat IgG (H+L) (min x Hn Sr Prot)	Rabbit	**31109:** 0.5 mg $122			**31302:** 0.5 ml	
Goat IgG (H+L) MsRbRt Sr Prot)	Donkey					**31860:** R-Phycoerythrin, 1 ml $201 **31980:** Allophycocyanin, 0.5 ml $193
Anti-Hamster						
Hamster IgG (H+L)	Goat	**31115:** 1.5 mg $100	**31750:** 1.5 mg			
Hamster IgG (H+L)	Rabbit	**31120:** 2 mg $82				**31587:** Fluorescein, 1.5 mg $99 **31652:** Rhodamine, 1.5 mg $93
Anti-Horse						
Horse IgG (H+L)	Goat	**31116:** 2 mg $82	**31760:** 1.5 mg			
Anti-Human						
Human IgG (H+L)	Goat	**31130:** 2 mg $88	**31770:** 1.5 mg	**31410:** 2 ml $126	**31310:** 1 ml	**31529:** Fluorescein, 2 mg $94 **31656:** Rhodamine, 2 mg $100 **31943:** Texas Red, 2 mg $102
Human IgG Gamma Chain Specific	Goat	**31118:** 0.5 mg $82				
Human IgG (H+L) (min x BvHsMs Sr Prot)*	Goat	**31119:** 1.5 mg $90	**31774:** 1.5 ml	**31412:** 1.5 ml $129		**31531:** Fluorescein, 1.5 mg $88 **31683:** Rhodamine, 1.5 mg $103 **31944**: Texas Red, 1.5 mg $99
Human IgG [F(ab')$_2$]	Goat	**31122:** 2 mg $108		**31482:** 2 ml $152	**31312:** 1 ml	**31628:** Fluorescein, 2 mg $115 **31684**: Rhodamine, 2 mg $112 **31945**: Texas Red, 2 mg $128
Human IgG [F(ab')$_2$] (min x BvHsMs Sr Prot)*	Goat	**31132:** 1.5 mg $100		**31414:** 1.5 ml $165		
Human IgM (Fc5µ)	Goat	**31136:** 2 mg $98		**31415:** 2 ml $164		**31575:** Fluorescein, 2 mg $128
Human IgM (µ)	Goat	**31124:** 0.5 mg $96	**31778:** 0.5 mg			
Human IgA (α)	Goat	**31140:** 2 mg $102		**31417:** 2 ml $149	**31314:** 1 ml	**31577:** Fluorescein, 2 mg $101
Human IgA + IgG + IgM (H+L)	Goat	**31128:** 2 mg $118	**31782:** 2 ml	**31418:** 2 ml $151	**31316:** 1 ml	
Human Kappa Chain	Goat	**31129:** 0.5 mg $91	**31780:** 0.5 mg			
Human Lambda Chain	Goat	**31131:** 0.5 mg $96				
Human IgG (H+L) (min x Ms Sr Prot)*	Mouse	**31135:** 2 mg $117		**31420:** 1.5 ml $129		
Human IgG (H+L) (min x BvHsMs Sr Prot)*	Mouse	**31137:** 1.5 mg $111	**31784:** 1 ml			
Human IgG (H+L)	Rabbit	**31143:** 2 mg $106	**31786:** 1.5 ml			
Human IgG (Fc)	Rabbit	**31142:** 2 mg $108	**31789:** 1.5 ml	**31423:** 1.5 ml $164	**31318:** 1 ml	**31535:** Fluorescein, 1.5 mg $112

** See the key to abbreviations on the following page*

Primary and Secondary Antibodies

Ordering Information

Specificity	Host	Product # / Pkg. Size Unconj.	Biotin-LC	Peroxidase	Alk. Phos.	Fluor Labeled
Anti-Human F(ab')$_2$ Fragment of Host Antibody						
Human IgG (Fc)	Goat	**31163:** 1 mg $126				
Human IgG (H+L)	Goat	**31165:** 1 mg $90				
Human IgA + IgG + IgM (H+L)	Goat					**31539:** Fluorescein, 1 mg $118
Anti-Mouse						
Mouse IgA (α) (min x Hn Sr Prot)	Goat	**31169:** 1 mg $153				
Mouse IgA + IgG + IgM (H+L)	Goat	**31171:** 2 mg $150				
Mouse IgG (H+L)	Goat	**31160:** 2 mg $91	**31800:** 2 ml	**31430:** 2 ml $141	**31320:** 1 ml	**31569:** Fluorescein, 2 mg $104 **31660:** Rhodamine, 2 mg $108 **31498:** Texas Red, 2 mg $103 **35502** DyLight 488, 1 mg $128 **35507:** DyLight 549, 1 mg $128 **35515:** DyLight 649, 1 mg $128 **35518:** DyLight 680, 1 mg $128 **35521:** DyLight 800, 1 mg $128
Mouse IgG (H+L) (min x BvHnHs Sr Prot)*	Goat	**31164:** 1.5 mg $86	**31802:** 1.5 mg	**31432:** 1.5 ml $139	**31322:** 1 ml	**31541:** Fluorescein, 1.5 mg $108 **31661:** Rhodamine, 1.5 mg $106 **31500:** Texas Red, 1.5 mg $117
Mouse IgG [F(ab')$_2$]	Goat	**31166:** 2 mg $118	**31803:** 2 ml	**31436:** 2 ml $152	**31324:** 1 ml	**31543:** Fluorescein, 2 mg $123
Mouse IgG (Fc)	Goat	**31168:** 2 mg $106	**31805:** 2 ml	**31437:** 2 ml $161	**31325:** 1 ml	**31547:** Fluorescein, 2 mg $123 **31663:** Rhodamine, 2 mg $118
Mouse IgG (Fc) (min x BvHnHs Sr Prot)*	Goat	**31170:** 1.5 mg $102		**31439:** 1.5 ml $179	**31327:** 1 ml	
Mouse IgM (μ)	Goat	**31172:** 2 mg $124	**31804:** 0.5 mg	**31440:** 2 ml $181	**31326:** 1 ml	**31992:** Fluorescein, 2 mg $149 **31662:** Rhodamine, 2 mg $148
Mouse IgG + IgM (H+L)	Goat	**31182:** 2 mg $97	**31807:** 2 ml	**31444:** 2 ml $146	**31328:** 1 ml	
Mouse IgG + IgM (H+L) (min x BvHnHs Sr Prot)*	Goat	**31184:** 1.5 mg $116		**31446:** 1.5 ml $179	**31330:** 1 ml	**31586:** Fluorescein, 1.5 mg $137
Mouse IgG (Fcγ) (subclasses 1+2a+2b+3) (min x BvHnRb Sr Prot)*	Goat	**31232:** 1 mg $102				**31630:** Fluorescein, 1 mg $136 **31861:** R-Phycoerythrin, 1 ml $201 **31981:** Allophycocyanin, 0.5 ml $193
Mouse IgG (Fcγ) subclass 1 specific (min x BvHnRb Sr Prot)*	Goat	**31236:** 1 mg $142				**31632:** Fluorescein, 0.5 mg $159 **31862:** R-Phycoerythrin, 0.5 ml $193 **31982:** Allophycocyanin, 0.3 ml $187
Mouse IgG (Fcγ) subclass 2a specific (min x BvHnRb Sr Prot)*	Goat	**31237:** 1 mg $142				**31634:** Fluorescein, 0.5 mg $159 **31863:** R-Phycoerythrin, 0.5 ml $187 **31983:** Allophycocyanin, 0.3 ml $187
Mouse IgG (H+L)	Horse	**31181:** 1.5 mg $95	**31806:** 1.5 mg			
Mouse IgG (H+L)	Rabbit	**31188:** 2 mg $113	**31810:** 1.5 ml	**31450:** 1.5 ml $143	**31329:** 1 ml	**31561:** Fluorescein, 1.5 mg $94 **31665:** Rhodamine, 1.5 mg $88 **31610:** Texas Red, 1.5 mg $103
Mouse IgG (H+L) (min x Hn Sr Prot)*	Rabbit	**31190:** 1.5 mg $106	**31812:** 1 ml	**31452:** 1 ml $150	**31334:** 0.5 ml	
Mouse IgG [F(ab')$_2$]	Rabbit	**31192:** 2 mg $112	**31811:** 1.5 ml	**31451:** 1.5 ml $175	**31331:** 1 ml	**31559:** Fluorescein, 1.5 mg $112 **31666:** Rhodamine, 1.5 mg $112
Mouse IgG (Fc)	Rabbit	**31194:** 2 mg $113	**31813:** 1.5 ml	**31455:** 1.5 ml $169	**31332:** 1 ml	**31555:** Fluorescein, 1.5 mg $106
Mouse IgM (μ)	Rabbit	**31196:** 2 mg $140	**31814:** 1.5 ml	**31456:** 1.5 ml $183	**31333:** 1 ml	**31557:** Fluorescein, 1.5 mg $133
Mouse IgG + IgM (H+L)	Rabbit	**31198:** 2 mg $133	**31815:** 1.5 ml	**31457:** 1.5 ml $156	**31335:** 1 ml	**31558:** Fluorescein, 1.5 mg $125

* Key to abbreviations for individual species.
Bv = Bovine Ch = Chicken Gt = Goat Gu = Guinea Pig Ha = Hamster Hs = Horse Hn = Human Ms = Mouse Rb = Rabbit Rt = Rat Sh = Sheep Sw = Swine

Ordering Information

Specificity	Host	Product # / Pkg. Size Unconj.	Biotin-LC	Peroxidase	Alk. Phos.	Fluor Labeled
Anti-Mouse F(ab')$_2$ Fragment of Host Antibody						
Mouse IgG (H+L) (min x BvHnHs Sr Prot)*	Goat	**31185:** 1 mg $127		**31438:** 0.5 ml $161		**31565:** Fluorescein, 1 mg $150
Mouse IgM (µ)	Goat	**31178:** 1 mg $158				
Mouse IgM (µ) (min x BvHnHs Sr Prot)*	Goat	**31186:** 1 mg $160		**31442:** 0.5 ml $173		
Mouse IgG + IgM (H+L) (min x BvHnHs Sr Prot)*	Goat			**31448:** 0.5 ml $143		
Anti-Rabbit						
Rabbit IgG (H+L) (min x BvChGtGuHaHn HsMsRtSh Sr Prot)*	Donkey	**31238:** 1 mg $95	**31821:** 0.5 ml	**31458:** 0.5 ml $124	**31345:** 0.5 ml	**31568:** Fluorescein, 0.5 mg $100 **31685:** Rhodamine, 0.5 mg $102 **31504:** Texas Red, 0.5 mg $116
Rabbit IgG (H+L)	Goat	**31210:** 2 mg $89	**31820:** 1.5 mg	**31460:** 2 ml $146	**31340:** 1 ml	**31635:** Fluorescein, 2 mg $105 **31670:** Rhodamine, 2 mg $122 **31506:** Texas Red, 2 mg $103 **35552:** DyLight 488, 1 mg $128 **35557:** DyLight 549, 1 mg $128 **35565:** DyLight 649, 1 mg $128 **35568:** DyLight 680, 1 mg $128 **35571:** DyLight 800, 1 mg $128
Rabbit IgG (H+L) (min x Hn Sr Prot)*	Goat	**31212:** 1.5 mg $101	**31822:** 1.5 ml	**31462:** 1.5 ml $175	**31342:** 1 ml	**31583:** Fluorescein, 1.5 mg $115 **31686:** Rhodamine, 1.5 mg $102 **31507:** Texas Red, 1.5 mg $113
Rabbit IgG [F(ab')$_2$]	Goat	**31234:** 2 mg $88	**31823:** 2 ml	**31461:** 2 ml $161	**31343:** 1 ml	**31573:** Fluorescein, 2 mg $123
Rabbit IgG (Fc)	Goat	**31216:** 2 mg $101		**31463:** 2 ml $162	**31341:** 1 ml	
Rabbit IgG (H+L) (min x GtHnMsSh Sr Prot)*	Mouse	**31213:** 1.5 mg $124	**31824:** 1 ml	**31464:** 1 ml $161		**31584:** Fluorescein, 1 mg $126 **31674:** Rhodamine, 1 mg $123
Anti-Rabbit F(ab')$_2$ Fragment of Host Antibody						
Rabbit IgG (H+L)	Goat	**31214:** 1 mg $95				**31579:** Fluorescein, 1 mg $129
Rabbit IgG (H+L) (min x HnMsRt Sr Prot)*	Goat	**31239:** 1 mg $132				**31636:** Fluorescein, 1 mg $151 **31864:** R-Phycoerythrin, 1 ml $203 **31984:** Allophycocyanin, 0.5 ml $193
Anti-Rat						
Rat IgG (H+L)	Goat	**31220:** 2 mg $101	**31830:** 2 ml	**31470:** 2 ml $122	**31350:** 1 ml	**31629:** Fluorescein, 2 mg $101 **31680:** Rhodamine, 2 mg $101 **31508:** Texas Red, 2 mg $103
Rat IgG [F(ab')$_2$]	Goat			**31474:** 2 ml $137		
Rat IgG (Fc)	Goat	**31226:** 2 mg $99	**31833:** 2 ml	**31475:** 2 ml $149	**31353:** 1 ml	**31621:** Fluorescein, 2 mg $108
Rat IgM (µ)	Goat	**31228:** 2 mg $137	**31832:** 2 ml	**31476:** 2 ml $204	**31354:** 1 ml	**31631:** Fluorescein, 2 mg $147
Rat IgG (H+L)	Rabbit	**31218:** 2 mg $103	**31834:** 1.5 mg			
Rat IgG (H+L) (min x Ms Sr Prot)*	Rabbit	**31219:** 0.5 mg $133	**31836:** 0.5 mg			
Anti-Sheep						
Sheep IgG (H+L)	Rabbit	**31240:** 2 mg $118	**31840:** 1.5 mg	**31480:** 1.5 ml $136	**31360:** 1 ml	**31627:** Fluorescein, 1.5 mg $95

* Key to abbreviations for individual species.
Bv = Bovine Ch = Chicken Gt = Goat Gu = Guinea Pig Ha = Hamster Hs = Horse Hn = Human Ms = Mouse Rb = Rabbit Rt = Rat Sh = Sheep Sw = Swine

Thermo Scientific Antibody Binding Proteins

Protein A

Binds specifically to the Fc region of immunoglobulin molecules, especially IgG.

Highlights:

- Isolated from native *Staphylococcus aureus* (MW = 42K)
- Contains four IgG-binding sites

Ordering Information

Product #	Description	Pkg. Size	U.S. Price
21181	Protein A	5 mg	$121
29989	Biotinylated Protein A	1 mg	$ 62

Protein A, Recombinant

No enterotoxins present, as there may be from Staphylococcus-*derived Protein A.*

Highlights:

- Harvested from a nonpathogenic form of *Bacillus*, which has been genetically designed to manufacture and secrete carboxy terminus truncated (MW ~ 44.6K) recombinant Protein A

Ordering Information

Product #	Description	Pkg. Size	U.S. Price
21184	Purified Protein A	5 mg	$110
32400	Pierce Purified Recombinant Protein A, Peroxidase Conjugated	1 mg	$114

Protein G, Recombinant

Useful for a variety of immunological and biochemical techniques.

Highlights:

- Protein G is a bacterial cell wall protein isolated from group G *Streptococci* (MW = 22K)
- Binds to most mammalian immunoglobulins through their Fc regions
- Albumin and cell surface binding sites have been removed from this recombinant form to reduce nonspecific binding when Protein G is used to purify, identify or locate immunoglobulins
- Useful for separating albumin from crude human or mouse IgG samples
- Binds with greater affinity to most mammalian immunoglobulins than Protein A, including human IgG_3 and rat IgG_{2a}
- Does not bind to human IgM, IgD and IgA

Ordering Information

Product #	Description	Pkg. Size	U.S. Price
21193	Pierce Purified Recombinant Protein G	5 mg	$282
29988	Biotinylated Protein G	0.5 mg	$145
31499	Protein G, Peroxidase Conjugated	0.5 mg	$122

Protein A/G, Recombinant

Produced by gene fusion of the Fc binding domains of Protein A and Protein G.

Highlights:

- Protein A/G is a 50,449 dalton protein containing 442 amino acids, 43 of which are lysines
- Binds well to immunoglobulins over a broad pH range (pH 4-9)
- Contains four Protein A Fc binding domains and two Protein G Fc binding domains
- Binds all IgG subclasses of mouse immunoglobulins, making it an excellent tool for purification and detection of mouse monoclonal antibodies

Ordering Information

Product #	Description	Pkg. Size
21186	Pierce Purified Recombinant Protein A/G	5 mg
32391	Protein A/G, Alkaline Phosphatase Conjugated	0.5 mg
32490	Protein A/G, Peroxidase Conjugated	0.5 mg

Protein L, Recombinant

Binds a wider range of Ig classes and subclasses, including all classes of IgG and single chain variable (ScFv) and Fab fragments.

Highlights:

- Protein L is an immunoglobulin-binding protein that was originally derived from the bacteria *Peptostreptococcus magnus* but now is produced recombinantly in *E. coli*
- Has the unique ability to bind through kappa light chain interactions, including kappa I, III and IV in human and kappa I in mouse, without interfering with an antibody's antigen-binding site

Ordering Information

Product #	Description	Pkg. Size
21189	Pierce Purified Recombinant Protein, Lyophilized	1 mg
32420	Protein L, Peroxidase Conjugated	0.5 mg
29997	Biotinylated Protein L	0.5 mg

Thermo Scientific Avidin-Biotin Products

The interaction between biotin (a vitamin) and avidin (hen egg white protein) has been exploited to produce a variety of applications. The noncovalent, high affinity of biotin for avidin ($K_a = 10^{15}$ M^{-1}) has allowed us to create a line of products that can help you develop nonradioactive assay systems. With four biotin-binding sites per avidin molecule, this system allows more signal to be concentrated at the detection site. A similar assay scenario can be developed for DNA or RNA hybridization assays when a probe is biotinylated instead of an antibody. Below are just a few of the applications exploiting the avidin-biotin interaction even beyond assay development.

- ELISA
- Immunohistochemical staining
- Western blotting
- DNA hybridization assays
- Immunoprecipitation
- Affinity chromatography
- Fluorescent activated cell sorting (FACS)

Comparison of Thermo Scientific NeutrAvidin Biotin-Binding Protein, Avidin and Streptavidin

Protein	MW	pI	Carbohydrate
Thermo Scientific NeutrAvidin Biotin-Binding Protein	60 kDa	6.3	No
Thermo Scientific Streptavidin	53 kDa	6.8-7.5	No
Thermo Scientific Avidin	67 kDa	10	Yes

Thermo Scientific NeutrAvidin Products

For ultralow nonspecific binding compared to avidin or streptavidin!

Achieve better assay results with the low nonspecific binding properties of NeutrAvidin Protein. NeutrAvidin Biotin-Binding Protein is a deglycosylated form of avidin, so lectin binding is reduced to undetectable levels without losing biotin-binding affinity ($K_a = 10^{15}$ M^{-1}).[1] NeutrAvidin Biotin-Binding Protein offers the advantage of a neutral pI to minimize nonspecific adsorption, along with lysine residues that remain available for derivatization or conjugation through amine-reactive chemistries. The molecular weight of NeutrAvidin Biotin-Binding Protein is approximately 60K. The specific activity for biotin-binding is approximately 14 µg/mg of protein, which is near the theoretical maximum activity.

Highlights:

- Near-neutral pI (6.3) and no glycosylation, unlike avidin
- No RYD recognition sequence like streptavidin
- Generally lower nonspecific binding than avidin and streptavidin
- Much lower price than streptavidin

References

Hiller, Y., *et al.* (1987). *Biochem. J.* **248**, 167-171.
Unson, M.D., *et al.* (1999). *J. Clin. Microbiol.* **37**, 2153-2157.
Wojciechowski, M., *et al.* (1999). *Clin. Chem.* **45**, 1690-1693.
Glover, B.P. and McHenry, C.S. (2001). *Cell* **105**, 925-934.
Guo, Y., *et al.* (2001). *J. Biol. Chem.* **276**, 45791-45799.
Claypool, S.M., *et al.* (2002). *J. Biol. Chem.* **27**, 28038-28050.

Ordering Information

Product #	Description	Features	Pkg. Size
31000	**NeutrAvidin Biotin-Binding Protein**	• pI that has been reduced to a neutral state • Deglycosylated, so lectin binding is reduced to undetectable levels • Can be used as a biotin blocking agent in tissues for histochemistry • 11-17 µg biotin bound/mg NeutrAvidin Protein	10 mg
31001	**NeutrAvidin Horseradish Peroxidase Conjugated**	• Better signal-to-noise ratio in assay systems • 1-2 moles HRP/mole NeutrAvidin Protein • 3-8 µg biotin bound/mg conjugate	2 mg
31002	**NeutrAvidin Alkaline Phosphatase Conjugated**	• Lower nonspecific binding than streptavidin conjugates • Better signal-to-noise ratio in assay systems • 3-8 µg biotin bound/mg conjugate	2 mg
31006	**NeutrAvidin Fluorescein Conjugated**	• Fluorescent-labeled NeutrAvidin Biotin-Binding Protein • Absorption: 490 nm; Emission 520 nm • ≥ 2 moles fluorescein/mole NeutrAvidin Protein	5 mg
31007	**EZ-Link Maleimide Activated NeutrAvidin Biotin-Binding Protein**	• Prepare NeutrAvidin conjugates of proteins/peptides • Reacts spontaneously with free sulfhydryls in the pH range of 6.5-7.5 • 4-8 moles maleimide/mole NeutrAvidin Protein	5 mg

Thermo Scientific Streptavidin Products

Wide selection of conjugates for almost any biotin-based assay.

Originally isolated from *Streptomyces avidinii*, streptavidin is a tetrameric biotin-binding protein that we produce and offer in recombinant form. Compared to the native protein, recombinant streptavidin is smaller that the native protein (MW 53K) and has a more neutral isoelectric point (pI 6.8-7.5). Streptavidin is carbohydrate-free and much less soluble in water than avidin, resulting in high binding affinity, capacity and specificity for biotinylated molecules. Streptavidin conjugates are useful for secondary detection in Western blotting, ELISA, and cell and tissue staining.

Ordering Information

Product #	Description	Features	Applications	Pkg. Size
21122	Streptavidin	• Lyophilized, stable powder • No carbohydrate	• Immunoassay reagent when bound to biotinylated enzymes or when conjugated to enzymes	1 mg
21125	Streptavidin	• Much less soluble in water than avidin • 13-22 µg biotin bound/mg of protein • Recombinant	• Blocking protein for biotin-rich tissue sections (use at 0.1% for inhibition of endogenous biotin) • Can be used with biotinylated enzymes (Product # 29339 or 29139)	5 mg
21126	Horseradish Peroxidase Conjugated	• 1-2 moles HRP/mole streptavidin	• Histochemistry	1 mg
21124	Horseradish Peroxidase Conjugated	• ≥ 100 peroxidase units/mg conjugate	• Western blotting	2 mg
21127	Horseradish Peroxidase Conjugated	• Lyophilized, stable powder • 6-9 µg biotin bound/mg conjugate	• Conti, L.R., *et al.* (2001). *J. Biol. Chem.* **276**, 41270-41278.	5 mg
21324	Alkaline Phosphatase Conjugated	• ≥ 3 µg biotin bound/mg conjugate	• Histochemistry	1 mg
21323	Alkaline Phosphatase Conjugated	• ≥ 100 phosphatase units/mg conjugate	• Western blotting • Harriman, G.R., *et al.* (1999). *J. Immunol.* **162**, 2521-2529. • Nielsen, P.K., *et al.* (2000). *J. Biol. Chem.* **275**, 14517-14523.	3 mg
21224	Fluorescein (FITC) Conjugated	• Fluorescently labeled streptavidin • Ex/Em: 490 nm and 520 nm • 3-5 moles FITC/mole streptavidin	• Histochemical staining • Fluorescence-activated cell sorting (FACS)	1 mg
21724	Rhodamine (TRITC) Conjugated	• Fluorescently labeled streptavidin • Excitation: 515-520 nm and 550-555 nm • Emission: 575 nm • 1-3 moles TRITC/mole streptavidin	• Histochemical staining • Fluorescence-activated cell sorting (FACS)	1 mg
21624	Texas Red Conjugated	• Fluorescently labeled streptavidin • Ex/Em: 595 nm and 615 nm	• Histochemical staining; can be used in double staining methods • Fluorescence-activated cell sorting (FACS)	1 mg
21627	R-Phycoerythrin Conjugated	• Fluorescently labeled streptavidin • Ex/Em: 480, 545 and 565 nm and 578 nm	• Histochemical staining • Fluorescence-activated cell sorting (FACS)	1 ml
21629	Allophycocyanin Conjugated	• Fluorescently labeled streptavidin • Ex/Em: 650 nm and 660 nm	• Histochemical staining • Fluorescence-activated cell sorting (FACS)	0.5 ml
21424	DyLight 547 Conjugated	• Fluorescently labeled streptavidin • Ex/Em: 557 nm and 574 nm	• Histochemical staining • Fluorescence-activated cell sorting (FACS) • Western blotting • ELISA	1 mg
21824	DyLight 647 Conjugated	• Fluorescently labeled streptavidin • Ex/Em: 652 nm and 673 nm	• Histochemical staining • Fluorescence-activated cell sorting (FACS) • Western blotting • ELISA	1 mg
21120	Hydrazide Activated	• Attaches streptavidin to oxidized carbohydrate residues on glycoproteins • ≥ 4 moles hydrazide/mole streptavidin	• Used to create immunoassay reagents • Localize glycoproteins on blot transfers, followed by detection with a biotinylated enzyme	2 mg

Thermo Scientific Avidin Products

Convenient conjugates for assay detection.

Avidin is a tetrameric glycoprotein (MW 67K) purified from chicken egg white. The highly specific interaction of avidin with biotin makes it a useful tool in designing nonradioactive detection systems. The extraordinary affinity of avidin for biotin ($K_a = 10^{15}\ M^{-1}$) allows biotin-labeled molecules to be detected with excellent sensitivity and specificity.

Avidin is more soluble than streptavidin and has an isoelectric point (pI) of 10.5. It is also more economical than streptavidin, and is commonly used in signal amplification systems such as the ABC system.

References

Chaiet, I. and Wolf, F.J. (1964). *Arch. Biochem. Biophys.* **106**, 1-5.
Savage, M.D., *et al.* (1992). *Avidin-Biotin Chemistry: A Handbook.* Rockford, Illinois: Pierce Chemical Company.
Wilchek, M. and Bayer, E.A. (1983). *Anal. Biochem.* **171**, 1-32.
Gitlin, G., *et al.* (1987). *Biochem. J.* **242**, 923-926.
Bruch, R.C. and White, III, H.B. (1982). *Biochemistry* **21**, 5334-5341.
Zuk, P.A. and Elferink, L.A. (2000). *J. Biol. Chem.* **275**, 26754-26764.

Ordering Information

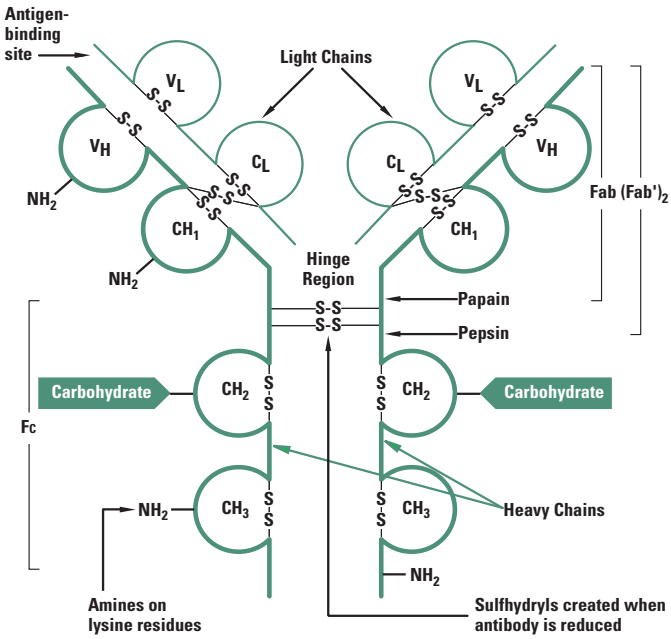
Product #	Description	Features	Applications	Pkg. Size
21121	**Avidin**	• Hen egg white glycoprotein, affinity-purified, salt-free, lyophilized powder • 11-14 µg biotin bound/mg avidin • Isoelectric point of 10-10.5 • Stable over a wide range of pH and temperatures	• Immunoassay reagent when bound to biotinylated enzymes or when conjugated to enzymes • Blocking protein for biotin-rich tissue sections (use at 0.1% for inhibition of endogenous biotin)	10 mg
21128	**Avidin**			20 mg
21123	**Horseradish Peroxidase Conjugated**	• Purified using special affinity techniques to eliminate nucleic acids • 1-2 moles HRP/mole avidin • 5-10 µg biotin bound/mg protein • ≥ 80 peroxidase units/mg protein	• Use in immunohistochemistry where endogenous phosphatase is a problem • Western blotting	2 mg
29994	**Horseradish Peroxidase Conjugated**			5 mg
21321	**Alkaline Phosphatase Conjugated**	• Homogeneous by SDS-PAGE • Purified using special affinity techniques to eliminate nucleic acids • ~1 mole alkaline phosphatase/mole avidin • One unit = 1.0 micromole of *p*-nitrophenol liberated from *p*-nitrophenylphosphate per minute at 37°C, pH 9.5	• Use for immunohistochemistry where high levels of endogenous peroxidase is a problem • Western blotting • ELISA	100 units
21221	**Fluorescein (FITC) Conjugated**	• Fluorescent-labeled avidin • Ex/Em: 490 nm and 52 nm • No free fluorescein • ~3.5 moles fluorescein/mole avidin	• Fluorescence-activated cell sorting (FACS) • Histochemical staining	5 mg
21021	**R-Phycoerythrin Conjugated**	• Fluorescent-labeled avidin • Ex/Em: 450-570 nm and 574 nm	• Fluorescence-activated cell sorting (FACS) • Histochemical staining	1 mg

Label Your Own Antibodies

Antibody Modification Sites Antibodies can be easily modified to contain labels such as biotin, fluorescent tags or enzymes to create reagents for Western blotting, ELISA, immunohistochemical staining and *in vivo* targeting. We offer tools for a variety of antibody modification strategies.

Most antibody labeling methods involve one of four common target strategies. The most common target is primary amines ($-NH_2$), which are primarily on lysine residues. Primary amines are abundant, widely distributed and easily modified because of their reactivity and location on the antibody surface. The second most common target are sulfhydryls, which are generated by reducing disulfide bonds. These disulfide bonds join heavy and light polypeptide chains together in a manner that ensures proper antibody structure and antigen-binding function. Therefore, complete reduction of antibody disulfides by treatment with reducing agents will usually inactivate the antibody. However, with the proper conditions, it is possible to selectively reduce only the more labile disulfides between heavy chains in the hinge region of IgG molecules; the result is functional half-antibodies with sulfhydryls available for labeling. Such partial reduction of antibody disulfides usually results in sulfhydryl group labeling points that will not sterically hinder antigen binding.

Two other common targets are carboxyls, present on glutamic and aspartic acid residues, and carbohydrates. Carboxyls are abundant and easily accessible, but they do not react as readily as amines and coupling to them requires the crosslinker EDC. Carbohydrate moieties are present on the Fc portion of most polyclonal antibodies. This region usually can be labeled without altering antibody activity. Labeling carbohydrates is a two-step process because the carbohydrates must first be oxidized to create reactive aldehydes.



Functional groups available on an antibody for labeling.

Primary amines ($-NH_2$) are on lysine residues and the N-terminus. These are abundant and distributed over the entire antibody.

Sulfhydryl groups (–SH) are on cysteine residues and are formed by selectively reducing disulfide bonds in the hinge region of the antibody.

Carbohydrate residues containing *cis*-diols can be oxidized (–CHO) to create active aldehydes. These are localized to the Fc region on antibodies and are more abundant on polyclonal antibodies.

Thermo Scientific Pierce Horseradish Peroxidase (HRP)

Its higher specific enzyme activity makes it the enzyme of choice.

Highlights:

- Superior to alkaline phosphatase and β-galactosidase conjugates because of its higher specific enzyme activity
- Small size (40 kDa) allows excellent cellular penetration
- Variety of substrates available
- Ideal in blotting and cytochemistry applications
- Used as the reporter enzyme for SuperSignal Chemiluminescent Substrates

References

Cordell, J.L., *et al.* (1984). *J. Histochem. Cytochem.* **32**, 219-229.
Hosoda, H., *et al.* (1987). *Chem. Pharm. Bull.* **35**, 3336-3342.
Passey, R.B., *et al.* (1977). *Clin. Chem.* **23(1)**, 131-139.
Porstmann, B., *et al.* (1985). *J. Immunol. Methods.* **79**, 27-37.
Samoszuk, M.K., *et al.* (1989). *Antibody, Immunoconjugates and Radiopharmaceuticals* **2**, 37-46.
Wordinger, R.J., *et al.* (1987). Manual of Immunoperoxidase Techniques, 2nd Edition. Chicago: American Society of Clinical Pathologists Press, pp. 23-24.
Yolken, R.H. (1982). *Rev. Infect. Dis.* **4(1)**, 35-68.

Thermo Scientific Pierce Alkaline Phosphatase (AP)

A highly sensitive enzyme for ELISA and immunohistochemical applications.

Highlights:

- Purified form – ready to conjugate without prior dialysis
- Activity is not affected by exposure to antibacterial agents, such as sodium azide or thimerosal
- Specific activity > 2,000 units/mg
- One unit is defined as the amount that will hydrolyze 1.0 µmol of *p*-nitrophenyl phosphate per minute at 37°C in 1.0 M diethanolamine, 0.5 mM $MgCl_2$, pH 7.8

Specific Activity per mg Protein

Buffer	25°C	37°C
0.1 M Glycine, 1.0 mM $ZnCl_2$, 1.0 mM $MgCl_2$, 6.0 mM *p*-Nitrophenyl phosphate, pH 10.4	> 500	> 1,000
1.0 M Diethanolamine, 0.5 mM $MgCl_2$, 15 mM *p*-Nitrophenyl phosphate, pH 9.8	> 1,000	> 2,000

References

Bulman, A.S. and Heyderman, E. (1981). *J. Clin. Pathol.* **34**, 1349-1351.
Cordell, J.L., *et al.* (1984). *J. Histochem. Cytochem.* **32**, 219-229.
Yolken, R.H. (1982). *Rev. Infect. Dis.* **4**, 35-68.

Ordering Information

Product #	Description	Pkg. Size
31490	Pierce Horseradish Peroxidase	10 mg
31491	Pierce Horseradish Peroxidase	100 mg
31391	Pierce Alkaline Phosphatase Calf intestinal. Supplied in Tris Buffer, pH ~7 Triethanolamine, 1 mM $MgCl_2$, 3 M NaCl, pH 7.6	20 mg
31392	Pierce Alkaline Phosphatase	100 mg

Label Your Own Antibodies

Thermo Scientific EZ-Link Enzyme Labeling Kits and Reagents

EZ-Link Pre-Activated Enzymes make it easy to convert almost any protein to a detection reagent. Simple protocols with each EZ-Link Kit or Activated Enzyme turn a chemical chore into a painless process.

Horseradish peroxidase (HRP) and alkaline phosphatase (AP) are the two most common enzymes used for immunoassay detection systems. Both of these enzymes catalyze reactions with substrates to form soluble color responses or colored precipitates, or to generate the chemical emission of light (chemiluminescence). Enzyme conjugates make stable assay reagents and can be stored for long periods at -20°C.

In one hour at room temperature, you can conjugate:

- HRP to a primary amine group (–NH_2) with the EZ-Link Plus Activated Peroxidase Kit (see next page)
- AP to a free sulfhydryl (–SH) group using the EZ-Link Maleimide Activated AP Kit
- HRP to a free sulfhydryl (–SH) group with the EZ-Link Maleimide Activated HRP Kit

Thermo Scientific EZ-Link Maleimide Activated Alkaline Phosphatase and Horseradish Peroxidase

Make quick and easy enzyme conjugates.

The EZ-Link Maleimide Activated Enzyme Kits can be used to directly prepare antibody conjugates via sulfhydryl groups. These kits eliminate the first step of the two-step maleimide method, making conjugate preparation much simpler. The kit is supplied with a stable, preactivated AP or HRP.

The AP or HRP conjugates can be purified by gel filtration chromatography, ultrafiltration or dialysis, depending upon the size of the conjugated protein. Alternatively, use Pierce Conjugate Purification Kit (Product # 44920) to separate unreacted enzyme from the conjugate.

Highlights:

- Prepare HRP or AP conjugates from proteins that contain a free sulfhydryl
- Includes 2-mercaptoethylamine to generate free sulfhydryls from disulfide bonds
- Includes SATA to add free sulfhydryls to lysine residues

Reference

Ishikawa, E., *et al.* (1983). *J. Immunoassay* **4**, 209-327.
Hashida, S., *et al.* (1984). *J. Appl. Biochem.* **6**, 56-63.
Imagawa, M., *et al.* (1982). *J. Appl. Biochem.* **4**, 41-57.
O'Sullivan, M.J., *et al.* (1979). *Anal. Biochem.* **100**, 100-108.

EZ-Link Maleimide Activated Peroxidase References

Choi, J.Y., *et al.* (2002). *J. Biol. Chem.* **277**, 21630-21638.
Seo, Y.R., *et al.* (2002). *Proc. Natl. Acad. Sci. USA* **99**, 14548-14553.
Yoo, J.H., *et al.* (2004). *J. Biol. Chem.* **279**, 848-858.

Ordering Information

Product #	Description	Pkg. Size
31486	**EZ-Link Maleimide Activated Alkaline Phosphatase**	2 mg
31493	**EZ-Link Maleimide Activated Alkaline Phosphatase Kit**	Kit
	Includes: EZ-Link Maleimide Activated Alkaline Phosphatase	2 mg
	Activation/Conjugation Buffer	20 ml
	BupH Tris Buffered Saline Pack	2 packs
	BupH Phosphate Buffered Saline Pack	1 pack
	Polyacrylamide Desalting Column	1 x 10 ml
	Mercaptoethylamine•HCl	6 mg
	SATA	2 mg
	Hydroxylamine	5 mg
	DMF	1 ml
	Column Extender	
31485	**EZ-Link Maleimide Activated Horseradish Peroxidase**	5 mg
31494	**EZ-Link Maleimide Activated Horseradish Peroxidase Kit**	Kit
	Includes: EZ-Link Maleimide Activated Horseradish Peroxidase	5 mg
	Activated Horseradish Peroxidase Conjugation Buffer	20 ml
	2-Mercaptoethylamine•HCl	6 mg
	SATA	2 mg
	Dimethylformamide	1 ml
	Hydroxylamine•HCl	5 mg
	Polyacrylamide Desalting Column	1 x 10 ml

Thermo Scientific EZ-Link Plus Activated Peroxidase Kit

Amine-reactive HRP with the highest conjugation yields.

EZ-Link Plus Activated Peroxidase is an amine-reactive HRP derivative that provides coupling efficiencies of greater than 95%. Other amine-reactive chemistries, like glutaraldehyde, tend to polymerize and produce lower amounts of viable conjugate. Sugar residues present on HRP have been oxidized with periodate to produce aldehydes that react with primary amines.

EZ-Link Plus Activated Peroxidase is mixed with the protein to be coupled at a pH compatible with your protein or antibody. After incubating the reaction for one to two hours, the conjugate linkage is reduced and the activated HRP quenched with ethanolamine. The conjugate is purified by desalting or by dialysis. If you are preparing an antibody-HRP conjugate, use the Pierce Conjugate Purification Kit (Product # 44920) to separate unreacted enzyme from the conjugate.

Highlights:

- Reacts with readily accessible primary amines to form a covalent secondary amine bond
- Can be stored for at least 12 months at -20°C
- Consistent conjugation yields reliable conjugates
- Enzyme activity is 120-200 units/mg

Suggested Antibody-HRP Dilutions for Colorimetric Substrates

Technique	Working Range
Immunohistochemistry	1:10-1:100
Immunoblotting	1:2,000-1:10,000
EIA	1:5,000-1:20,000

Active Aldehyde Reaction Scheme

Thermo Scientific EZ-Link Activated Peroxidase and Antibody Labeling Kit

The easy way to make HRP-antibody conjugates.

Primary amines on the EZ-Link Activated HRP have been converted to active aldehydes. This chemistry can be used to couple HRP to primary amines on another protein such as an antibody. The EZ-Link Activated Peroxidase Kit is designed to purify HRP-antibody conjugates by using Immobilized Protein A/G to separate unreacted enzyme from the antibody conjugate. Using EZ-Link Activated HRP produces an HRP-antibody conjugate in which both the enzyme activity and the antigen-binding activity are preserved.

Highlights:

- Reacts with primary amines to form a covalent amide bond
- No reduction step is necessary to secure the linkage
- Can be stored for at least 12 months at -20°C
- One mg produces about 0.5 ml of conjugate with a working dilution of 1:1,000 when coupled to a high titer antibody
- Enzyme activity is > 200 units/mg

EZ-Link Plus Activated Peroxidase References

Glover, L., *et al.* (2002). *Eur. J. Biochem.* **269**, 4607-4616.
Nawa, M., *et al.* (2000). *Clin. Diagn. Lab. Immunol.* **7**, 774-777.
Völkel, T., *et al.* (2001). *Protein Eng.* **14**, 815-823.

EZ-Link Activated Peroxidase References

Sandt, C.H. and Hill, C.W. (2001). *Infect. Immun.* **69**, 7293-7303.
Turpin, E.A., *et al.* (2003). *J. Clin. Microbiol.* **41**, 3579-3583.

Ordering Information

Product #	Description	Pkg. Size
31487	EZ-Link Plus Activated Peroxidase (Periodate Activated)	1 mg
31488	EZ-Link Plus Activated Peroxidase (Periodate Activated)	5 x 1 mg
31489	EZ-Link Plus Activated Peroxidase Kit (Periodate Activated)	Kit
	Includes: EZ-Link Plus Activated Peroxidase	5 x 1 mg
	Sodium Cyanoborohydride Solution	1 x 0.5 ml
	Quenching Buffer	25 ml
	BupH Phosphate Buffered Saline Pack	500 ml
	BupH Carbonate Buffer Pack	500 ml
31496	EZ-Link Activated Peroxidase (Glutaraldehyde Activated)	1 mg
31495	EZ-Link Activated Peroxidase (Glutaraldehyde Activated)	5 mg
31497	EZ-Link Activated Peroxidase Antibody Labeling Kit (Glutaraldehyde Activated)	Kit
	Includes: EZ-Link Activated Peroxidase	5 mg
	Conjugation Buffer	50 ml
	Lysine	250 mg
	Immobilized Protein A/G Column	0.5 ml
	Gentle Ag/Ab Binding Buffer	200 ml
	Gentle Ag/Ab Elution Buffer	200 ml

Label Your Own Antibodies

Thermo Scientific Pierce Fluorescent Labeling Kits

Fluor-label your own antibody in less than two hours!

Pierce Flourescent Labeling Kits are designed for labeling any size protein – even if you have only a small amount of your protein. Protein sample volumes ranging from 50 µl to 1 ml can be used, with protein concentration up to 10 mg/ml for each reaction. Pierce Flourescent Labeling Kits were specially developed and optimized for the most efficient labeling.

Pierce Flourescent Labeling Kits contain everything you need to successfully label your antibody or protein:

- Fluorescent dye provided in individual microtubes, eliminating the need to weigh dye
- Conveniently packaged dimethylformamide (DMF) to prepare the fluorescent dye solution
- Pre-made borate and phosphate buffers – just add water to the powder and they are ready to use
- Pre-packed, ready-to-use desalting columns for fast buffer exchange when your protein sample is greater than 100 µl
- Slide-A-Lyzer® MINI Dialysis Units† for easy buffer exchange when your protein sample is less than or equal to 100 µl
- Amber reaction tubes – no handling in the dark required

Thermo Scientific Pierce Flourescent Labeling Kit	Excitation Wavelength (nm)	Emission Wavelength (nm)
Fluorescein Protein Labeling Kit	491	518
Rhodamine Protein Labeling Kit	544	576
Fluorescein Isothiocyanate (FITC) Protein Labeling Kit	494	520

These kits contain sufficient reagents to perform five fluorescent labeling reactions, which use up to 10 mg/ml of protein for each reaction (50 µl-1 ml of protein).

Ordering Information

Product #	Description	Pkg. Size
53000	**Pierce Fluorescein Protein Labeling Kit**	Kit
	Sufficient for five coupling reactions.	
	Includes: No-Weigh™ Fluorescein Microtubes	6 x 1 mg microtubes
	Dimethylformamide (DMF)	1 ml
	BupH Borate Buffer Packs	5 packs
	BupH Phosphate Buffered Saline Packs	5 packs
	D-Salt Dextran Desalting Columns	5 columns
	Slide-A-Lyzer MINI Dialysis Unit Pack	5 units
	Reaction Tubes	5 tubes
53002	**Pierce Rhodamine Protein Labeling Kit**	Kit
	Sufficient for five coupling reactions.	
	Includes: No-Weigh Rhodamine Microtubes	6 x 0.5 mg microtubes
	Dimethylformamide (DMF)	1 ml
	BupH Borate Buffer Packs	5 packs
	BupH Phosphate Buffered Saline Packs	5 packs
	D-Salt Dextran Desalting Columns	5 columns
	Slide-A-Lyzer MINI Dialysis Unit Pack	5 units
	Reaction Tubes	5 tubes
53004	**Pierce Fluorescein Isothiocyanate (FITC) Protein Labeling Kit**	Kit
	Sufficient for five coupling reactions.	
	Includes: No-Weigh FITC Microtubes	6 x 1 mg microtubes
	Dimethylformamide (DMF)	1 ml
	BupH Borate Buffer Packs	5 packs
	BupH Phosphate Buffered Saline Packs	5 packs
	D-Salt Dextran Desalting Columns	5 columns
	Slide-A-Lyzer MINI Dialysis Unit Pack	5 units
	Reaction Tubes	5 tubes

† See patent information on inside back cover.

The fluorescent labeling procedure is easy with the use of Thermo Scientific Pierce Kits.

Step 1. Preparation of Protein

For salt-free lyophilized protein:
Dissolve in borate buffer.

For proteins in buffers or salt solutions:
a.) Samples of 100 µl or less:
Exchange into borate buffer using Slide-A-Lyzer MINI Dialysis Unit.

b.) Samples greater than 100 µl:
Exchange into borate buffer using a D-Salt Dextran Column.

Step 2. Labeling Reaction

Reconstitute fluorescent dye with DMF.

Add dye to the protein solution. Incubate for 1 hour.

Step 3. Removal of Excess Fluorescent Dye

For samples of 100 µl or less:
Exchange into PBS buffer using Slide-A-Lyzer MINI Dialysis Unit.

For samples greater than 100 µl:
Exchange into PBS buffer using a D-Salt Dextran Column.

Thermo Scientific DyLight 488, 549, 649, 680 and 800 Reactive Fluorescent Dyes

Bright new alternatives to Alexa Fluor, CyDye and LI-COR Fluorescent Dyes.

The DyLight Dyes have absorption spectra ranging from 493 nm to 770 nm (Table 1) and match the principal output wavelengths of common fluorescence instrumentation. They exhibit higher fluorescence intensity and photostability than Alexa Fluor, CyDye and LI-COR Dyes in many applications and remain highly fluorescent over a broad pH range (pH 4-9). Additionally, DyLight Dye water solubility allows a high dye-to-protein ratio without precipitation during conjugation.

Highlights:

- Available in both amine- and sulfhydryl-reactive chemistries for fast and efficient labeling of IgG or other proteins
- High water solubility
- Excellent photostability
- pH insensitive (pH 4-9)
- Compatible with common fluorescence instrumentation

Ordering Information

Amine-Reactive Dyes

Product #	Description	Pkg. Size
46402	DyLight 488 NHS Ester	1 mg
46407	DyLight 549 NHS Ester	1 mg
46415	DyLight 649 NHS Ester	1 mg
46418	DyLight 680 NHS Ester	1 mg
46421	DyLight 800 NHS Ester	1 mg

Sulfhydryl-Reactive Dyes

Product #	Description	Pkg. Size
46602	DyLight 488 Maleimide	1 mg
46607	DyLight 549 Maleimide	1 mg
46615	DyLight 649 Maleimide	1 mg
46618	DyLight 680 Maleimide	1 mg
46621	DyLight 800 Maleimide	1 mg

Table 1. Thermo Scientific DyLight Fluorescent Dyes spectral characteristics.

Emission	Color	Thermo Scientific DyLight Dye	Ex/Em*	ε†	Spectrally Similar Dyes
Green		488	493/518	70,000	Alexa Fluor 488, fluorescein and FITC
Yellow		549	550/568	150,000	Alexa Fluor 546, Alexa Fluor 555, Cy3 and TRITC
Red		649	646/674	250,000	Alexa Fluor 647 and Cy5
Near Infrared		680	682/715	140,000	Alexa Fluor 680 and Cy5.5
		800	770/794	270,000	IRDye 800

*Excitation and emission maxima in nanometers
†Molar extinction coefficient (M^{-1} cm^{-1})

Label Your Own Antibodies

Featured Product: **Thermo Scientific DyLight Antibody Labeling Kits**

Label and purify antibodies in one hour!

Antibody Labeling

The DyLight Antibody Labeling Kits were specifically developed for fast, efficient labeling of antibodies. Two convenient kit formats are available to accommodate varied labeling requirements. The DyLight Antibody Labeling Kits contain all necessary components to perform three separate labeling reactions using 1 mg of IgG or similar quantities of other proteins. The DyLight Microscale Antibody Labeling Kits contain all the necessary components to perform five separate labeling reactions using 100 µg of IgG.

Highlights:

- **Fast** – label and purify protein in approximately one hour
- **Amine-reactive dyes** – label virtually any protein
- **Pre-measured fluorescent dye** – eliminates the time, waste and hassle associated with weighing dye

Antibody Recovery

The Labeling Kits use high-performance spin desalting columns to provide exceptional dye removal and antibody recovery (Figure 1).

Highlights:

- Efficient non-reacted dye removal
- Minimal sample dilution
- Spin column format eliminates the need for column preparation, fraction screening and waiting for protein to emerge from a gravity-flow column (Figure 2)

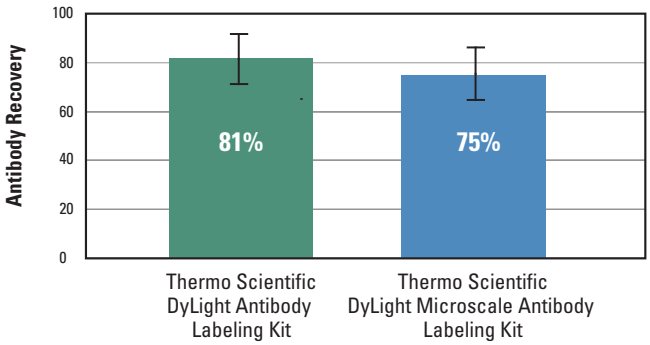


Figure 1. Thermo Scientific DyLight Antibody Labeling Kits provide outstanding recovery. The percent recovery for DyLight Antibody Labeling Kits is the average for 16 labeling reactions using three different antibodies. The percent recovery for DyLight Microscale Antibody Labeling Kits is the average for 13 labeling reactions using three different antibodies.

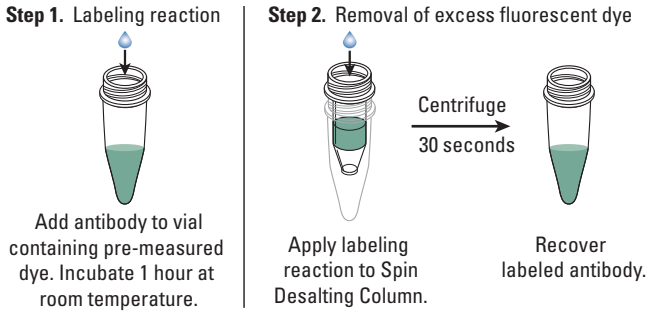


Figure 2. Thermo Scientific DyLight Antibody Labeling Kit protocol summary.

Antibody Labeling Kits

The Antibody Labeling Kits contain sufficient reagents to label and purify 3 x 1 mg of IgG or similar quantities of other proteins. In addition to contents listed below, all Antibody Labeling Kits include:

- Reaction Buffer, 1 ml
- Spin Columns, 6 ea.
- Microcentrifuge Collection Tubes, 12 ea.
- Purification Resin, 5 ml

Ordering Information

Product #	Description	Pkg. Size
53024	DyLight 488 Antibody Labeling Kit DyLight 488 NHS Ester	Kit 3 vials
53034	DyLight 549 Antibody Labeling Kit DyLight 549 NHS Ester	Kit 3 vials
53050	DyLight 649 Antibody Labeling Kit DyLight 649 NHS Ester	Kit 3 vials
53056	DyLight 680 Antibody Labeling Kit DyLight 680 NHS Ester	Kit 3 vials
53062	DyLight 800 Antibody Labeling Kit DyLight 800 NHS Ester	Kit 3 vials

Microscale Kits

The DyLight Microscale Kits contain sufficient reagents to label and purify 5 x 100 µg of IgG. In addition to contents listed below, all Microscale Kits include:

- Reaction Buffer, 1 ml
- Spin Columns, 5 ea.
- Microcentrifuge Collection Tubes, 10 ea.
- Purification Resin, 5 ml

Ordering Information

Product #	Description	Pkg. Size
53025	DyLight 488 Microscale Antibody Labeling Kit DyLight 488 NHS Ester	Kit 5 vials
53035	DyLight 549 Microscale Antibody Labeling Kit DyLight 549 NHS Ester	Kit 5 vials
53051	DyLight 649 Microscale Antibody Labeling Kit DyLight 649 NHS Ester	Kit 5 vials
53057	DyLight 680 Microscale Antibody Labeling Kit DyLight 680 NHS Ester	Kit 5 vials
53063	DyLight 800 Microscale Antibody Labeling Kit DyLight 800 NHS Ester	Kit 5 vials

Thermo Scientific EZ-Link Biotinylation Kits

Everything you need to rapidly and successfully biotinylate purified proteins.

Choose one of these easy-to-use kits and be confident that you have all the tools you need to efficiently biotinylate an antibody or other protein. The kits provide sufficient biotinylation reagent for eight to 10 labeling reactions of 1-10 mg of protein each. Each kit also includes an appropriate labeling reaction buffer and 10 Zeba Desalt Spin Columns for efficient clean-up of the labeled protein. Finally, the HABA dye and avidin kit components allow one to determine the extent of labeling (i.e., the biotin:protein molar ratio).

Highlights:

- NHS-ester biotins react specifically with primary amines (N-terminus and side chain of lysine residues), resulting in stable amide bond
- Sufficient reagents for 10 labeling reactions (8 reactions for Product # 21455)
- Labels 1-10 mg protein in 0.5-2 ml per reaction
- Sulfonate form of NHS ester (or the PEG spacer arm for Product # 21455) enhances water solubility of the biotin reagent
- Zeba Desalt Spin Columns (5 ml) provide rapid sample clean up and excellent protein recovery (faster than dialysis and gravity-flow gel filtration, and much better protein recovery and desalting efficiency than diafiltration)
- Includes reagents and protocol for determining labeling efficiency

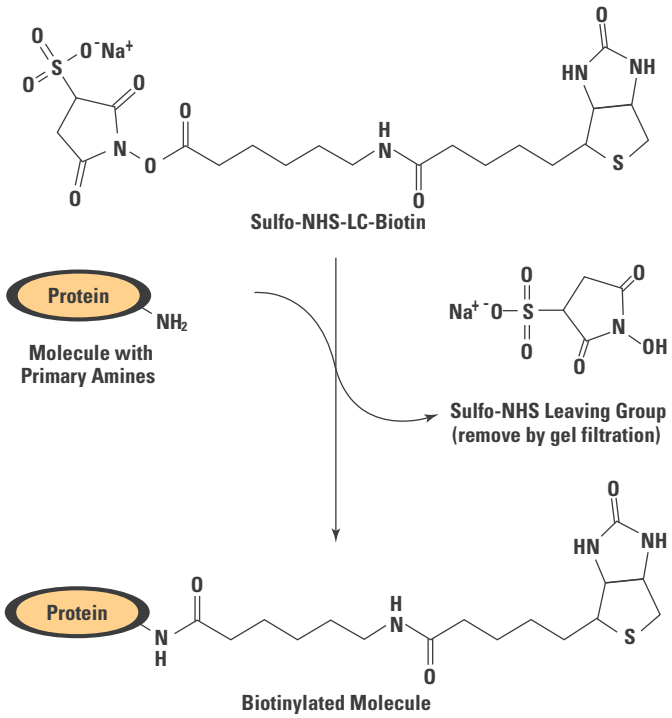


Figure 3. Sulfo-NHS-LC-Biotin reaction scheme.

Sulfo-NHS-Biotin (Product # 21425)

- Shortest spacer arm and simplest biotin reagent
- Adds smallest possible mass to labeled protein

Sulfo-NHS-LC-Biotin (Product # 21435, Figure 3)

- Historically the most popular and widely applied biotin reagent
- Extended spacer arm (compared to Sulfo-NHS-Biotin) minimizes possibility of steric hindrance for avidin or streptavidin binding
- Useful for other applications (e.g., cell-surface labeling) besides labeling purified proteins

Sulfo-NHS-SS-Biotin (Product # 21445)

- Cleavable disulfide bond in spacer arm (can be reduced with DTT or other reducing agent to release the labeled protein avidin or streptavidin in protein interaction or affinity applications)

NHS-PEO_4-Biotin (Product # 21455)

- Polyethylene glycol (PEG) spacer arm enhances water solubility
- Hydrophilic PEG spacer confers solubility to labeled protein (i.e., antibodies labeled with this reagent are less likely to aggregate and precipitate during long-term storage than those labeled with other biotin reagents)

Ordering Information

Product #	Description	Pkg. Size
21425	EZ-Link Sulfo-NHS-Biotinylation Kit	Kit
	Includes: EZ-Link Sulfo-NHS-Biotin	25 mg
	BupH PBS (pack makes 500 ml)	1 pack
	Zeba Desalt Spin Columns, 5 ml	10 each
	HABA (10 mM)	1 ml
	Avidin, Affinity-purified	10 mg
21435	EZ-Link Sulfo-NHS-LC-Biotinylation Kit	Kit
	Includes: EZ-Link Sulfo-NHS-Biotin	25 mg
	Non-reagent contents same as Product # 21425	
21445	EZ-Link Sulfo-NHS-SS-Biotinylation Kit	Kit
	Includes: EZ-Link Sulfo-NHS-SS-Biotin	25 mg
	Non-reagent contents same as Product # 21425	
21455	EZ-Link NHS-PEO_4-Biotinylation Kit	Kit
	Includes: No-Weigh NHS-PEO_4-Biotin	8 x 2 mg
	Non-reagent contents same as Product # 21425	

Label Your Own Antibodies

Thermo Scientific EZ-Link Micro Biotinylation Kits

Biotinylate 50-200 µg antibody or other protein.

Finally! Biotinylation kits that provide reagents and accessories suited for labeling small amounts of protein. These Micro Biotinylation Kits are similar to the kits described on the previous page, the reagent in these kits is supplied in a convenient No-Weigh Microtube format and the kits include smaller Zeba Desalt Spin Columns that are ideal for small-scale labeling of commercial antibodies and other samples that are available in limited amounts.

Highlights:

- NHS-ester biotins react specifically with primary amines (N-terminus and side chain of lysine residues), resulting in stable amide bond
- Sufficient reagents for eight labeling reactions
- Labels 50-200 µg protein in 200-700 µl per reaction
- Sulfonate form of NHS ester (or the PEG spacer arm for Product # 21955) enhances water solubility of the biotin reagent
- Zeba Desalt Spin Columns (2 ml) provide rapid sample clean up and excellent protein recovery (faster than dialysis and gravity-flow gel filtration, and much better protein recovery and desalting efficiency than diafiltration)
- Micro Biotinylation Kits do NOT include reagents and protocol for determining labeling efficiency because it would use up most of the small amount of labeled sample

Ordering Information

Product #	Description	Pkg. Size
21925	EZ-Link Micro Sulfo-NHS-Biotinylation Kit	Kit
	Includes: No-Weigh Sulfo-NHS-Biotin	8 x 1 mg
	BupH PBS (pack makes 500 ml)	1 pack
	Zeba Desalt Spin Columns, 2 ml	10 each
21935	EZ-Link Micro Sulfo-NHS-LC-Biotinylation Kit	Kit
	Includes: No-Weigh Sulfo-NHS-LC-Biotin	8 x 1 mg
	Non-reagent contents same as Product # 21925	
21945	EZ-Link Micro Sulfo-NHS-SS-Biotinylation Kit	Kit
	Includes: No-Weigh Sulfo-NHS-SS-Biotin	8 x 1 mg
	Non-reagent contents same as Product # 21925	
21955	EZ-Link Micro NHS-PEO_4-Biotinylation Kit	Kit
	Includes: No-Weigh NHS-PEO_4-Biotin	8 x 2 mg
	Non-reagent contents same as Product # 21925	

Thermo Scientific EZ-Link NHS-Chromogenic-Biotinylation Kit

A protein labeling kit with a unique biotinylation reagent.

This convenient kit includes all components needed to label five purified protein samples (1-10 mg each) with NHS-Chromogenic-Biotin, a distinctive reagent with a long spacer arm and built-in chromophore for measuring labeling efficiency. Antibodies and other proteins are easily labeled where primary amines occur on their surface. After sample cleanup with a Zeba Desalt Spin Column that is included in the kit, the sample absorbance at 354 nm can be used to directly calculate the extent of biotinylation.

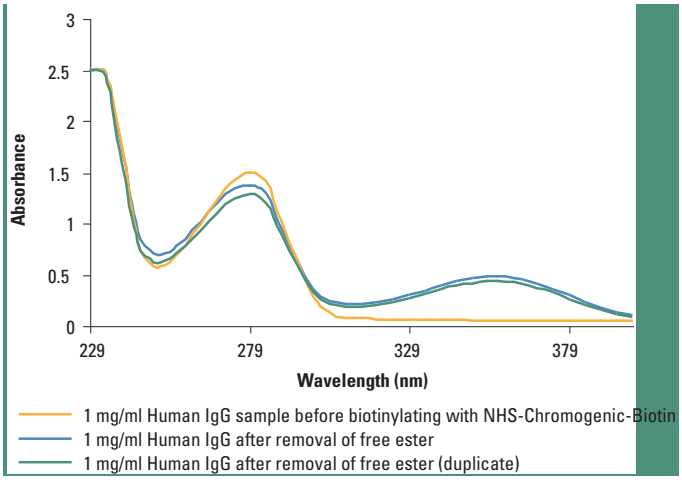
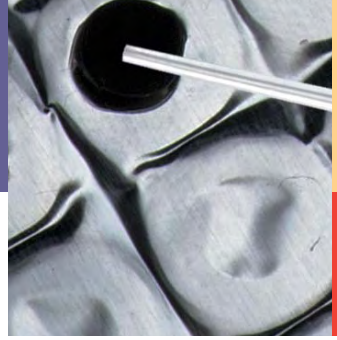


Figure 4. Labeling of human IgG with Thermo Scientific EZ-Link NHS-Chromogenic-Biotin. A 1 mg/ml human IgG sample in 1 ml total volume of phosphate-buffered saline was modified with EZ-Link NHS-Chromogenic-Biotin. Samples were prepared in duplicate and desalted using 5 ml Zeba Desalt Spin Columns. Note the absorption at 354 nm from the covalent addition of the chromogenic biotinylation agent and the minimal loss of protein as illustrated by the 280 nm absorption maxima.

Ordering Information

Product #	Description	Pkg. Size
21625	EZ-Link NHS-Chromogenic-Biotinylation Kit	Kit
	Includes: NHS-Chromogenic-Biotin	10 mg
	BupH PBS (pack makes 500 ml)	1 pack
	Zeba Desalt Spin Columns, 5 ml	5 each
	DMF (N,N-dimethylformamide)	4 ml



Thermo Scientific EZ-Link Solid-Phase Biotinylation Kits

An easier way to biotinylate IgG antibodies.

This innovative antibody-labeling system uses nickel-chelated agarose to temporarily immobilize antibody molecules via their histidine-rich Fc regions. Once held in place on the gel, the antibody can be biotinylated at either sulfhydryl groups (after mild reduction or disulfide bonds) or primary amines. Excess labeling reagent and byproducts are then washed away before recovering the labeled and purified antibody from the gel using a mild imidazole solution. No gel filtration or dialysis are needed. Four kits are available for small (0.1-1 mg) or large (1-10 mg) antibody samples using either amine-directed (NHS ester) or sulfhydryl-directed (maleimide) labeling reagents.

These kits contain our unique No-Weigh Single-Dose Microtube Packaging. A single sealed microtube containing 2 mg of reagent is reconstituted for each biotinylation. The exclusive No-Weigh Packaging allows access to fresh reagent on-demand for each solid-phase biotinylation reaction.

Highlights:

- **Fast labeling and purification** – the entire procedure takes only one hour (two hours for sulfhydryl labeling kits) (Figure 5)
- **Easy removal of spent and excess labeling reagent** – simply wash away the reaction byproducts – no need for dialysis or gel filtration
- **No dilution effects** – solid-phase method allows initially dilute antibodies to be recovered in a smaller volume after labeling
- **Optimized protocols** – specific protocols for antibody ensure appropriate level of labeling (2-5 biotins per antibody molecule), minimizing possibility of inactivation caused by overlabeling
- **Sufficient reagents for eight biotin-labeling experiments** – No-Weigh Single-Dose Microtube Packaging ensures that the biotin reagent is fully active for eight separate experiments
- **Convenient kit sizes and labeling chemistries available**:

Antibody Sample Size	Amine-directed Labeling (NHS-PEO_4-Biotin)	Sulfhydryl-directed Labeling (Maleimide-PEO_2-Biotin)
0.1-1 mg IgG	Product # 21450	Product # 21930
1-10 mg IgG	Product # 21440	Product # 21920

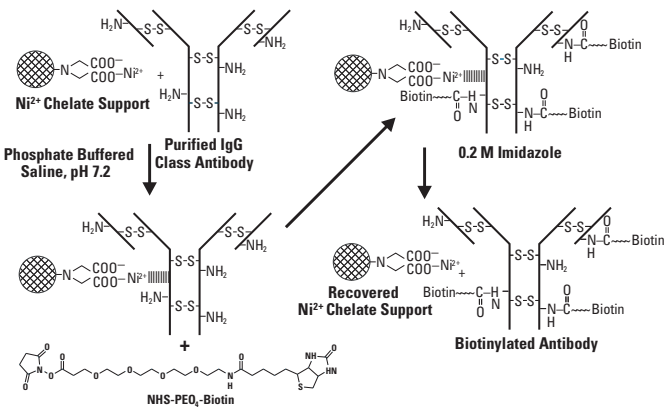


Figure 5. Solid-phase protocol summary for labeling amines.

Step 1. Immobilize the IgG
- a. 1 ml Ni-IDA column (for 1-10 mg of IgG)
- b. SwellGel® Nickel Chelated Disc (for 0.1-1 mg IgG)†

Step 2. Add the labeling reagent(s) to the immobilized IgG
- a. NHS-PEO_4-Biotin for amine-directed reactions
- b. TCEP, followed by Maleimide-PEO_2-Biotin for sulfhydryl-directed reactions

Step 3. Elute the biotinylated IgG with 0.2 M imidazole

Ordering Information

Product #	Description	Pkg. Size
21440	**EZ-Link NHS-PEO Solid-Phase Biotinylation Kit – Pre-Packed Column**	Kit
	Biotinylates antibodies and other proteins that bind to the nickel-chelated support provided. A 1 ml column biotinylates 1-10 mg of antibody and can be re-used 10 times.	
	Includes: Immobilized Nickel Chelated Column	1 ml, pre-packed
	BupH Phosphate Buffered Saline (makes 500 ml)	1 pack
	No-Weigh NHS-PEO_4-Biotin	8 x 2 mg
	4 M Imidazole Stock Solution	5 ml
21450	**EZ-Link NHS-PEO Solid-Phase Biotinylation Kit – Mini-Spin Columns**	Kit
	Biotinylate antibodies and other proteins that bind to the rehydrated nickel-chelated discs provided. Each disc can biotinylate 100-1,000 µg of antibody.	
	Includes: SwellGel Nickel Chelated Discs	10 pack
	Handee Mini-Spin Columns	10 pack
	Handee Microcentrifuge Tubes (2 ml)	30 pack
	BupH Phosphate Buffered Saline (makes 500 ml)	1 pack
	No-Weigh NHS-PEO_4-Biotin	8 x 2 mg
	4 M Imidazole Stock Solution	5 ml
21920	**EZ-Link Maleimide-PEO Solid-Phase Biotinylation Kit – Pre-Packed Column**	Kit
	Reduces and biotinylates IgG class antibodies and other proteins that bind to the nickel-chelated support provided. A 1 ml column biotinylates 1-10 mg of antibody and can be re-used 10 times.	
	Includes: Bond-Breaker® TCEP Solution, Neutral pH	5 ml
	Immobilized Nickel Chelated Column	1 ml pre-packed
	BupH Tris Buffered Saline (makes 500 ml)	1 pack
	No-Weigh Maleimide-PEO_2-Biotin	8 x 2 mg
	4 M Imidazole Stock Solution	5 ml
21930	**EZ-Link Maleimide-PEO Solid-Phase Biotinylation Kit – Mini-Spin Columns**	Kit
	Reduces and biotinylates IgG class antibodies and other proteins that bind to the nickel-chelated support provided. Each disc can biotinylate 100-1,000 µg of antibody.	
	Includes: SwellGel Nickel Chelated Discs	10 pack
	Bond-Breaker TCEP Solution, Neutral pH	5 ml
	Handee Mini-Spin Columns	10 pack
	Handee Microcentrifuge Tubes (2 ml)	30 pack
	BupH Tris Buffered Saline (makes 500 ml)	1 pack
	No-Weigh Maleimide-PEO_2-Biotin	8 x 2 mg
	4 M Imidazole Stock Solution	5 ml

† See patent information on inside back cover.

Label Your Own Antibodies

Thermo Scientific ProFound Sulfo-SBED Biotin Label Transfer Kit– Western Blot Application

Now it's easier than ever to discover a protein interaction.

Label transfer has increased in popularity as an *in vitro* method for protein interaction discovery. A growing number of publications feature ProFound Sulfo-SBED Biotin Label Transfer Reagent to study a variety of issues relating to protein interactions. To simplify use of this exceptional reagent, we have assembled the key components frequently cited in publications featuring Sulfo-SBED. The resulting easy-to-use kit also contains streptavidin-HRP for subsequent detection of the biotinylated complex or biotinylated prey protein on a Western blot. The ProFound Sulfo-SBED Biotin Label Transfer Kit is of value to both the first-time user and experienced user of Sulfo-SBED and other label transfer reagents.

The label transfer method requires a purified "bait" protein and enables capture and detection of previously unknown interacting "prey" proteins (Figure 6). The amine groups of the bait protein are modified via the sulfo-NHS moiety of Sulfo-SBED. This derivatized bait protein is then incubated in the dark with a lysate or with a purified putative "prey" protein. When the bait-prey complex has formed, the reaction is exposed to UV-light, thereby activating the phenylazide moiety of Sulfo-SBED to covalently link together the bait and prey proteins. Upon reduction of this complex, the biotin label that first resided with the bait protein transfers to the prey protein, which may then be detected by Western blot analysis using streptavidin-HRP.

Highlights:

- Ready-to-use kit eliminates the need to prepare the necessary buffers or purchase the critical reagents necessary to perform a label transfer experiment
- Sulfo-SBED and DTT components are supplied in single-use packaging, minimizing waste and preserving the integrity of the reagents for the lifetime of the kit
- Special packaging and bundling of components offer reduced costs over purchasing or preparing components individually (a calculated minimum savings of nearly 40%)
- Incorporates the experience of those who have successfully published their results using Sulfo-SBED in the label transfer mode
- Guidelines are provided to aid in critical aspects of reagent use
- Twenty-reference literature review supplied with the kit

Ordering Information

Product #	Description	Pkg. Size
33073	**ProFound Sulfo-SBED† Biotin Label Transfer Kit – Western Blot Application** *Sufficient reagents to perform 8 label transfer reactions for subsequent Western blot analysis.*	Kit
	Includes: No-Weigh Sulfo-SBED Label Transfer Reagent	8 x 1 mg
	BupH Phosphate Buffered Saline	1 pack (makes 500 ml)
	Label Transfer Buffer (20X)	200 ml (makes 4 L)
	Streptavidin-HRP Conjugate	0.1 mg
	No-Weigh Dithiothreitol (DTT)	8 x 7.7 mg
	Slide-A-Lyzer MINI Dialysis Units† Plus Float, 10K MWCO, 10-100 µl	10 units/pack

†See patent information on inside back cover.

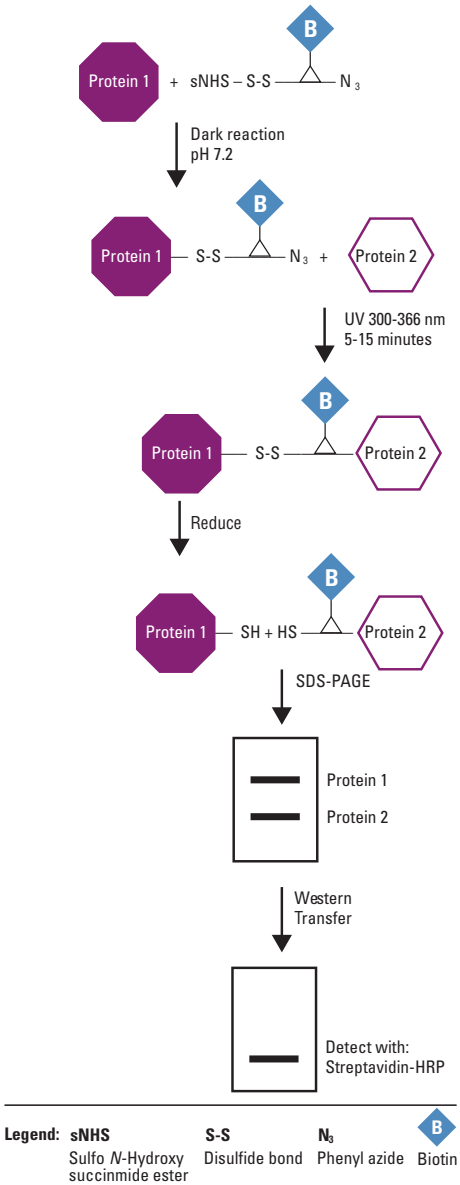
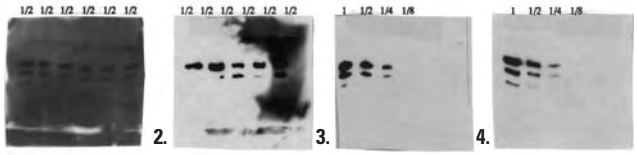


Figure 6. Thermo Scientific ProFound Sulfo-SBED Biotin Label Transfer Kit protocol summary.

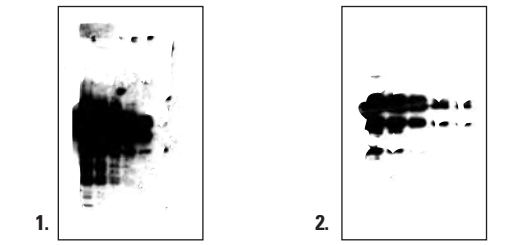
Optimize Antibody Concentration

Because every new Western blot is unique, there is no "perfect" antibody concentration for every blot. Therefore, every new Western blot needs to be optimized to determine the antibody concentration that is most appropriate for a particular combination of membranes, proteins and antibodies. Optimization is even more crucial when key components of a system are changed, such as switching from a colorimetric substrate like chloronaphthol (CN) to more sensitive chemiluminescent substrates such as SuperSignal West Products. Antibodies must be used at the optimal concentrations with chemiluminescent substrates to achieve low background and high band resolution (Figures 1-2). The first step of optimizing the blotting conditions usually involves optimizing the antibody concentrations (or dilutions) through the use of a dot blot protocol. The next step is typically the optimization of the blocking buffer by testing cross-reactivity of several different buffers with the blotting system's key components (see page 11).



Mouse anti-Human p53	1:500 (1 µg/ml)	1:500 (1 µg/ml)	1:1,000 (0.5 µg/ml)	1:1,000 (0.5 µg/ml)
Goat anti-Mouse HRP	1:1,000 (1 µg/ml)	1:5,000 (0.2 µg/ml)	1:10,000 (0.1 µg/ml)	1:20,000 (0.05 µg/ml)
Exposure Time	30 seconds	30 seconds	1 minute	1 minute

Figure 1. Example of signal intensity on a Western blot when using Thermo Scientific SuperSignal West Pico Substrate and antibodies at various concentrations. Recombinant Human Wild-Type p53 Baculovirus lysate at various concentrations was electrophoretically separated and transferred to nitrocellulose membrane. The membrane was blocked with BSA and then incubated with various dilutions of mouse anti-human p53 starting at the manufacturer's recommended dilution. HRP-labeled goat anti-mouse was added at different concentrations and the signal was developed with SuperSignal West Pico Substrate. The exposure times were also varied as indicated. In **Blot 1**, the blot was totally black due to both the primary and secondary antibody concentrations being too high. In **Blot 2**, the background is inconsistent but very dark, again a result of too much primary and secondary antibody. In **Blots 3 and 4**, the signal-to-noise was much better because both the primary and secondary antibody concentrations were reduced. Neither blot 3 nor 4 had background signal.



Primary Antibody 1:500
Secondary Antibody 1:5,000

Primary Antibody 1:5,000
Secondary Antibody 1:50,000

Figure 2. Example of signal intensity on a Western blot using Thermo Scientific SuperSignal West Dura Substrate and antibodies at various concentrations. Blots were optimized with SuperSignal West Dura Chemiluminescent Substrate. **Blot 1** primary and secondary antibody concentrations are too high. The bands are too intense and blur together, resulting in poor resolution. A large number of nonspecific bands are also visible.

Optimize Antibody Concentration

Dot Blot Protocol for Optimization of Antigen and Antibody Concentrations

The optimal antibody concentrations to use with a given antigen are dependent on the antigen and antibody themselves. The affinity/avidity of the antibody for the antigen and the specific activity of both the primary and secondary antibody will vary. The optimal antigen and antibody concentrations can be determined by performing complete Western blots with varying concentrations of antigen and antibody. Alternatively, a faster and easier method is to perform a dot blot procedure. The following is a dot blot protocol using SuperSignal West Pico Substrate. When using other Thermo Scientific Substrates, refer to the product instructions for recommended antigen/antibody concentrations.

Note: All antibody dilutions assume a starting concentration of ~1 mg/ml.

1. Prepare dilutions of the protein sample in either TBS or PBS. The proper dilution will depend on the antigen concentration present in the sample, but because the concentration of the antigen of interest often is not known, it is necessary to test a wide range of dilutions. SuperSignal West Pico Substrate has picogram-level detection sensitivity so sample dilutions can range from the low microgram to low picogram levels. If too much antigen is applied, the results may have any or all of the following: detection of nonspecific bands, blurred banding patterns and rapid signal deterioration.
2. Prepare membranes. The number of membrane pieces needed depends on how many different dilutions of primary and/or secondary antibody will be screened. Typically, one or two dilutions of the primary antibody are tested with two or three different dilutions of the secondary antibody. For example: 1/1,000 primary with 1/50,000 secondary, 1/1,000 primary with 1/100,000 secondary, 1/5,000 primary with 1/50,000 secondary, and 1/5,000 primary with 1/100,000 secondary.
3. Place membranes on a paper towel. Dot antigen dilutions onto the membranes. Apply the smallest possible volume to the membranes (2-5 µl works well) because the greater the volume that is applied, the more diffuse the signal will be. Allow the antigen dilutions to dry on the membranes for 10-30 minutes or until no visible moisture remains.
4. Block the nonspecific sites on the membranes by incubating them in blocking buffer that contains 0.05% Tween-20 (blocker/Tween-20 Detergent) for 1 hour at RT with shaking.
5. Prepare the primary antibody dilutions in blocker/Tween-20 Detergent and apply to the membranes. Incubate for 1 hour at RT with shaking.

Thermo Scientific Pierce Substrate	Recommended Primary Antibody Dilutions (from 1 mg/ml stock)
Pierce ECL Substrate	1:100-1:5,000 or 0.2-10 µg/ml
SuperSignal West Pico Substrate	1:1,000-1:5,000 or 0.2-1.0 µg/ml
SuperSignal West Femto Substrate	1:5,000-1:100,000 or 0.01-0.2 µg/ml
SuperSignal West Dura Substrate	1:1,000-1:50,000 or 0.02-1.0 µg/ml
Lumi-Phos WB Substrate	1:200-1:2,000 or 0.5-5.0 µg/ml

6. Wash the membrane four to six times in TBS or PBS, using as large a volume of wash buffer as possible. Add 0.05% Tween-20 Detergent to the wash buffer to help reduce nonspecific background. For each wash, suspend the membrane in wash buffer and agitate for approximately 5 minutes. Pour off the wash buffer and repeat. Brief rinses of the membranes before incubation in the wash buffer may increase the wash step efficiency.
7. Prepare dilutions of the secondary antibody/HRP conjugate in blocker/Tween-20 Detergent. Add the secondary antibody dilutions to the membranes and incubate for 1 hour with shaking.

Thermo Scientific Pierce Substrate	Recommended Secondary Antibody Dilutions (from 1 mg/ml stock)
Pierce ECL Substrate	1:1,000-1:15,000 or 0.067-1 µg/ml
SuperSignal West Pico Substrate	1:20,000-1:100,000 or 10-50 ng/ml
SuperSignal West Femto Substrate	1:100,000-1:500,000 or 2.0-10 ng/ml
SuperSignal West Dura Substrate	1:50,000-1:250,000 or 4.0-20 ng/ml
Lumi-Phos WB Substrate	1:5,000-1:25,000 or 40-200 ng/ml

8. Wash the membrane again as described in Step 6.
9. Prepare the substrate working solution by mixing equal volumes of the Luminol/Enhancer Solution and the Stable Peroxide Solution. Prepare a sufficient volume to ensure that the blot is completely wetted with substrate and the blot does not dry out during incubation. **Recommended volume: 0.1 ml/cm^2 of blot surface.**
10. Incubate the membrane in the SuperSignal West Pico Substrate Working Solution for 5 minutes.
11. Remove the membrane from the substrate and place in a plastic sheet protector or other protective wrap.
12. Place the blot against the film – protein side up – and expose. Any standard or enhanced autoradiographic film can be used. A recommended first exposure is 30-60 seconds. Exposure time can be varied to obtain optimum results. Alternatively, use a CCD camera or other imaging device; however, these devices may require longer exposure times.
13. On an optimized blot, the SuperSignal West Pico Substrate-generated signal should last for up to eight hours. The blot can be re-exposed to film or an imaging device as needed to obtain the optimal results. Longer exposure times may be necessary as the blot ages. If optimal results are not achieved, repeat this procedure using different antigen and/or antibody dilutions.

Chromogenic Substrates

As with the other components in a Western blotting system, there are many substrate choices available. The appropriate substrate choice depends on the enzyme label (AP or HRP), desired sensitivity, and desired form of signal or method of detection. Chromogenic substrates are widely used and offer perhaps the simplest and most cost-effective method of detection. When these substrates come in contact with the appropriate enzyme, they are converted to insoluble, colored products that precipitate onto the membrane and require no special equipment for processing or visualizing. Substrates such as TMB (3,3´,5,5´-tetramethylbenzidine), 4-CN (4-chloro-1-naphthol) and DAB (3,3´-diaminobenzidine tetrahydrochloride) are available for use with HRP. For use with AP, NBT (nitro-blue tetrazolium chloride), BCIP (5-bromo-4-chloro-3´-indolylphosphate *p*-toluidine salt) and Fast Red (naphthol AS-MX phosphate + Fast Red TR Salt) are available. The performance of a particular substrate may vary dramatically when obtained from different suppliers because performance can be affected by the concentration and purity of the substrate and by other additives and buffer components that are a part of the formulation.

Peroxide must be added to a substrate for colorimetric detection with HRP. Because of its extremely short shelf life at the desired concentration, hydrogen peroxide traditionally was added to a buffer, along with the substrate, immediately before use. As a result, these substrates typically have a useful shelf life of only a few hours. Many of our precipitating HRP substrates are supplied with, or come prepared in, Stable Peroxide Substrate Buffer (Product # 34062). The Stable Peroxide Substrate Buffer is a 10X concentrate that offers several advantages. It is less corrosive than the traditional 30% stock solution of hydrogen peroxide and, because fewer preparation steps are involved, it provides more consistent results. Although the Stable Peroxide Substrate Buffer is provided as a 10X concentrate, it is also stable at a 1X concentration.

Ordering Information

Product #	Description	Pkg. Size
34062	Pierce Stable Peroxide Buffer (10X)	100 ml

Substrates for HRP

TMB, with a molecular weight of 240.4, is most often used as a substrate for HRP in ELISAs. However, in the presence of HRP and peroxide, a water-soluble blue product is generated that can be precipitated onto a membrane. Pierce TMB – Blotting (Product # 34018) is a single-component peroxidase substrate for Western blotting and immunohistochemistry. Precipitating the product results in dark blue bands where the enzyme is located. Pierce TMB – Blotting is well suited to applications that require a high signal-to-noise ratio.

Ordering Information

Product #	Description	Pkg. Size
34018	Pierce TMB – Blotting	250 ml

4-CN has a molecular weight of 178.6 and can be used for chromogenic detection of HRP in blotting and histochemistry. This precipitate is not as sensitive or as stable as TMB and DAB, but the alcohol-soluble precipitate photographs well and has a distinct blue-purple color that can be useful in double-staining applications.

Ordering Information

Product #	Description	Pkg. Size
34012	Pierce CN	250 ml
34010	Pierce 4-Chloro-1-Napthol Powder	25 g powder
34011	Pierce 4-Chloro-1-Napthol Tablets (30 mg/tablet)	50 tablets

Chromogenic Substrates

DAB has a molecular weight of 214.1 and yields a brown precipitate in the presence of HRP and peroxide. The brown, insoluble product can be readily chelated with osmium tetroxide. This property makes DAB ideal for electron microscopy. The color produced by DAB can be intensified with the addition of metals such as nickel, copper, silver and cobalt that form complexes. The color produced by the metal complexes is darker than the color produced by DAB alone, enhancing the sensitivity in staining applications.

Ordering Information

Product #	Description	Pkg. Size
34002	Pierce DAB Substrate Kit	275 ml
	Includes: DAB (10X)	25 ml
	Stable Peroxide Buffer	250 ml
34065	Pierce Metal Enhanced DAB Substrate Kit	275 ml
	Includes: 10X Metal Enhanced DAB	25 ml
	Stable Peroxide Buffer	250 ml

The individual benefits of 4-CN and DAB are often combined into a single substrate mixture, CN/DAB Substrate. The CN/DAB Substrate has excellent sensitivity, yielding a dark black precipitate that photographs well. The CN/DAB Substrate works well in Western blotting and dot blotting applications.

Ordering Information

Product #	Description	Pkg. Size
34000	Pierce CN/DAB Substrate Kit	275 ml
	Includes: CN/DAB (10X)	25 ml
	Stable Peroxide Buffer	250 ml

Substrates for Alkaline Phosphatase

NBT, with a molecular weight of 817.6, is a member of a class of heterocyclic organic compounds known as tetrazolium salts. Upon reduction, the compound yields NBT-formazan, a highly colored, water-insoluble product. The substrate is widely used for immunochemical assays and techniques because the color produced by the formazan is linear and stable over a wide dynamic range.

Ordering Information

Product #	Description	Pkg. Size
34035	Pierce Nitro-Blue Tetrazolium Chloride	1 g powder

BCIP has a molecular weight of 433.6, and hydrolysis by AP results in a blue-purple precipitate. BCIP can be used as a chromogenic substrate for both immunoblotting and immunohistochemical studies.

Ordering Information

Product #	Description	Pkg. Size
34040	Pierce 5-Bromo-4-chloro-3'-indolyphosphate *p*-toluidine Salt	1 g powder

An ideal system for blotting or staining applications with AP is the combination of NBT and BCIP (Figure 1). Together, they yield an intense, black-purple precipitate that provides much greater sensitivity than either substrate alone. This reaction proceeds at a steady rate, allowing accurate control of its relative sensitivity. NBT/BCIP characteristically produces sharp band resolution with minimal background.

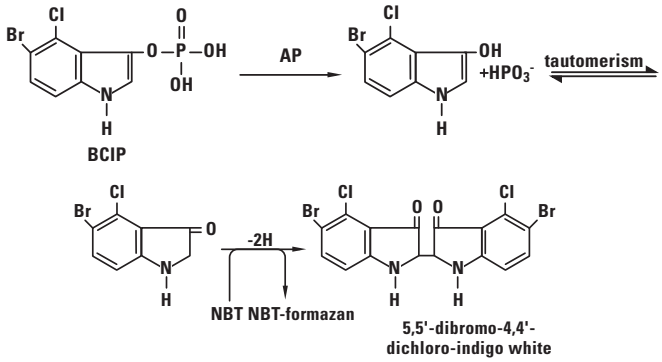


Figure 1. Reaction of AP with BCIP and NBT.

Ordering Information

Product #	Description	Pkg. Size
34042	Pierce NBT/BCIP	250 ml
34070	Pierce NBT/BCIP Plus Suppressor	100 ml

Chemiluminescent Substrates

When energy in the form of light is released from a substance because of a chemical reaction, the process is called chemiluminescence. Luminol is one of the most widely used chemiluminescent reagents and its oxidation by peroxide results in creation of an excited state product called 3-aminophthalate. This product decays to a lower energy state by releasing photons of light (Figure 1).

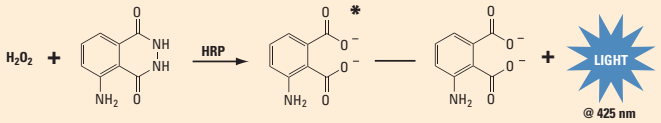


Figure 1. Luminol is oxidized in the presence of HRP and hydrogen peroxide to form an excited state product (3-aminophthalate). The 3-aminophthalate emits light at 425 nm as it decays to the ground state.

Chemiluminescent substrates have steadily gained in popularity because they offer several advantages over other detection methods (Table 1). These advantages have allowed chemiluminescence to become the detection method of choice in most protein laboratories. Using chemiluminescence allows multiple exposures to obtain the best image. The detection reagents can be removed and the entire blot reprobed to visualize another protein or to optimize detection of the first protein. A large linear response range allows detection and quantitation for a large range of protein concentrations. Most importantly, chemiluminescence yields the greatest sensitivity of any available detection method. Using HRP as the enzyme label and SuperSignal West Femto Chemiluminescent Substrate (Product # 34095), detection limits as low as 1 femtogram are possible because the enhancers in this substrate greatly intensify the emitted light and extend the signal duration.

Chemiluminescent substrates differ from other substrates in that the light detected is a transient product of the reaction that is only present while the enzyme-substrate reaction is occurring. This is in contrast to substrates that produce a stable, colored product; these colored precipitates remain on the membrane after the enzyme-substrate reaction has terminated. On a chemiluminescent Western blot, the substrate is the limiting reagent in the reaction; as it is exhausted, light production decreases and eventually ceases. A well-optimized procedure using the proper antibody dilutions will produce a stable output of light for several hours, allowing consistent and sensitive detection of proteins. When the antibody is not diluted sufficiently, a stable output of light will never be achieved. Too much enzyme in the system will rapidly oxidize the substrate and terminate the signal. This is the single greatest cause of symptoms such as variability, dark background with clear bands and decreased sensitivity in Western blotting experiments with chemiluminescence. To avoid this problem, it is crucial to optimize the amount of antibody used for detection. Antibody suppliers typically suggest a dilution range for using their antibody on a Western blot. This dilution range is often appropriate for blots detected with a relatively insensitive chromogenic substrate, but a much greater dilution is generally required for optimum performance with a sensitive chemiluminescent substrate such as SuperSignal West Chemiluminscent Substrates.

To view a comparison of our chemiluminescent substrates, see Table 4 on page 47.

Table 1. Advantages of enhanced chemiluminescence.

Sensitive
- Intense signal with low background
- Requires less antigen and antibody

Fast
- Rapid substrate processing of blot
- Signal generated within seconds

Stable
- Unlike radioisotopes, the shelf life is long
- Store at room temperature or 4°C

Hard-copy results
- Results are captured on X-ray film
- No fading or tearing of brittle membrane over time
- Permanent record

Film results
- Signal output continues for a long time (i.e., 8-24 hours)
- Can expose blot to film multiple times
- Can optimize the developing method

Can reprobe the blot
- Can remove nonisotopic probes from the membrane
- Can repeat immunodetection

Large linear response
- Can detect a large range of protein concentrations

Quantitative
- The X-ray film can be scanned using a reflectance densitometer or using an imaging device, such as a CCD camera

Featured Product: Thermo Scientific Pierce ECL Western Blotting Substrate

A reliable ECL formulation without inflated prices.

Paying twice what you should for an enhanced chemiluminescent (ECL) substrate? For researchers interested in a quality product at a fair price, there is a new option available. Pierce ECL Western Blotting Substrate is an entry-level Western blotting substrate that is value-priced.

If you are currently using a needlessly expensive ECL substrate, you can switch to Pierce ECL Western Blotting Substrate without any optimization. Simply switch out the substrates and save a bundle

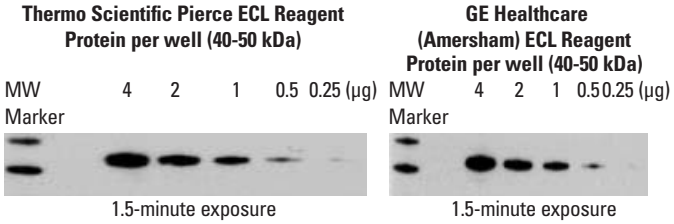


Figure 2. Thermo Scientific Pierce ECL Substrate Western blot detection of actin (beta) from HeLa cell lysate. Dilutions of HeLa cell lysate were prepared and separated by electrophoresis. The proteins were transferred to nitrocellulose membranes (Product # 88025). Membranes were blocked with 5% skim milk and then incubated with Mouse Anti-Human Actin (US Biological, Swampscott, MA) at 1 µg/ml. The membranes were washed and then incubated with 0.2 µg/ml of HRP-conjugated Goat Anti-Mouse IgG (Product # 31430) and then washed again. Working solutions of the substrates were prepared according to the manufacturers' instructions and added to the membranes for 1 minute. The membranes were placed in plastic sheet protectors and exposed to CL-XPosure Film (Product # 34090) for 90 seconds.

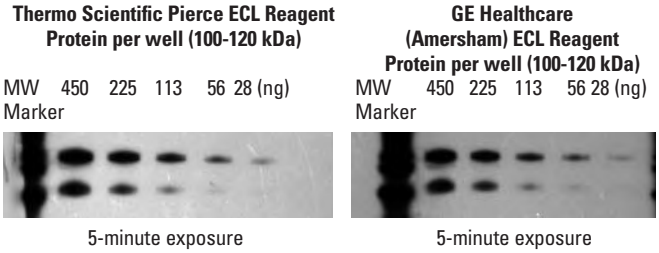


Figure 3. Thermo Scientific Pierce ECL Substrate Western blot detection of β-galactosidase (expressed) from *Escherichia coli* lysate. Dilutions of *E. coli* cell lysate were prepared and separated by electrophoresis. The proteins were transferred to PVDF membranes (Product # 88585). Membranes were blocked with 5% skim milk and then incubated with Mouse Anti-β galactosidase AB-1 (Lab Vision, Fremont, CA) at 1 µg/ml. The membranes were washed and then incubated with 0.2 µg/ml of HRP-conjugated Goat Anti-Mouse IgG (Product # 31430) and then washed again. Working solutions of the substrates were prepared according to the manufacturers' instructions and added to the membranes for 1 minute. The membranes were placed in plastic sheet protectors and exposed to CL-XPosure Film (Product # 34090) for five minutes.

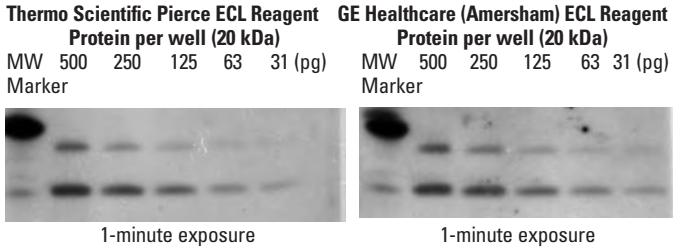


Figure 4. Thermo Scientific Pierce ECL Substrate Western blot detection of recombinant bovine TNF-α. Dilutions of recombinant bovine TNF-α (Product # RBOTNFAI) were prepared and electrophoresed. The proteins were transferred to nitrocellulose membranes (Product # 88025). Membranes were blocked with 5% skim milk and then incubated with rabbit anti-bovine TNF-α at 4 µg/ml. The membranes were washed and then incubated with 0.4 µg/ml of HRP-conjugated Goat Anti-Rabbit IgG (Product # 31460) and then washed again. Working solutions of the substrates were prepared according to the manufacturers' instructions and added to the membranes for 1 minute. The membranes were placed in plastic sheet protectors and exposed to Hyperfilm® Film (GE Healthcare, Piscataway, NJ).

Highlights:

- **Half the price of other ECL Substrates** – low overhead and a commitment to customer value enables us to offer this product for half the price other companies charge (these claims are based on the 2007 U.S. list prices)
- **No optimization required** – switch to our ECL substrate without the need for optimization or protocol changes
- **A product you can rely on** – we put both our strong technical support and reputation behind this product

Ordering Information

Product #	Description	Pkg. Size
32106	Pierce ECL Western Blotting Substrate	500 ml kit
32209	Pierce ECL Western Blotting Substrate	250 ml kit
32109	Pierce ECL Western Blotting Substrate	50 ml kit

Featured Product: Thermo Scientific SuperSignal West Pico Chemiluminescent Substrate

Twice as much signal for about 40% less than the price of the GE Healthcare Amersham ECL System.

In side-by-side comparisons using identical conditions, blots incubated in SuperSignal West Pico Chemiluminescent Substrate exhibit at least twice the intensity of blots treated with the GE Healthcare Amersham ECL System.

More stable
SuperSignal West Pico Substrate is room temperature (RT)-stable for months, with no discernable loss in activity. RT stability frees up valuable cold-room space and saves time because there is no need to wait for the reagents to warm up.

Long signal
With signal duration of more than six hours, there is adequate time to optimize the exposure conditions. In most cases, there is no need to rerun samples and repeat the blotting procedure.

Highlights:

- **Economy** – costs less per ml than other chemiluminescent substrates (Table 2)
- **Long light emission** – strong light emission over a working day allows you to make several exposures
- **High intensity** – signal is twice as intense as other compatibly priced luminol-based systems (Figure 5)
- **Picogram sensitivity** – highly sensitive for the rapid development of a wide range of protein levels (Figure 6)
- **Excellent stability** – 24-hour-plus working solution stability; kit is stable for at least one year at room temperature
- **Saves antibody** – primary and secondary antibodies are used highly diluted so they can be used for more blots

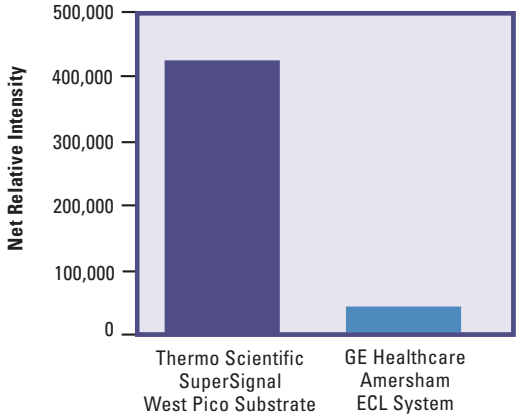


Figure 5. Enhanced light emission kinetics: Thermo Scientific SuperSignal Substrate vs. GE Healthcare Amersham ECL System. Net relative intensity six hours after incubation is much greater for SuperSignal West Pico Substrate than for the ECL System.

Table 2. Cost comparisons between SuperSignal West Pico Substrate and competitors' substrates.

Substrate Cost Comparison	SuperSignal West Pico Substrate[1]	GE Healthcare Amersham ECL Substrate[2]	Perkin-Elmer Western Lightning™ Substrate[3]
Membrane (10 x 10)	$ 7.96	$10.20	$ 7.96
TBS Wash Buffer	$ 1.29	$ 1.29	$ 1.29
SuperBlock Blocking Buffer	$ 4.86	$ 4.86	$ 4.86
Primary Antibody*	**$ 3.35**	**$33.46**	**$ 6.70**
Secondary Antibody	$ 0.04	$ 0.56	$ 0.56
Substrate	**$ 4.00**	**$ 7.13**	**$ 6.12**
Film	$ 0.93	$ 3.54	$ 2.91
Total Blotting Cost	**$22.29**	**$61.04**	**$30.37**

**Endogen Anti-CD54 (Product # MA5407, 500 µg) was used at the substrate manufacturer's recommended starting dilution.*
Costs are based on January 2007 U.S. list prices for an 8 x 10 cm mini gel following manufacturer's instructions.
1. Using Thermo Scientific products where applicable
2. Using GE Amersham Biosciences products where applicable
3. Using Perkin-Elmer products where applicable

Chemiluminescent Substrates

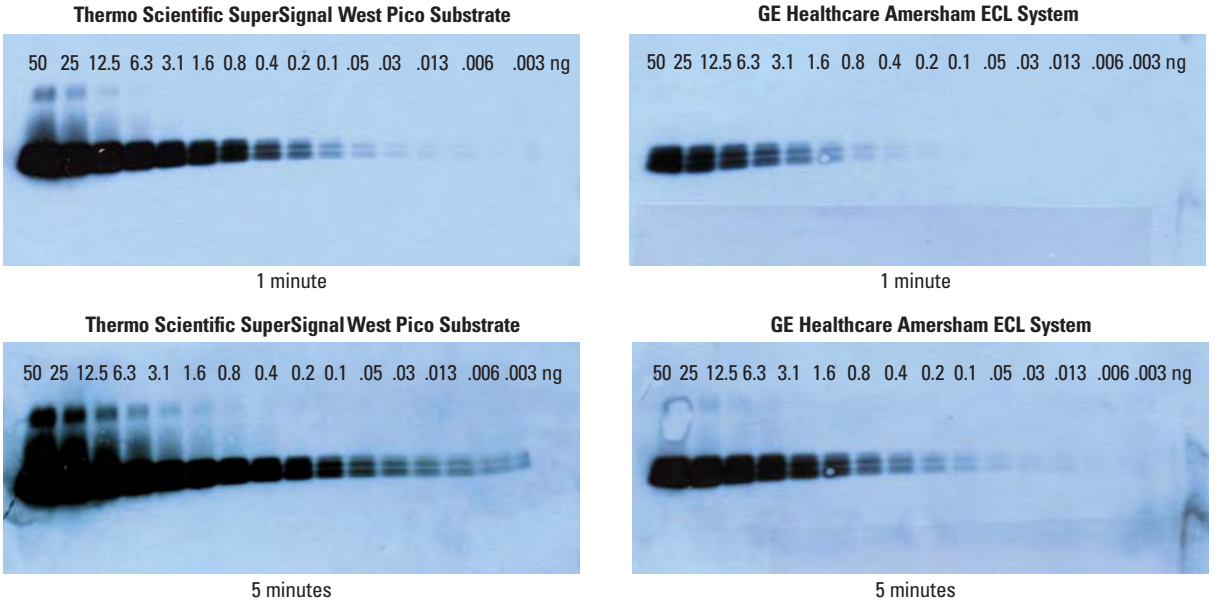


Figure 6. Thermo Scientific SuperSignal West Pico is more sensitive than GE Healthcare Amersham ECL Substrate. Recombinant mouse IL-2 was serially diluted (50-0.003 ng) and electrophoresis was performed. The gels were transferred to nitrocellulose membranes, blocked and incubated with a 1 µg/ml dilution of Rat Anti-Mouse IL-2. After washing, the membranes were incubated with 20 ng/ml dilutions of HRP-conjugated Goat Anti-Rat antibody. The membranes were washed again and then incubated with substrate that was prepared according to the manufacturers' instructions. Blots were exposed to film for one- and five-minute exposures.

Table 3. A conversion protocol for using Thermo Scientific SuperSignal West Pico Substrate.

Step-by-step Conversion Protocol	GE Healthcare Amersham ECL Substrate	Thermo Scientific SuperSignal West Pico Substrate
1. Perform standard electrophoresis and blotting.	Use their Hybond™ Nitrocellulose Membrane.	Use any nitrocellulose or PVDF membrane.
2. Block the nonspecific sites.	Add blocking reagent, incubate and wash.	Add blocking reagent, incubate and skip the wash!
3. Add diluted primary antibody; incubate for 1 hour, then wash.	Optimization Range: **1:100-1:1,500 dilution**	Optimization Range: **1:1,000-1:5,000 dilution**
4. Add diluted secondary antibody (HRP-labeled); incubate for 1 hour, then wash.	Optimization Range: **1:1,500-1:50,000 dilution**	Optimization Range: **1:20,000-1:100,000 dilution**
5. Prepare chemiluminescent substrate.	Mix equal volumes of both solutions.	Mix equal volumes of both solutions.
6. Incubate the substrate on the blot.	Incubate blot with Working Solution without agitation for precisely 1 minute.	Incubate blot with Working Solution with agitation for ~5 minutes.
	It's recommended that you work quickly once GE's ECL Working Solution has been added to the membrane.	The signal lasts for hours, so take your time!
7. Expose to film.	Immediately expose to film for 1 minute.	Expose to film for 1 minute.

References

Ju, T., *et al.* (2002). *J. Biol. Chem.* **277**, 178-186.
Kagan, A., *et al.* (2000). *J. Biol. Chem.* **275**, 11241-11248.
Messenger, M.M., *et al.* (2002). *J. Biol. Chem.* **277**, 23054-23064.

Ordering Information

Product #	Description	Pkg. Size
34078	SuperSignal West Pico Chemiluminescent Substrate *Sufficient materials for 10,000 cm² membrane.* Includes: Luminol/Enhancer Stable Peroxide Buffer	1 L 500 ml 500 ml
34080	SuperSignal West Pico Chemiluminescent Substrate *Sufficient materials for 5,000 cm² membrane.* Includes: Luminol/Enhancer Stable Peroxide Buffer	500 ml 250 ml 250 ml
34077	SuperSignal West Pico Chemiluminescent Substrate *Sufficient materials for 1,000 cm² membrane.* Includes: Luminol/Enhancer Stable Peroxide Buffer	100 ml 2 x 25 ml 2 x 25 ml
34079	SuperSignal West Pico Chemiluminescent Substrate Trial Kit *Sufficient materials for 500 cm² membrane.* Includes: Luminol/Enhancer Stable Peroxide Buffer	50 ml 25 ml 25 ml

Thermo Scientific SuperSignal Western Blotting Kits

For convenience and ease of use, nothing beats a complete Western blotting kit!

The Standard Detection Kits provide:

- HRP-conjugated Anti-Rabbit IgG, Anti-Mouse IgG or NeutrAvidin Biotin-Binding Protein
- SuperSignal West Pico Substrate

The Complete Detection Kits provide:

- HRP-conjugated Anti-Rabbit IgG, Anti-Mouse IgG or NeutrAvidin Biotin-Binding Protein
- SuperBlock Blocking Buffer
- TBS Wash Buffer
- SuperSignal West Pico Substrate

Ordering Information

Product #	Description	Pkg. Size
Standard Detection Kits		
34082	SuperSignal West Pico Mouse IgG Detection Kit	Kit
34083	SuperSignal West Pico Rabbit IgG Detection Kit	Kit
34085	SuperSignal West Pico Biotinylated Protein Detection Kit	Kit
Complete Detection Kits		
34081	SuperSignal West Pico Complete Mouse IgG Detection Kit	Kit
34084	SuperSignal West Pico Complete Rabbit IgG Detection Kit	Kit
34086	SuperSignal West Pico Complete Biotinylated Protein Detection Kit	Kit

For a list of kit components, visit our website and search on the product #.

Featured Product: Thermo Scientific SuperSignal West Dura Extended Duration Substrate

Specially formulated for use with CCD cameras.

SuperSignal West Dura Extended Duration Substrate meets the needs of researchers using cooled charge-coupled device (CCD) technology. Cooled CCD cameras, which offer the advantages of instant image manipulation, higher sensitivity, greater resolution and a larger dynamic range than film, eliminate the need for film processing equipment and a darkroom. However, this technology requires a substrate that produces an intense signal that is strong enough, and of long enough duration, to be captured by the cameras. By combining 24-hour light emission with ultraintensity, SuperSignal West Dura Substrate allows researchers to take full advantage of all the features offered by imaging instruments. SuperSignal West Dura Substrate provides the maximum light duration, allowing multiple extended exposures. We performed an experiment to compare SuperSignal West Dura Substrate with GE Healthcare Amersham ECL Plus Substrate using the manufacturers' protocols. Recombinant mouse IL-2 (0.003-50 ng) was applied to a polyacrylamide gel and electrophoresed. The proteins were transferred to PVDF for the GE Healthcare ECL Plus Substrate and to nitrocellulose for the SuperSignal Substrate. The primary antibody for both substrates was used at a 1 µg/ml.

The secondary antibodies were used at 10 ng/ml for SuperSignal West Dura Substrate and 20 ng/ml for GE's ECL Plus Substrate. A five-minute film exposure produced a high signal-to-noise ratio for the SuperSignal West Dura System with detection down to 3 pg (Figure 7A) but produced high background for the ECL Plus Substrate and detection down to only 800 pg (Figure 7C). A 30-minute exposure at F1.6 on the CCD camera demonstrated detection down to 12.5 pg with the SuperSignal Product (Figure 7B). When the GE Healthcare ECL Plus Blot was exposed to the CCD camera at F1.6, the exposure was stopped at 15 minutes because of the intense background. Signal was difficult to distinguish above background (Figure 7D).

Highlights:

- **24-hour light emission** – 10 times longer than other enhanced chemiluminescent substrates for HRP; make multiple exposures for publication-quality blots
- **Great sensitivity** – see bands you've never been able to see before with femtogram-level sensitivity
- **Maximize your antibody** – antibodies can be diluted much further when using SuperSignal West Dura Extended Duration Substrate than with other chemiluminescent substrates; perform 25- to 50-times more blots
- **Intense signal** – generated immediately and easily detected on film or chemiluminescent imager systems
- **Stable** – working solution stable for at least 24 hours; kit stable for at least one year and shipped at ambient temperature

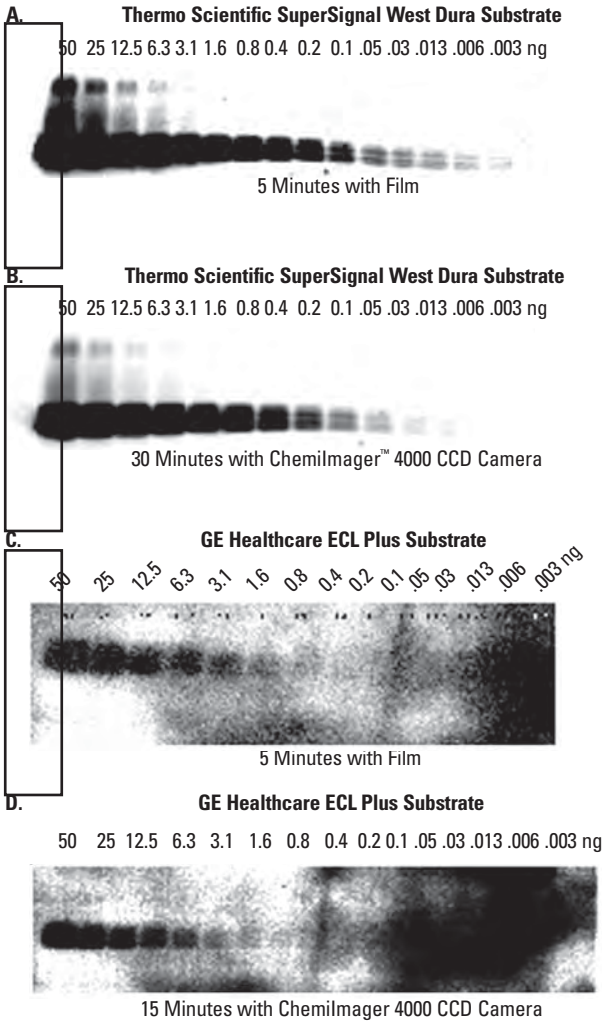


Figure 7. Better sensitivity and less background. The membranes were blocked and incubated with Anti IL-2 antibody (1 µg/ml). After washing, the membranes were incubated with secondary antibody (10 ng/ml). The membranes were washed and incubated with substrates that were prepared according to the manufacturer's instructions. Each membrane was exposed to X-ray film for 5 minutes. The SuperSignal West Dura Substrate membrane was exposed to the ChemiImager 4000 for 30 minutes (7B) and the GE Healthcare ECL Plus Blot was exposed for 15 minutes (7D).

Reference

Tokumaru, H., *et al.* (2001). *Cell* **104**, 421-432.

Ordering Information

Product #	Description	Pkg. Size
34076	**SuperSignal West Dura Extended Duration Substrate** *Sufficient materials for 2,000 cm² membrane.* Includes: Luminol/Enhancer Stable Peroxide Buffer	200 ml 100 ml 100 ml
34075	**SuperSignal West Dura Extended Duration Substrate** *Sufficient materials for 1,000 cm² membrane.* Includes: Luminol/Enhancer Stable Peroxide Buffer	100 ml 50 ml 50 ml
37071	**SuperSignal West Dura Extended Duration Substrate Trial Kit** *Sufficient materials for 200 cm² membrane.* Includes: Luminol/Enhancer Stable Peroxide Buffer	20 ml 10 ml 10 ml

Featured Product: Thermo Scientific SuperSignal West Femto Maximum Sensitivity Substrate

True femtogram detection.

SuperSignal West Femto Maximum Sensitivity Substrate provides the ultimate sensitivity for Western blotting, allowing you to see protein bands that were never before visualized.

Highlights:

- **Sensitive** – reach low-femtogram detection limits (Figure 8), that's zeptomole-level detection
- **Economical** – conserve precious antibodies with up to 1:100,000 primary antibody dilutions and 1:500,000 secondary antibodies dilutions
- **Intense** – releases the most intense signal generated by chemiluminescent systems, making it easy to capture an image on film or via an imager system
- Quantitative over two orders of magnitude*

*Feissner, R., *et al.* (2003). *Anal. Biochem.* **315**, 90-94.

Lower detection limit

- Low-femtogram (10^{-15})
- Mid-zeptomole (10^{-20})

Signal duration

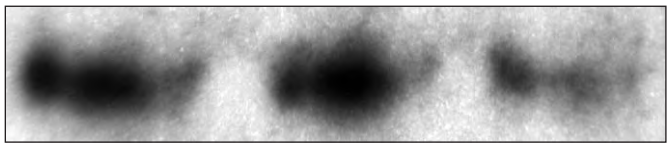
- 8 hours

Suggested antibody dilutions (from 1 mg/ml stock)

- Primary: 1:5,000-1:100,000
- Secondary: 1:100,000-1:500,000

Reagent stability

- 1 year at 4˚C or 6 months at RT



100 fg 10 fg 1 fg

Figure 8. True femtogram detection of IκBα using Thermo Scientific SuperSignal West Femto Maximum Sensitivity Substrate. Serially diluted samples from 100 to 1 fg were run on 4-20% Precise Precast Gels. The protein was then transferred to PVDF membrane and blocked with StartingBlock Blocking Buffer for 1 hour at room temperature (RT). The blot was incubated in Rabbit Anti-IκBα (1 mg/ml) at 1:1,000 dilution overnight at 4°C, followed by incubation in Goat Anti-Rabbit HRP (1 mg/ml) at 1:200,000 dilution for 1 hour at RT. The membrane was exposed to CL-XPosure Film for 1 minute.

Ordering Information

Product #	Description	Pkg. Size
34096	**SuperSignal West Femto Maximum Sensitivity Substrate** *Sufficient materials for 2,000 cm² membrane.* Includes: Luminol/Enhancer Solution Stable Peroxide Solution	200 ml 100 ml 100 ml
34095	**SuperSignal West Femto Maximum Sensitivity Substrate** *Sufficient materials for 1,000 cm² membrane.* Includes: Luminol/Enhancer Solution Stable Peroxide Solution	100 ml 50 ml 50 ml
34094	**SuperSignal West Femto Maximum Sensitivity Substrate Trial Kit** *Sufficient materials for 200 cm² membrane.* Includes: Luminol/Enhancer Solution Stable Peroxide Solution	20 ml 10 ml 10 ml

References

Adilakshmi, T. and Laine, R.O. (2002). *J. Biol. Chem.* **277**, 4147-4151.
Conti, L.R., *et al.* (2001). *J. Biol. Chem.* **276**, 41270-41278.
Guo, Y., *et al.* (2001). *J. Biol. Chem.* **276**, 45791-45799.

Table 4. A comparison of Thermo Scientific Chemiluminescent Substrates.

	Pierce ECL Substrate	SuperSignal West Pico Chemiluminescent Substrate	SuperSignal West Dura Extended Duration Substrate	SuperSignal West Femto Maximum Sensitivity Substrate
Primary Benefit	• The same signal intensity at half the price of competing ECL Substrates	• Twice the signal for about half the price of competing products	• Extended signal duration is ideal for use with imaging equipment	• The most sensitive chemiluminescent substrate for HRP detection available
Lower Detection Limit*	• Low-microgram (10^{-6}) • High-picomoles (10^{-11})	• Low-picogram (10^{-12}) • Mid-attomole (10^{-17})	• Mid-femtogram (10^{-14}) • High-zeptomole (10^{-19})	• Low-femtogram (10^{-15}) • Mid-zeptomole (10^{-20})
Signal Duration	• 30 minutes-2 hours	• 6-8 hours	• 24 hours	• 8 hours
Suggested Antibody Dilutions**	• Primary: 1:100-1:5,000 • Secondary: 1:1,000-1:15,000	• Primary: 1:1,000-1:5,000 • Secondary: 1:20,000-1:100,000	• Primary: 1:1,000-1:50,000 • Secondary: 1:50,000-1:250,000	• Primary: 1:5,000-1:100,000 • Secondary: 1:100,000-1:500,000
Room Temperature (RT) Working Solution Stability	• 1 hour	• 24 hours	• 24 hours	• 8 hours
Stock Solution Shelf Life	• 1 year at 4°C	• 1 year at RT	• 1 year at RT	• 1 year at 4°C or 6 months at RT

*Lower detection limits were determined using Streptavidin-HRP or Biotinylated-HRP as the ligand.
**Please follow recommended antibody dilutions. SuperSignal Substrates are much more sensitive than other substrates, so it is critical that you follow these guidelines. Failure to do so could result in unsatisfactory results. Dilutions are from a 1 mg/ml stock solution.

Chemiluminescent Substrates

Featured Product: Lumi-Phos WB Chemiluminescent Substrate

A chemiluminescent substrate for AP detection that provides the best of both worlds – high sensitivity and low background.

Lumi-Phos WB Substrate provides sensitivity in the low picogram range (Figure 9), enabling you to detect mere attomoles of your target ligand. Lumi-Phos WB Substrate also produces less background noise than other popular chemiluminescent substrates for AP, providing a better signal-to-noise ratio and a clearer image. Because signal generation is immediate, there's no need to wait 15 to 30 minutes for a measurable signal.

Figure 9. Lumi-Phos Substrate provides high sensitivity and low background. Serial dilutions of recombinant mouse IL-2 were separated electrophoretically on a 4-20% SDS-polyacrylamide gel. The separated protein was then transferred to nitrocellulose membrane followed by blocking. The membranes were subsequently incubated in a 1:500 (1 µg/ml) dilution of purified Rat Anti-Mouse IL-2, followed by a 1:5,000 (200 ng/ml) dilution of AP-labeled Goat Anti-Rat IgG. The membranes were washed and then incubated in Lumi-Phos WB Substrate for five minutes before film exposure.

Highlights:

- **High sensitivity** – able to detect 1.2 pg or 71 attomoles of the target ligand mouse IL-2
- **Low background** – high signal-to-noise ratios produce clear blots
- **Inexpensive** – less expensive than other AP substrates (based on 2007 U.S. list prices) and there is no need to purchase additional enhancers for nitrocellulose membranes
- **Long signal duration** – allows you to redevelop blots over and over
- **Immediate strong signal** – no more waiting 15 to 30 minutes for the signal to become strong enough to detect
- **Ready to use** – no mixing required with this one-component system

Ordering Information

Product #	Description	Pkg. Size
34150	Lumi-Phos WB Chemiluminescent Substrate *Sufficient materials for 1,000 cm² membrane.*	100 ml

References

Capasso, J.M., *et al.* (2003). *P. Natl. Acad. Sci. USA.* **100**, 6428-6433.
Ha, S-A., *et al.* (2003). *Mol. Biol. Cell.* **14**, 1319-1333.
Liu, R.Y., *et al.* (2000). *J. Biol. Chem.* **275**, 21086-21093.
Tikhonov, I., *et al.* (2003). *J. Virol.* **77**, 3157-3166.

Table 5. Thermo Scientific Pierce Substrates guide.

Substrate	Product #	Measurement / Color	Dilution Range of Antibody (From 1 mg/ml stock)	Approximate Sensitivity*	Enzyme
Pierce ECL Substrate	32106	425 nm chemiluminescent	1° 1:100-1:5K 2° 1:1K-15K	10 pg	HRP
SuperSignal West Pico Substrate	34080	425 nm chemiluminescent	1° 1:1K-1:5K 2° 1:20K-100K	1 pg	HRP
SuperSignal West Dura Substrate	34075	425 nm chemiluminescent	1° 1:1K-1:50K 2° 1:50K-250K	250 fg	HRP
SuperSignal West Femto Substrate	34095	425 nm chemiluminescent	1° 1:5K-1:100K 2° 1:100K-500K	1 fg	HRP
Lumi-Phos Substrate	34150	440 nm chemiluminescent	1° 1:200-1:2K 2° 1:5K-1:25K	15 pg	AP
Pierce TMB-Blotting Substrate	34018	Dark blue PPT	1° 1:500 2° 1:2K-20K	1 ng	HRP
Pierce 4-CN Substrate	34012	Blue-purple PPT	1° 1:500 2° 1:2K-20K	1 ng	HRP
Pierce CN/DAB Substrate	34000	Black PPT	1° 1:500 2° 1:2K-20K	1 ng	HRP
Pierce DAB Substrate	34001	Brown PPT	1° 1:500 2° 1:2K-20K	1 ng	HRP
Pierce Metal Enhanced DAB Substrate	34065	Brown-black PPT	1° 1:500 2° 1:2K-20K	20 pg	HRP
Pierce BCIP Substrate	34040	Blue-purple PPT	1° 1:250 2° 1:2.5K	100 pg	AP
Pierce NBT Substrate	34035	Blue-purple PPT	1° 1:250 2° 1:2.5K	100 pg	AP
Pierce NBT/BCIP Substrate	34042	Black-purple PPT	1° 1:500 2° 1:2.5K	30 pg	AP
Pierce NBT/BCIP + Suppressor Substrate	34070	Black-Purple PPT	1° 1:500 2° 1:2.5K	30 pg	AP

* Actual sensitivity is unique to each antibody-antigen pair. The approximate sensitivities listed are conservative amounts that should be easily detectable for most antigens.
1°= Primary, 2°= Secondary, PPT = precipitate, HRP = horseradish peroxidase, AP = alkaline phosphatase

Data Imaging

There are several methods for capturing data generated from chemiluminescent Western blots, including X-ray film, cooled CCD cameras and phosphorimagers that detect chemiluminescence. Cooled CCD cameras, which offer the advantages of instant image manipulation, greater resolution and a larger dynamic range than film, also eliminate the need for a darkroom and film-processing equipment.

Although electronic data capture with digital cameras and imagers is growing in popularity as the technologies improve and equipment prices decline, most of the data obtained from Western blotting with chemiluminescence is still captured on film. Often, it is necessary to expose several films for different time periods to obtain the proper balance between signal and background. The goal is to time the exposure of the membranes to the film so that the desired signal is clearly visible while the background remains low. This is difficult to accomplish because the process cannot be observed and stopped when the desired endpoint is reached. If the film is not exposed long enough (underexposed), the signal will not be visible. If the film is exposed too long (overexposed), the signal may be lost in the background or separate bands may become blurred together. An overexposed film can be "fixed" by incubating it in Pierce Background Eliminator Solution (Product # 21065), which effectively decreases the background without altering the integrity of the data. This is done at the lab bench while watching the film and the process can be halted when the signal is clearly visible and background is at a minimum. For more information on this method, see page 63.

Most instrument companies know and recommend SuperSignal West Substrates over other chemiluminescent substrates for use in their instruments.

Troubleshooting tips for chemiluminescence and cooled CCD cameras

- SuperSignal West Dura and SuperSignal West Femto Substrates are the recommended substrates for use in imaging instruments.
- SuperSignal West Pico Substrate will work in imaging instruments, but sensitivity may not be as good as it is with film.
- Imagers sometimes require longer exposure times than required by film to obtain similar images.
- Background is less of an issue in many of these instruments; therefore, higher antibody concentrations may be used to achieve the best image in the shortest exposure time.
- No darkroom is necessary when using imaging instruments. The instruments have their own light-proof boxes.
- Refer to the instrument manufacturer's instructions for more information on an individual instrument.

Featured Product: **Thermo Scientific CL-XPosure Radiography Film**

Save 65-75% on film!

Highlights:

- Up to one-third the price of competitive products (Table 1)
- Provides the same detection sensitivity as other commercially available films (Figure 1)
- Available in 5 x 7", 8 x 10", 9.5 x 11.8", 14 x 17" or 18 x 24 cm sheets, in packages of 25, 50 or 100 non-interleaved sheets

Reference

Tikhonov, I., *et al.* (2003). *J. Virol.* **77**, 3157-3166.

Thermo Scientific CL-XPosure Film | Kodak® BioMax® MR-1 Film | Kodak X-Omat® Blue (XB) Film

Figure 1. Thermo Scientific CL-XPosure Film vs. Kodak Film. Three types of X-ray film were tested using identical Western blotting conditions (2 blue, 1 grey). The results showed no appreciable difference between any of these films. The only significant difference is the cost-per-sheet of film (Table 1).

Table 1. Cost comparison of 5 x 7" sheets.

Product	Cost-per-sheet (U.S. Price)
Thermo Scientific CL-XPosure Film (Blue X-ray Film)	**$0.93**
Kodak X-Omat Blue (XB) Film (Blue X-ray Film) (Perkin Elmer)	**$2.91**
Kodak BioMax MR-1 (Gray X-ray Film) (GE Healthcare)	**$3.70**

Source: 2007 Online Catalogs

Ordering Information

Product #	Description	Pkg. Size
34090	CL-XPosure Film, 5 x 7 in (13 x 18 cm)	100/pkg.
34092	CL-XPosure Film, 5 x 7 in (13 x 18 cm)	25/pkg.
34089	CL-XPosure Film, 7 x 9.5 in (18 x 24 cm)	100/pkg.
34091	CL-XPosure Film, 8 x 10 in (20 x 25 cm)	100/pkg.
34093	CL-XPosure Film, 8 x 10 in (20 x 25 cm)	50/pkg.
34097	CL-XPosure Film, 9.5 x 11.8 in (24 x 30 cm)	100/pkg.
34099	CL-XPosure Film, 14 x 17 in (35 x 43 cm)	100/pkg.

Specialized Western Blotting Kits

Specialized Western Blotting Kits

In additional to our traditional SuperSignal Western Blotting Substrates and kits, we offer specialized kits for the detection of histidine-tagged proteins, phosphoproteins, *O*-Glc-NAc post-translational modifications, multiple target proteins on a single Western blot, and target proteins to verify siRNA Reagent gene knockdown. Reach for Thermo Scientific Pierce Protein Detection Products for specificity, sensitivity, speed and convenience.

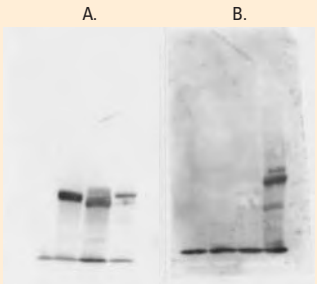
Featured Product: Thermo Scientific SuperSignal West Pico HisProbe™ Kit

Specific detection of histidine-tagged fusion proteins.

This chemiluminescent system uses HisProbe-HRP chemistry to overcome the limitations of anti-histidine antibodies and other detection strategies. HisProbe-HRP is more specific for polyhistidine tags, reducing background problems. Unlike anti-His antibodies, HisProbe-HRP can recognize polyhistidine tags independent of adjacent tags.

Highlights:

- **Specific** – more specific for the detection of histidine-tagged fusion proteins than anti-His antibodies (Figure 1)
- **Fast** – one-step probe incubation eliminates the lengthy two-step primary/secondary antibody sequential reaction protocol
- **Sensitive** – when used in combination with SuperSignal West Chemiluminescent Substrates, this kit allows the detection of even low-expression histidine-tagged clones



- More versatile than anti-polyHis antibody-based systems; the HisProbe Kit detects polyhistidine fusion proteins that are undetectable using some monoclonal anti-polyHis antibodies
- Sufficient reagents for fifty 7.5 x 10 cm blots

Figure 1. Specificity comparison of polyhistidine-tagged (PHT) fusion protein detection methods. Panel A using HisProbe-HRP shows high specific binding and low background. Panel B using anti-polyHis failed to recognize two of the three fusion proteins.

References

Adler, J. and Bibi, E. (2004). *J. Biol. Chem.* **279**, 8957-8965.
Kanaya, E., *et al.* (2001). *J. Biol. Chem.* **276**, 7383-7390.
Kiick, K.L., *et al.* (2002). *P. Natl. Acad. Sci. USA.* **99**, 19-24.
Sylvester, S.R. and Roy, A. (2002). *Biol. Reprod.* **67**, 895-899.

Ordering Information

Product #	Description	Pkg. Size	U.S. Price
15165	HisProbe-HRP	2 mg	$153
15168	SuperSignal West Pico HisProbe Kit	Kit	$366
	Includes: HisProbe-HRP	2 mg	
	SuperSignal West Pico Chemiluminescent Substrate	500 ml	
	Blocker™ BSA in TBS (10X)	1 x 125 ml	
	BupH Tris Buffered Saline Packs	10 x 500 ml	
	Surfact-Amps 20 (10%) Ampules	6 x 10 ml	

Featured Product: Thermo Scientific Phosphoprotein Detection Reagent and Kit

Novel chemistry enables specific detection of phosphorylated protein.

PhosphoProbe™-HRP is an iron (Fe^{3+})-activated derivative of horseradish peroxidase (HRP). PhosphoProbe-HRP exhibits two distinct binding specificities, one of which is phosphate ($R\text{-}PO_3$)-specific. The other binding specificity is related to a carboxyl-containing binding motif that is common to most proteins and some peptides. This carboxyl motif binding specificity can be used in a total protein detection application. A novel treatment, reactive chemical blocking (RCB), may be used to eliminate this carboxyl-binding motif, thus imparting exclusive specificity toward phosphate groups. PhosphoProbe-HRP, in conjunction with RCB, is a universal phosphate detection probe. PhosphoProbe-HRP has been optimized for direct detection of phosphoester molecules such as nucleotides or protein/peptides containing phosphoserine, phosphothreonine and phosphotyrosine.

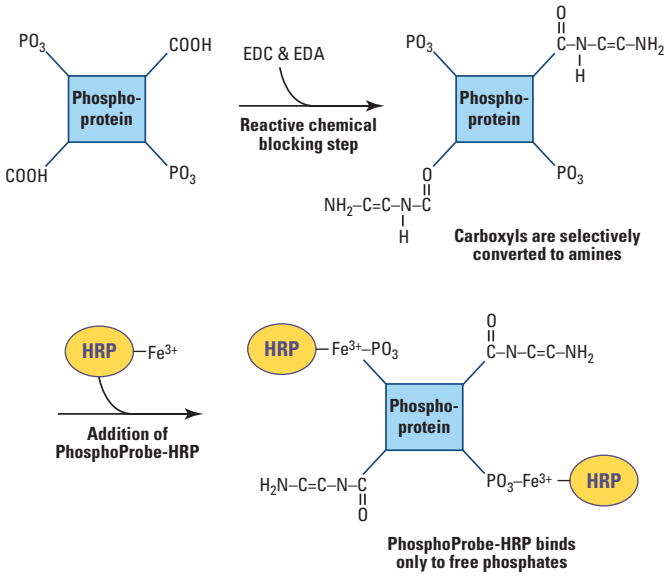


Figure 2. Chemistry for PhosphoProbe-HRP specificity toward phosphate groups. EDC and ethylenediamine are used to eliminate the carboxyl-binding motif on proteins, resulting in phosphate-specific reactivity of the iron-derivatized HRP.

Ordering Information

Product #	Description	Pkg. Size
15167	PhosphoProbe Phosphorylated Protein Detection Kit	Kit
	Includes: PhosphoProbe-HRP	2 mg
	EDC	5 g
	Ethylenediamine	10 g
	Tween-20	1 vial

Thermo Scientific Pierce *O*-GlcNAc Western Blot Detection Kit

High specificity monoclonal against O*-GlcNAc.*

The Thermo Scientific Pierce *O*-GlcNAc Western Blot Detection Kit contains the most highly specific mouse monoclonal antibody available for the detection of the *O*-GlcNAc post-translational modification. Reaction of the monoclonal antibody in this Western blotting kit is confined to the β-*O*-linked serine or threonine GlcNAc modification. There is no cross-reactivity with the α-*O*-GlcNAc linkage, the α/β-*O*-GalNAc modification or the other *N*-linked oligosaccharides (Figure 3).

Speed and sensitivity of chemiluminescent detection

Chemiluminescent detection with SuperSignal West Dura Extended Duration Substrate allows visualization of *O*-GlcNAc-modified proteins in less than one minute after exposure of the blot to X-ray film. In addition to speed, this kit is sensitive to the low picomole range.

Performance validated on Jurkat cell lysates

This Western blot kit also includes the popular Thermo Scientific M-PER® Mammalian Cell Lysis Reagent; an HRP-labeled, anti-IgM antibody conjugate; blocking buffer; and wash buffer components all validated to perform as specified.

Highlights:

- Kit includes MAb CTD 110.6, the most specific monoclonal antibody for the detection of β-*O*-linked *N*-acetylglucosamine (*O*-GlcNAc)
- Detection of the target modification confined to only β-*O*-linked serine or threonine
- No cross-reactivity with the α-*O*-GlcNAc linkage

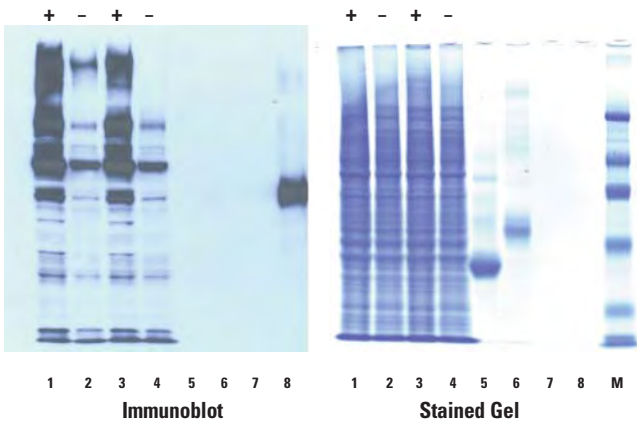


Figure 3. Western blot detection of *O*-GlcNAc-modified proteins after SDS-PAGE. Lanes 1-4 are proteins from the Jurkat cell extract. Lanes 5, 6 and 7 are the negative controls: ovalbumin (5 µg), fetuin (5 µg) and *O*-β-GalNAc-modified BSA (10 ng). Lane 8 is *O*-β-GlcNAc-modified BSA (5 ng, positive control). The (+) and (-) refer to plus and minus treatment with PUGNAc and glucosamine, and M represents the molecular weight marker (Pierce Blue Prestained Protein Molecular Weight Marker Mix, Product # 26681).

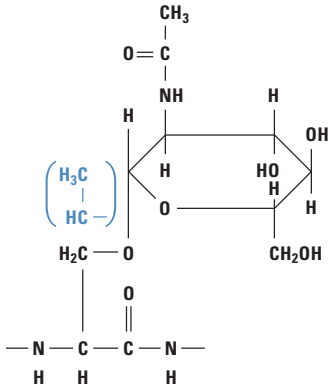


Figure 4. β-*O*-GlcNAc Modified Serine/ Threonine in Peptide Linkage

Ordering Information

Product #	Description	Pkg. Size
24565	***O*-GlcNAc Western Blot Detection Kit** *Sufficient material to develop up to 10 mini-blots.*	Kit
	Includes: M-PER Mammalian Protein Extraction Reagent Dilution	25 ml
	Buffer (10X) – Blocking Buffer	2 x 50 ml
	BupH Phosphate Buffered Saline	17 packs
	Surfact-Amps 20 (10% Tween-20 Solution)	3 x 10 ml
	Anti O-GlcNAc Monoclonal Antibody (MAb CTD 110.6) in ascites	100 µl
	Goat anti-Mouse IgM(µ), HRP Conjugate	75 µg
	SuperSignal West Dura Extended Duration Substrate	100 ml

Note: This Western blot kit is shipped in a single box as a two-part kit. **Part A** contains some components that require storage at 4°C upon arrival. **Part B** contains only the *O*-GlcNAc-specific monoclonal antibody. This MAb is shipped on dry ice to ensure it maintains integrity during transit. Upon its arrival, store it at -20°C.

Thermo Scientific DyLight 549/649 Western Blotting Kit

Fluorescent Western blotting made easy!

The fluorescent detection of two different targets on a single Western blot is easy to perform with the Thermo Scientific DyLight 549/649 Western Blotting Kit. This highly optimized and convenient format saves you time and the frustration of having to evaluate reagents for compatibility with fluorescent Western blotting (Table 1). The kit contains sufficient reagents for 10 Western blots and includes Pierce Dual-Labeled Protein Molecular Weight Markers† and secondary antibodies conjugated to DyLight 549 and 649 Fluorescent Dyes.

Highlights:

- **DyLight 549 excitation/emission maxima** – 550/568 nm
- **DyLight 649 excitation/emission maxima** – 646/674 nm
- **Optimized format** – provides low background and high signal intensity (Figure 5, next page)
- **Convenient** – saves time and money associated with optimizing fluorescent Western blots

Specialized Western Blotting Kits

Table 1. Recommended instruments for in-gel and Western blot detection using Thermo Scientific DyLight Fluors.

Company	Instrument	Excitation (nm)	Emission (nm)	Thermo Scientific DyLight 549 Dye	Thermo Scientific DyLight 649 Dye
Kodak	Image Station[1] 2000MM[1]	535/625	600/700	✓	✓
	Image Station 4000MM	535/625	600/700	✓	✓
Bio-Rad	Molecular Imager® FX (FX Pro)	532/635	605/695	✓	✓
Amersham	Typhoon 9410[1]	532/633	580/670	✓	✓
	Typhoon 9400	532/633	580/670	✓	✓
	Typhoon 9210	532/633	580/670	✓	✓
	Typhoon 9200	532/633	580/670	✓	✓
	Storm® 830	635	670	Not compatible	✓
	Storm 860	635	670	Not compatible	✓
Fuji	FLA-3000	532/633	570/675	✓	✓
	FLA-5100	532/633	570/675	✓	✓
	FLA-8000	532/633	570/675	✓	✓

1. Thermo Scientific DyLight Dye performance has been evaluated with this instrument. Compatibility of other instruments is based on manufacturers' specifications.

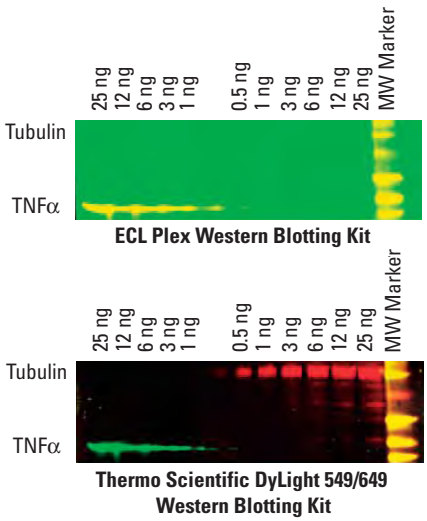


Figure 5. The Thermo Scientific DyLight 549/649 Western Blotting Kit provides lower background and higher signal in two-color Western blot detection compared to a competing fluorescent Western blotting kit. Proteins were separated in 4-20% Precise Protein Gels and transferred to low-fluorescence PVDF membrane. The membranes were blocked overnight in 1% BSA and target proteins were detected following manufacturer-recommended protocols. Membranes were imaged with the Typhoon 9410.

Ordering Information

Product #	Description	Pkg. Size
22854	**DyLight 549/649 Western Blotting Kit** *Sufficient reagents for 10 Western blots.* Includes: DyLight 549 Goat Anti-Mouse IgG (H+L) DyLight 649 Goat Anti-Rabbit IgG (H+L) Fluorescent Dual-labeled Protein MW Markers Wash Buffer (30X) Blocker BSA in PBS (10X) Low-Fluorescence PVDF Transfer Membrane	Kit 50 µl 50 µl 60 µl 200 ml 50 ml 10 each
Complementary Product		
22860	**Low-Fluorescence PVDF Transfer Membrane** 0.2 µm, 7 cm x 8.4 cm	10/pkg.

Thermo Scientific DyLight 680/800 Near Infrared Western Blotting Kit

Compatible with LI-COR Odyssey and other fluorescent imaging systems.

Near infrared fluorescent detection of two different targets on a single Western blot is easy to perform with the Thermo Scientific DyLight 680/800 Western Blotting Kit. The kit provides highly optimized reagents and a convenient format to save you the time and frustration of having to evaluate reagents for compatibility with fluorescent Western blotting (Figure 6). Each kit contains sufficient reagents for 10 Western blots and includes Pierce Dual-Labeled Protein Molecular Weight Markers† and secondary antibodies conjugated to DyLight 680 and 800 Fluorescent Dyes.

Highlights:

- **DyLight 680 excitation/emission maxima** – 682/715 nm
- **DyLight 800 excitation/emission maxima** – 770/794 nm
- **Optimized format** – provides low background and high signal intensity
- **Convenient** – saves time and money associated with optimizing fluorescent Western blots

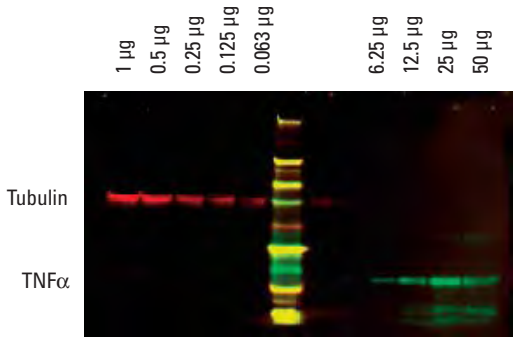


Figure 6. The Thermo Scientific DyLight 680/800 Western Blotting Kit provides low background and high signal in two-color Western blot detection. Proteins were separated in a 4-20% Precise Protein Gel and transferred to a low-fluorescence PVDF membrane. The membrane was blocked overnight in SEA BLOCK Blocking Buffer and target proteins were detected according to the manufacturer's protocol. Membranes were imaged with the LI-COR Odyssey Infrared Imaging System. Tubulin was detected from the indicated quantity of HeLa cell lysate. Purified TNFα was detected at the indicated quantity.

Ordering Information

Product #	Description	Pkg. Size
22855	**DyLight 680/800 Western Blotting Kit** *Sufficient reagents for 10 Western blots.* Includes: DyLight 680 Goat Anti-Mouse IgG (H+L) DyLight 800 Goat Anti-Rabbit IgG (H+L) Fluorescent Dual-labeled Protein MW Markers Wash Buffer (30X) SEA BLOCK Blocking Buffer Low-Fluorescence PVDF Transfer Membrane	Kit 120 µl 120 µl 30 µl 200 ml 500 ml 10 each
Complementary Product		
22860	**Low-Fluorescence PVDF Transfer Membrane** 0.2 µm, 7 cm x 8.4 cm	10/pkg.

† See patent information on inside back cover.

For more information, or to download product instructions, visit www.thermo.com/pierce

Thermo Scientific SuperSignal siRNA Chemiluminescent Detection Modules

Optimized tools for human gene-specific knockdown and accurate protein detection.

Thermo Scientific scientists behind Pierce Western blotting technology and Dharmacon RNAi technology have teamed up to create siRNA/antibody modules and a chemiluminescent detection module optimized for the specific knockdown of mRNA transcripts and detection of the effect on protein expression via Western blotting. The team's efforts enable you to focus on the science rather than worrying about time-consuming RNAi method validation and optimization work.

Highlights:

- Complete, validated solutions for human gene-specific knock-down by siRNA and subsequent protein detection by antibody in a Western blot
- Dharmacon ON-TARGET*plus*® *SMART*Pool® siRNA Reagent for potent gene knockdown and greatly reduced off-target effects
- SuperSignal Chemiluminescent Substrate for sensitive detection of target protein
- Modules utilize the highest quality antibodies available, providing the best results
- SuperSignal siRNA/Antibody Modules are optimized for use with the SuperSignal siRNA Chemiluminescent Detection Module
- Quickly growing number of modules for relevant genes in cellular pathway analysis and drug discovery

siRNA/Antibody Module Components:

Each module contains the following components for the specific target X

- ON-TARGET*plus SMART*Pool siRNA for X, 5 nmol
- ON-TARGET*plus* si*CONTROL*® Non-Targeting Pool, 5 nmol
- si*GLO*® Green Transfection Indicator, 5 nmol
- 5X siRNA Buffer, 1.5 ml
- Anti-X Protein Antibody, (10 mini blots)
- Detailed Protocol

siRNA Chemiluminescent Detection Module Components:

Sufficient materials for 10 (10 cm x 10 cm) blots.

- SuperSignal Luminol/Enhancer, 50 ml
- SuperSignal Stable Peroxide, 50 ml
- Blocking Buffer (1X), 500 ml
- Wash Buffer Concentrate (30X), 50 ml
- HRP Conjugate, 120 µl
- Detailed Protocol

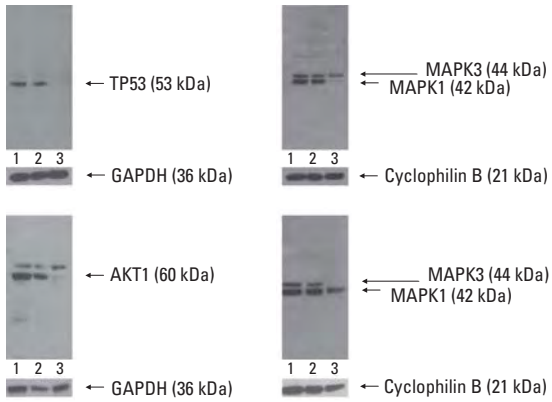


Figure 7. Western blot results for RNAi experiments with four different Thermo Scientific SuperSignal siRNA Chemiluminescent Modules. A549 cells were transfected and then lysed after 72 hours. The lysates were then run on a gel, transferred to nitrocellulose membrane and subjected to a typical Western blot protocol. Cells were transfected with Transfection Reagent alone (Lane 1), 100 nM ON-TARGET*plus* si*CONTROL* Non-Targeting Pool (Lane 2) or 100 nM ON-TARGET*plus SMART*Pool siRNA (Lane 3). Western blot data for GAPDH or Cyclophilin B are included as a control for equal protein loading.

Ordering Information

Product #	Description	Pkg. Size
82200	SuperSignal siRNA Chemiluminescent Detection Module	Kit

See table below for Product # and Targets

SuperSignal siRNA/Antibody Module for Target X — Kit

Product #	Target X	Product #	Target X	Product#	Target X
82217	AKT1	82271	CDKN2A	82210	MAPK3
82247	AKT2	82241	CHEK1	82258	MDM2
82262	ARF6	82227	CHUK	82228	MET
82211	ATM	82272	CSNK2A1	82202	MYC
82246	ATR	82218	CTNNB1	82259	NCK1
82263	AURKB	82254	E2F1	82216	PLK1
82264	BAX	82219	EGFR	82260	PPP2CA
82209	BCL2	82255	EIF2AK2	82276	PRKCA
82223	BCL2L1	82205	EP300	82277	PRKDC
82283	BID	82239	ERBB2	82233	PTEN
82250	BIRC5	82273	FOXO1A	82242	PTK2
82238	BRCA1	82243	FRAP1	82278	RB1
82251	CASP3	82249	GSK3B	82222	RELA
82265	CASP8	82226	HDAC1	82235	RHOA
82266	CASP9	82240	HIF1A	82282	RIPK1
82231	CAV1	82207	HRAS	82232	SMAD2
82267	CCNB1	82256	HSPB1	82279	SMAD3
82215	CCND1	82221	IKBKB	82280	SMAD4
82268	CCNE1	82274	ILK	82213	SP1
82229	CDC2	82206	JUN	82204	SRC
82237	CDK2	82257	KIF11	82245	STAT1
82212	CDK4	82236	MAP2K1	82225	STAT3
82269	CDK5	82275	MAP3K7	82201	TP53
82252	CDK6	82203	MAPK1	82281	TTK
82270	CDK9	82208	MAPK14	82261	VIL2

Please visit www.thermo.com/Pierce for an up-to-date list of the targets.

Far-Western Blotting

Far-Western Blotting

Studying protein interactions by far-Western blotting

Far-Western blotting was developed to screen protein expression libraries with ^{32}P-labeled glutathione S-transferase (GST)-fusion protein. Far-Western blotting is now used to identify protein:protein interactions. In recent years, far-Western blotting has been used to determine receptor:ligand interactions and to screen libraries for interacting proteins. With this method of analysis, it is possible to study the effect of post-translational modifications on protein:protein interactions, examine interaction sequences using synthetic peptides as probes and identify protein:protein interactions without using antigen-specific antibodies.

Far-Western blotting vs. Western blotting

Far-Western blotting is quite similar to Western blotting. In a Western blot, an antibody is used to detect the corresponding antigen on a membrane. In a classical far-Western analysis, a labeled or antibody-detectable "bait" protein is used to probe and detect the target "prey" protein on the membrane. The sample (usually a lysate) containing the unknown prey protein is separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) or native PAGE and then transferred to a membrane. When attached to the surface of the membrane, the prey protein becomes accessible to probing. After transfer, the membrane is blocked and then probed with a known bait protein, which usually is applied in pure form. Following reaction of the bait protein with the prey protein, a detection system specific for the bait protein is used to identify the corresponding band (Table 1).

Specialized far-Western analysis

By creative design of bait protein variants and other controls, far-Western blotting can be adapted to yield highly specific information about protein:protein interactions. For example, Burgess, *et al.* used a modified far-Western blotting approach to determine sites of contact among subunits of a multi-subunit complex. By an "ordered fragment ladder" far-Western analysis, they were able to identify the interaction domains of *E. coli* RNA polymerase β' subunit. The protein was expressed as a polyhistidine-tagged fusion, then partially cleaved and purified using a Ni^{2+}-chelate affinity column. The polyhistidine-tagged fragments were separated by SDS-PAGE and transferred to a nitrocellulose membrane. The fragment-localized interaction domain was identified using a ^{32}P-labeled protein probe.

Importance of native prey protein structure in far-Western analysis

Far-Western blotting procedures must be performed with care and attention to preserving as much as possible the native conformation and interaction conditions for the proteins under study. Denatured proteins may not be able to interact, resulting in a failure to identify an interaction. Alternatively, proteins presented in non-native conformations may interact in novel, artificial ways, resulting in "false-positive" interactions. The prey protein in particular is subjected to preparative processing steps for far-Western blotting that can have significant effects on detection of protein:protein interactions. This is not to imply that identification of valid interactions is not possible, but only to stress the importance of appropriate validation and use of controls.

Table 1. Comparison of Western blotting and far-Western blotting methods.

Step	Western Blotting	Far-Western Analysis
Gel Electrophoresis	Native or denaturing (usually)	Native (usually) or denaturing
Transfer System	Optimal membrane and transfer system determined empirically	Optimal membrane and transfer system determined empirically
Blocking Buffer	Optimal blocking system determined empirically	Optimal blocking system determined empirically
Detection (several possible strategies)* (Arrows designate sequence of steps in the detection strategy)	Unlabeled primary antibody→ Enzyme-labeled secondary antibody→ Substrate reagent	Unlabeled bait protein→ Enzyme-labeled bait-specific antibody→ Substrate reagent
	Enzyme-labeled primary antibody→ Substrate reagent	Radiolabeled bait protein→ Exposure to film
	Biotinylated antibody→ Enzyme-labeled streptavidin→ Substrate reagent	Biotinylated bait protein→ Enzyme-labeled streptavidin→ Substrate reagent
		Fusion-tagged bait protein→ Tag-specific antibody→ Enzyme-labeled secondary antibody→ Substrate reagent

* Antibodies are generally enzyme-labeled (either HRP or AP). By contrast, bait proteins generally are not enzyme-labeled because enzymes are large and likely to sterically hinder unknown binding sites between bait and prey proteins. Other labeling and detection schemes are possible.

Critical Steps in Far-Western Analysis

Gel electrophoresis

Separation of proteins by SDS-PAGE (i.e., denaturing conditions with or without a reducing agent) offers more information about molecular weight, presence of disulfides and subunit composition of a prey protein, but may render the prey protein unrecognizable by the bait protein. In these cases, the proteins may need to be subjected to native electrophoresis; i.e., nondenaturing and without reducing agent.

Transfer to membrane

After separation on the gel, proteins are electrophoretically transferred from the gel to a membrane in two to 16 hours. The type of membrane (e.g., nitrocellulose or PVDF) used is critical, as some proteins bind selectively or preferentially to a particular membrane. The efficiency and rate of protein transfer is inversely proportional to the molecular weight of the protein. In some cases, transfer conditions alter the shape of the protein and destroy or sterically hinder the protein interaction site. For Far-Western analysis, it is essential that at least the interaction domain of the prey protein is not disrupted by the transfer or is able to re-fold on the membrane to form a three-dimensional (3-D) structure comprising an intact interaction site. Generally, a significant percentage of the protein population renatures upon removal of SDS. When SDS is eliminated during the transfer process, transferred proteins generally renature with greater efficiency and are, therefore, more easily detected by far-Western blotting. In the event that the protein is unable to re-fold to create an intact binding site, it may be necessary to add a denaturation/renaturation step to the procedure or to perform the protein:protein interaction in-gel without transfer. (See In-Gel Far-Western Detection section that follows.) Denaturation/renaturation is typically accomplished using guanidinium hydrochloride.

Blocking buffer

After protein transfer, unreacted binding sites on the membrane are blocked with a non-relevant protein solution. In addition to blocking all remaining binding sites on the membrane, a blocking buffer reduces nonspecific binding and aids in protein renaturation during the probing procedure. A variety of different protein blockers may be used, and no one blocking protein solution will work for all blotting experiments. Any given protein blocker may cross-react or otherwise disrupt the specific probing interaction being studied. Determination of an effective blocking buffer must be made empirically. Often, bovine serum albumin (BSA) is used as a starting point for many membrane-probing reactions. Insufficient blocking may result in high background, whereas prolonged blocking could result in a weak or masked signal. Renaturation of the protein also appears to occur during the blocking step so it is important to optimize the blocking conditions to obtain the best signal-to-noise ratio for each application and then not deviate from the method.

Binding and wash conditions

Protein:protein interactions vary, depending on the nature of the interacting proteins. The strength of the interactions may depend on the pH, salt concentrations and presence of certain co-factors during incubation with the bait protein. Some protein:protein interactions may also require the presence of additional proteins. Whatever the necessary conditions, they will need to be maintained throughout the procedure to sustain the interaction until it can be detected. This may influence the formulation of washing buffer used between probing steps.

Controls

The appropriate controls are essential to distinguish true protein:protein interaction bands from nonspecific artifactual ones. For example, experiments involving detection with recombinant GST fusion proteins should be replicated with GST alone. A bait protein with a mutation in the predicted interaction domain can be processed as a control to determine specificity of the protein:protein interaction.

A non-relevant protein processed alongside the prey protein sample acts as a negative control. Ideally, the control protein would be of similar size and charge to the protein under investigation and would not interact nonspecifically with the bait protein.

In approaches that use a secondary system for detection of the prey protein, such as enzyme-labeled streptavidin with a biotinylated bait protein, it is important to include a duplicate control membrane that is probed only with the labeled streptavidin. This reveals any bands resulting from endogenous biotin in the sample or nonspecific binding of the labeled streptavidin. When a fusion tag is used with a corresponding antibody, it is critical to probe one of the control membranes with the labeled antibody alone. This control helps to confirm that the relevant band is not from nonspecific binding of the labeled secondary antibody. To obtain meaningful results, appropriate test and control experiments are subjected to gel electrophoresis, transfer and probing in parallel.

In-Gel Far-Western Detection

Advantages of in-gel detection

Because of restrictions associated with the transfer process, blocking and the possibility of nonspecific binding of bait proteins to unrelated bands on the membranes, it is sometimes advantageous to perform far-Western detection within the gel. In this procedure, the gels are pre-treated with 50% isopropyl alcohol and water to remove SDS and to allow the prey protein to renature. The gel is then incubated with the bait protein, which is then detected with an HRP-tagged antibody or biotin-binding protein.

The same controls and experimental conditions necessary for optimization of membrane-based far-Westerns apply to in-gel detection. With in-gel detection, the blocking step can be eliminated but the "bait" protein and the labeled detection protein must be diluted in the blocking buffer to reduce nonspecific binding. Also, higher amounts of prey and bait proteins are often required for detection compared to membrane detection with the equivalent chemiluminescent substrate.

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Far-Western Blotting

Featured Product: Thermo Scientific Pierce Far-Western Protein:Protein Interaction Kits

Our two Far-Western Analysis Kits are optimized for detection both on-membrane or in-gel. One kit allows the detection of biotinylated bait proteins (Product # 23500) and the other allows for the detection of GST-tagged bait proteins (Product # 23505). Both kits include blocking and wash buffers, HRP-labeled detection protein (Streptavidin-HRP or Anti-GST- HRP), and an extremely sensitive formulation of Thermo Scientific Pierce In-Gel Chemiluminescent Substrate† optimized for both on-membrane and in-gel use.

Highlights:

- **On-membrane or in-gel detection options** – on-membrane detection offers greater sensitivity; in-gel detection offers speed and prevents problems associated with incomplete or inefficient transfer
- **Nonradioactive alternative for far-Western analysis** – reliable and sensitive biotin/streptavidin-HRP or anti-GST-HRP chemistry combined with chemiluminescent detection offers a practical and safe alternative to radiolabeling the bait protein
- **Useful interaction range** – kit targets moderate to strong associations between a prey and the biotinylated bait protein or GST-tagged probe protein
- **Primary antibody-free detection** – kit uses a biotinylated or GST-tagged protein as the probe, eliminating the need for antibody production
- **Compatible with both SDS-PAGE and native gels** – provides option to probe for prey proteins in a more native environment because reduced or denaturing systems may not always present an interface that promotes the intended interaction
- **Reduced nonspecific binding** – biotin/streptavidin-HRP systems demonstrate less nonspecific binding compared to antibodies directed against the bait protein; the anti-GST antibody conjugate is highly specific for the GST tag
- **Compatible with protein staining** – can be used for total protein staining after the chemiluminescent detection step, eliminating the need to run two gels

Ordering Information

Product #	Description	Pkg. Size
23500	**Pierce Far-Western Biotinylated-Protein:Protein Interaction Kit** *Materials and methods for the discovery, in-gel or on-membrane, of protein interactions using a biotinylated bait protein as the probe.*	10 mini gels
	Includes: Streptavidin-HRP	0.1 mg
	Dilution Buffer (10X)	50 ml
	BupH Phosphate Buffered Saline	17 packs
	10% Tween-20 Ampules	6 x 10 ml
	Pierce In-Gel Stable Peroxide	55 ml
	Pierce In-Gel Luminol Enhancer	55 ml
	Cellophane Exposure Sheets	10 pack
23505	**Pierce Far-Western GST-Protein:Protein Interaction Kit** *Materials and methods for the discovery, in-gel or on-membrane, of protein interactions using a GST-tagged bait protein as the probe.*	10 mini gels
	Includes: Anti-Glutathione S-Transferase (GST)-HRP	0.25 mg
	Dilution Buffer (10X)	50 ml
	BupH Phosphate Buffered Saline	17 packs
	10% Tween-20 Ampules	6 x 10 ml
	Pierce In-Gel Stable Peroxide	55 ml
	Pierce In-Gel Luminol Enhancer	55 ml
	Cellophane Exposure Sheets	10 pack

† See patent information on inside back cover.

In-Gel Western Detection

Detection of Difficult-to-transfer Proteins

The major reason that proteins are blotted or adsorbed onto a membrane for detection with an antibody is that the proteins on a membrane are more accessible to immunochemical reagents (antibodies, etc.) than are proteins within polyacrylamide gels. A recent advance in the field of Western blotting involves immunodetection of proteins directly in the gel. This method, Thermo Scientific Pierce In-Gel Detection, circumvents the transfer and blocking steps entirely, enabling immunoblotting techniques to be applied to proteins that cannot be transferred efficiently from a gel to a membrane. Because there's no transfer step, no protein is lost in the process (Figure 1) and no artifacts are introduced into the data. This makes Pierce In-Gel Detection an ideal control experiment to confirm results obtained by Western blotting and to study proteins that cannot be transferred to a membrane.

Another feature of the Pierce In-Gel System is that it does not require a blocking step, eliminating the chance of cross-reactivity with the blocking buffer. This saves time because no blocking buffer optimization is necessary and background is often lower than with traditional Western blotting.

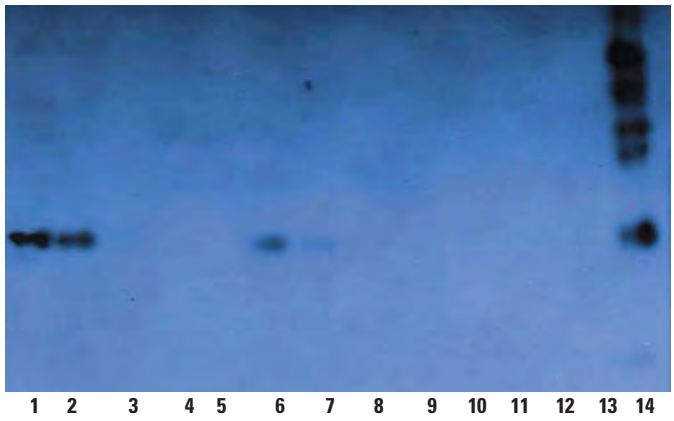

Figure 1. Protein left in a gel after transfer to a nitrocellulose membrane. Pure GFP/6xHis-tagged protein and *E. coli* bacterial GFP/6xHis-tagged lysate were separated by SDS-PAGE (Novex 10-20% Tris-Glycine gels). Gels were transferred to nitrocellulose membrane using the Bio-Rad® Mini Gel Transfer Unit. Following the transfer, the protein left in the gel was detected using the Thermo Scientific Pierce system with a 1:500 dilution of anti-Penta His antibody followed by a 1:250 dilution of HRP-labeled goat anti-mouse antibody. **Lanes 1-5**. *E. coli* bacterial GFP/6xHis-tagged lysate diluted 1:100, 1:250, 1:1,000, 1:2,000 and 1:4,000, respectively. **Lanes 6-13.** Pure GFP/6xHis-tagged protein at 12.5, 6.25, 3.12, 1.56, 1.0, 0.5, 0.1 and 0.05 ng, respectively. **Lane 14.** 6xHis-tagged ladder (1:16 dilution).

Featured Product: Thermo Scientific Pierce In-Gel Chemiluminescent Detection

Highlights

- Uniform representation of antigen(s) not skewed by inefficient transfer
- Compatible with stripping and reprobing protocols
- Compatible with protein staining
- Sensitive to 1 ng – comparable to an ECL Substrate

Benefits

- Many proteins, such as membrane proteins, do not transfer well to membranes; the Pierce In-Gel Detection Method prevents any problems associated with incomplete transfer
- When transferring to membranes, low MW proteins transfer more efficiently than higher MW proteins, often skewing results
- Transfer units, buffers, membranes and filter paper are eliminated
- Procedure can be optimized by stripping and reprobing without running another gel
- After immunodetection, the gel can be used for total protein staining; there's no need to run two gels
- The blocking step is omitted because the antibodies bind only specific antigens in the gel

In-Gel Western Detection

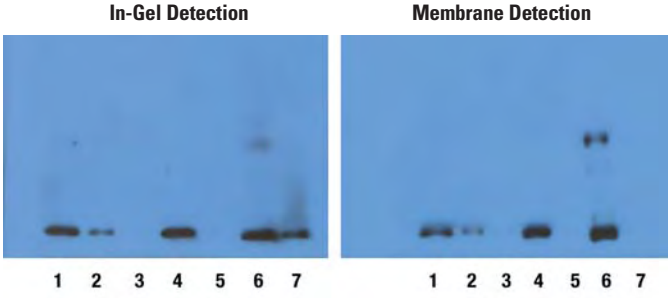

Figure 2. High sensitivity using Thermo Scientific Pierce In-Gel Detection. Pure GFP 6xHis-tagged and yeast GFP extract were separated by SDS-PAGE. One gel was transferred to nitrocellulose membrane. After transfer, the membrane was blocked overnight in 1% BSA. The other gel was pre-treated with 50% isopropanol. Antigens were detected using a 1:1,000 dilution of polyclonal Anti-Living Color Peptide Antibody, Rabbit (Clontech) and the Pierce In-Gel Chemiluminescent Detection Kit – Rabbit (Product # 33500). Signal was detected using Pierce In-Gel Detection Substrate. The lanes on the gel and in the membrane are as follows: **Lanes 1, 2 and 3** correspond to 10, 5 and 1 ng pure GFP/6xHis-tagged, respectively. **Lanes 4 and 5** correspond to *E. coli* bacterial GFP lysate diluted 1:100 and 1:1,000, respectively. **Lanes 6 and 7** correspond to yeast GFP Lysate diluted 1:10 and 1:100, respectively.

Note: The Pierce In-Gel Chemiluminescent Detection Kit has been tested successfully with Novex, FMC-BioWhittaker and Bio-Rad Criterion™ brand gels.

- The Pierce In-Gel Chemiluminescent Detection Kit does not perform well with Bio-Rad Ready Gel®, Precise Protein Gels or Gradipore iGel™ Gels; studies showed 25 times lower sensitivity and require individual optimization.
- The recommended gel thickness for use with this kit is 0.75-1.5 mm.
- The recommended crosslinking of gel is 8-18%, 4-20% or 10-20% gradient.

When using Pierce In-Gel Detection Technology with homemade gels, the glass plates must be siliconized before pouring the gel. Please visit our website to review the protocol and see other tips on optimizing the Pierce In-Gel Detection Method.

Ordering Information

Product #	Description	Pkg. Size
33500	**Pierce In-Gel Chemiluminescent Detection Kit – Rabbit**†	Kit
	Sufficient reagents to perform 10 mini-gel detections.	
	Includes: Pierce In-Gel Substrate	110 ml
	Stabilized Goat Anti-Rabbit-HRP	10 µl
	Dilution Buffer	50 ml
	BupH Pack PBS Buffer	17 packs
	10% Tween-20	5 x 10 ml
	Incubation Colander	1 unit
	Pre-cut Cellophane	10 sheets
	CL-XPosure Film (5" x 7")	25 sheets
33505	**Pierce In-Gel Chemiluminescent Detection Kit – Mouse**	Kit
	Includes same components as Product # 33500 except it contains Goat Anti-Mouse-HRP instead of Goat Anti-Rabbit-HRP	10 µl
33510	**Pierce In-Gel Chemiluminescent Detection Kit for Biotinylated Antibody Probes**	Kit
	Includes: Streptavidin-HRP	0.1 mg
	Dilution Buffer	50 ml
	Phosphate Buffered Saline	17 packs
	10% Tween-20	6 x 10 ml
	Pierce In-Gel Substrate	110 ml
	Cellophane Exposure Sheets	10 pack
33515	**Pierce In-Gel Chemiluminescent Detection Kit for GST-Tagged Proteins**	Kit
	Includes: Anti-Glutathione S-Transferase (GST)-HRP	0.25 mg
	Dilution Buffer	50 ml
	Phosphate Buffered Saline	17 packs
	10% Tween-20	6 x 10 ml
	Pierce In-Gel Substrate	110 ml
	Cellophane Exposure Sheets	10 pack
33550	**Pierce In-Gel Detection Chemiluminescent Substrate**	110 ml
33499	**Incubation Colander**	1 unit

† See patent information on inside back cover.

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Optimize the Signal-to-Noise Ratio

Signal-to-noise ratio (S/N ratio) refers to how much relevant content (signal) something has as opposed to non-relevant content (noise). The term is from the radio industry, but is often applied to Western blotting. In Western blotting, the signal is the density of the specific protein band being probed for; the noise is the density of the background. Optimizing the S/N ratio is often more important than increasing the sensitivity of the system. The sensitivity of the system is irrelevant if the signal cannot be distinguished from the noise.

The General Troubleshooting Guide in the next section contains many tips on optimizing the S/N, including a method of increasing the signal and lowering the background by optimizing antibody concentration. This process is made much easier by stripping and reprobing the membrane instead of starting from the beginning.

Stripping and reprobing a membrane

One of the major advantages offered by chemiluminescent detection is the ability to strip reagents from a blot and then reprobe the same blot. This is possible because the product detected is light rather than a colored precipitate on the membrane. A blot may be stripped and reprobed several times to visualize other proteins or to optimize detection of a protein (i.e., antibody concentrations) without the need for multiple gels and transfers. The key to this process is to use conditions that cause the release of antibody from the antigen without removing a significant amount of antigen from the membrane. Various protocols have been proposed to accomplish this task and they generally include some combination of detergent, reducing agent, heat and/or low pH. During the stripping procedure, some amount of antigen is inevitably removed from the membrane. It is important to minimize this effect by stripping the antibody under gentle conditions. Because each antibody-antigen pair has unique characteristics, there is no guaranteed method to remove every antibody while preserving the antigen. Restore Western Blot Stripping Buffer (Product # 21059) and Restore PLUS Western Blot Stripping Buffer (Product # 46430) were designed to achieve maximum removal of antibodies while preserving the integrity of the antigen. They are unique among stripping buffers because they are odor-free and can often strip a membrane in as little as 15 minutes (Figure 1).

Stripping and reprobing a Western blot instead of preparing an entirely new blot may be preferable because it:

- **Conserves sample**

  When the protein mixture is rare or valuable, reprobing conserves the sample and allows the membrane to be analyzed with the same or different antibodies.

- **Saves time**

  It is time-consuming to run an SDS-polyacrylamide gel and then transfer the proteins to a membrane. By using the same blot for several different detections, you save time.

- **Makes it easy to optimize**

  The light emission intensity of SuperSignal West Pico Substrate, and the increased sensitivity of SuperSignal West Dura and SuperSignal West Femto Substrates, often require antibody concentration optimization to achieve the highest quality blot. Optimization is achieved easily by stripping the membrane and reprobing with a different antibody concentration.

- **Saves money**

  By reusing the same blot, you save money on the costs of membrane, buffers and protein sample.

- **Makes it easy to confirm atypical results**

  When immunoblot results are not as expected, reprobing allows the use of the same protein sample without going back to gel electrophoresis.

- **Makes it easy to correct mistakes**

  Immunoblotting requires many steps, providing ample opportunity for mistakes to occur. By stripping the membrane, the blot can be reused.

After any stripping procedure, the blot should be tested to ensure that all of the detection reagents were removed. The membrane should be washed several times with blocking agent, incubated with secondary antibody, then reincubated with chemiluminescent substrate. If the primary antibody was effectively removed by the stripping procedure, no secondary antibody will bind to the membrane and no signal will be produced. If bands are still visible on the blot, the stripping conditions must be intensified. Often a simple increase of the reaction time or temperature will complete the stripping process. However, it is sometimes necessary to alter the composition of the stripping buffer or change methods entirely.

Optimize the Signal-to-Noise Ratio

Protocol for Stripping an Immunoblot

Note 1: Optimization of both incubation time and temperature is essential for best results.

Note 2: If the blot cannot be stripped immediately after chemiluminescent detection, store the blot in PBS at 4°C until ready to perform the stripping procedure.

1. Place the blot to be stripped in Restore Western Blot Stripping Buffer and incubate for 5-15 minutes at RT. Use a sufficient volume of buffer to ensure that the blot is completely wetted (i.e., approximately 20 ml for an 8 x 10 cm blot). Alternatively, the blot can be incubated with a solution of 2% w/v SDS, 62.5 mM Tris•HCl, 100 mM 2-mercaptoethanol, pH 6.8 for 30-90 minutes at 50-70°C. However, these reaction conditions are much harsher than Restore Western Blot Stripping Buffer and are more likely to interfere with future ligand:antibody interactions.

Note: In general, high-affinity antibodies will require at least 15 minutes of stripping and may require an incubation temperature of 37°C. Alternatively, use Restore PLUS Stripping Buffer, which is optimized for high-affinity antibodies.

2. Remove the blot from the Restore Western Blot Stripping Buffer and wash in Wash Buffer.

3. Test for the removal of the immunodetection reagents.

   A. To test for complete removal of the HRP label, incubate the membrane with SuperSignal West Working Solution and expose to film. If no signal is detected with a 5-minute exposure, the HRP conjugate has been successfully removed from the antigen or primary antibody.

   B. To test for complete removal of the primary antibody, incubate the membrane with the HRP-labeled secondary antibody, followed by a wash in wash buffer. Apply SuperSignal West Working Solution. If no signal is detected with a 5-minute exposure, the primary antibody has been successfully removed from the antigen.

   C. If signal is detected with experiment A or B, place the blot back into Restore Western Blot Stripping Buffer for an additional 5-15 minutes. Some antigen/antibody systems require an increase in temperature and/or longer incubation periods.

After determining that the membrane is free of immunodetection reagents, a second immunoprobing can begin.

Note 1: The Western blot can be stripped and reprobed several times, but it may require longer exposure times or a more sensitive chemiluminescent substrate. Subsequent reprobings may result in a decrease in signal if the antigen is labile in Restore Western Blot Stripping Buffer. Analysis of the individual system is required.

Note 2: Reblocking of the membrane is not critical, but it may be required in some applications.

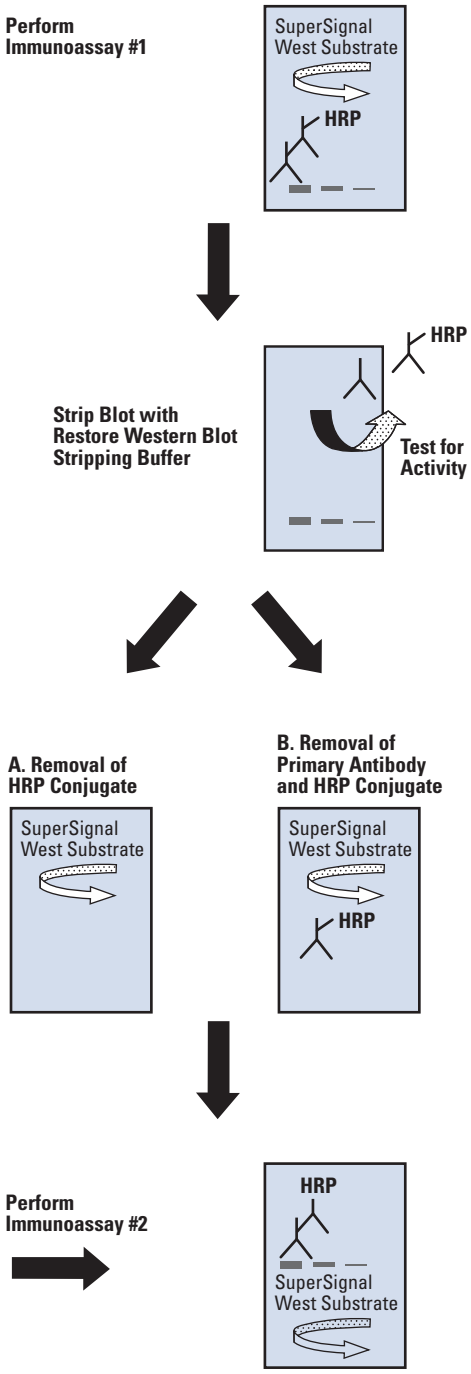


Figure 1. Thermo Scientific Restore Western Blot Stripping Buffer protocol.

Featured Product: **Thermo Scientific Restore Western Blot Stripping Buffer**

Strip time off your research with our stripping buffer.

Tired of re-running electrophoresis gels and waiting to see your results? Although optimizing assay conditions is best, re-performing the gel electrophoresis process to test each new primary antibody or antibody concentration is time-consuming and expensive. You can forget about starting over when you use Restore Western Blot Stripping Buffer!

Optimize assay conditions

Using Pierce SuperSignal West Substrates, the secondary antibody concentrations are optimized after a single stripping and re-probing cycle (Figure 2).

Test different primary antibodies

There's no need to waste precious sample and re-run a gel to test different primary antibodies. Simply strip the membrane with Restore Stripping Buffer to remove the antibodies. It takes only 5-15 minutes, depending on the affinity of the primary antibody. After stripping, re-probe with a new primary antibody and detect with SuperSignal Chemiluminescent Substrate (Figure 3).

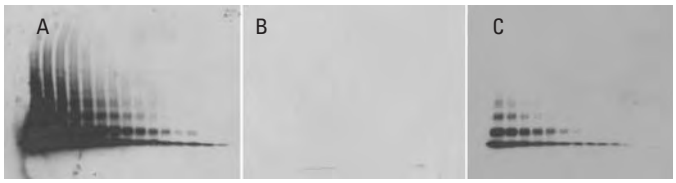


Figure 2. Antibody optimization study. Western blots of Interleukin-2 (diluted 20-0.156 ng) were detected using SuperSignal West Pico Chemiluminescent Substrate. The first blot (A) used the primary antibody diluted to 1:1,000 (0.5 µg/ml) of Rat Anti-Mouse IL-2 (BD PharMingen; San Jose, CA) and the horseradish peroxidase (HRP)-labeled Goat Anti-Rat secondary antibody (Product # 31470) diluted 1:5,000. The same blot was stripped with Restore Western Blot Stripping Buffer (B) for 5 minutes at room temperature and re-probed (C) with the primary antibody at 1:5,000 and the HRP-secondary conjugate at 1:20,000. SuperBlock Blocking Buffer was used for blocking.

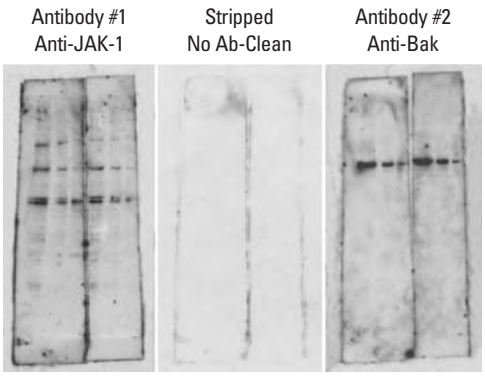


Figure 3. Re-probing with different antibodies. Western blots of HeLa cell lysate protein (diluted 750-83.3 ng) were detected with SuperSignal West Dura Chemiluminescent Substrate. The first blot used polyclonal rabbit anti-JAK-1 primary antibody (BD PharMingen; San Jose, CA) at 1:2,000 dilution with an HRP-secondary conjugate diluted at 1:350,000. The same blot was stripped for 5 minutes at room temperature in Restore Western Blot Stripping Buffer and then re-probed with purified Mouse Anti-Human Bak monoclonal primary antibody at 1:1,000 with the HRP-secondary conjugate at 1:100,000. Five-percent nonfat milk with 0.05% Tween-20 was used for blocking.

Highlights:

- **Saves time** – no need to re-run gels
- **Saves precious sample** – re-probe the membrane using the same target sample
- **Provides efficient removal** – proprietary formulation works better than "homemade" buffers
- **Gentle formulation** – does not damage target protein after stripping and re-probing
- **Odor-free** – no mercaptans means no acrid odors
- **Economical** – less expensive than other competing stripping buffers

References

Baolin Zhang, B., *et al.* (2003). *Mol. Cell. Biol.* **23**, 5716-5725.
Kaufmann, S.H., *et al.* (1987). *Anal. Biochem.* **161**, 89-95.
Kaufmann, S.H. and Kellner, U. (1998). Erasure of Western blots after autoradiographic or chemiluminescent detection. In Immunochemical Protocols. Ed. Pound, J.D. Humana Press, Totowa, NJ, 223-235.
Lanying Wen, L., *et al.* (2003). *Genetics.* **165**, 771-779.
Schrager, J.A., *et al.* (2002). *J. Biol. Chem.* **277**, 6137-6142.
Skurk, C., *et al.* (2004). *J. Biol. Chem.* **279**, 1513-1525.

Ordering Information

Product #	Description	Pkg. Size
21059	Restore Western Blot Stripping Buffer *Sufficient for stripping 25 (8 cm x 10 cm) blots.*	500 ml
21062	Restore Western Blot Stripping Buffer *Sufficient for stripping one (8 cm x 10 cm) blot.*	30 ml

Optimize the Signal-to-Noise Ratio

Featured Product: **Thermo Scientific Restore PLUS Western Blot Stripping Buffer**

A new formulation for high-affinity antibodies that require special treatment.

When researchers require a robust but gentle Western blotting stripping buffer, the original Restore Western Blot Stripping Buffer has been the buffer of choice. However, some antibodies remain difficult to remove from Western blots and require longer incubation times or incubation temperatures greater than 22°C. Restore PLUS Western Blot Stripping Buffer was developed to reduce incubation times while keeping incubations at room temperature. High-affinity antibodies can be quickly and effectively stripped from Western blots without removing transferred proteins, thereby allowing multiple reprobes of the target.

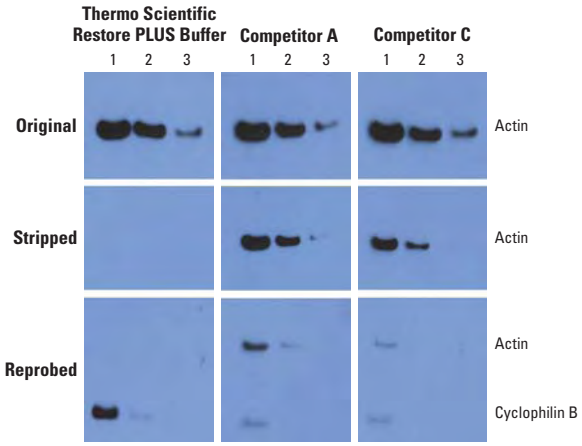


Figure 4. Reprobing with different antibodies. HeLa cell lysate was probed for actin and detected with Pierce ECL Substrate (Original panel). Blots were then stripped with either Restore PLUS Stripping Buffer or competitive stripping buffers (Stripped panel). The blots were then re-blocked and reprobed for cyclophilin B and detected with Pierce ECL Substrate (Reprobed panel).

Highlights:

Ready and easy to use
- No dilution necessary
- No offensive odors
- Store at room temperature

Compatible with commonly used Western blotting reagents and other materials
- Use on nitrocellulose and PVDF membranes, stored wet or dry
- Works with blocking buffer, enzyme conjugate and chemiluminescent substrate of choice

Cost effective
- Save valuable time and samples
- Strip blots effectively the first time

Robust, but gentle
- Transferred proteins remain viable
- Strip the same blot up to five times

Flexible
- Strip and re-probe to optimize antibody concentrations
- Strip and re-probe for new antigen of interest (Figure 4)

Ordering Information

Product #	Description	Pkg. Size
46428	Restore PLUS Western Blot Stripping Buffer *Sufficient reagent to strip one to two (8 cm x 10 cm) blots.*	30 ml
46430	Restore PLUS Western Blot Stripping Buffer *Sufficient reagent to strip 25 (8 cm x 10 cm) blots.*	500 ml
Complementary Products		
32106	Pierce ECL Substrate	500 ml
34080	SuperSignal West Pico Chemiluminescent Includes: Luminol/Enhancer Stable Peroxide Buffer	500 ml 250 ml 250 ml
34075	SuperSignal West Dura Chemiluminescent Substrate Includes: Luminol/Enhancer Solution Stable Peroxide Buffer HRP-Conjugated Goat Anti-Rabbit HRP HRP-Conjugated Goat Anti-Mouse HRP	100 ml 50 ml 50 ml 1 ml 1 ml
34095	SuperSignal West Femto Chemiluminescent Substrate Includes: Luminol/Enhancer Solution Stable Peroxide Solution HRP-Conjugated Goat Anti-Rabbit HRP-Conjugated Goat Anti-Mouse	100 ml 50 ml 50 ml 1 ml 1 ml

Featured Product: **Thermo Scientific Pierce Background Eliminator**

Another method by which the signal-to-noise (S/N) ratio can be improved is to "erase" the background on exposed film, leaving just the signal with little to no interference. Pierce Background Eliminator does just that without altering the integrity of the data. The Pierce Solution works on overexposed film, lightening the entire film evenly. This is done directly in the lab while viewing the film. No darkroom is required. The process can be halted when the signal is clearly visible and the background is at a minimum, thereby increasing the S/N ratio without altering the data's integrity (Figure 5).

Pierce Background Eliminator provides fast, easy removal of background image on exposed X-ray film for Western, Northern or Southern blots, so you can see your results clearly.

High background, shading, overexposed bands and speckling are problems inherent to film exposure. High background and shading can be caused by overexposure, poor use of blocking buffer or inappropriate enzyme-labeled probe or antibody concentration. Overexposed bands are a common occurrence when the enzyme-labeled probe or antibody used is too concentrated or if the film was exposed for too long. Speckling and shading occur when enzyme conjugates form complexes and precipitate on the blot. The Pierce Background Elimination Kit can correct all these problems without the need to re-expose your blot to film or re-do the experiment, allowing you to visualize your data within minutes (Figures 6-8). The Pierce Solution can be used with newly exposed films or exposed films that have been stored for years. In addition, the Pierce Kit can be used with any brand of film.

For applications requiring densitometric measurement, the Pierce Background Eliminator reduces signal evenly over the film so that relative densitometry values are consistent (Figure 7).

The procedure is simple. Immerse your exposed film in Pierce Background Eliminator Working Solution, watch for desired image and stop the reaction by rinsing the film in water. The Pierce Solution works quickly, with ideal signal level typically attained in just a few minutes.

A. Before using Thermo Scientific Pierce Background Eliminator

B. After using Thermo Scientific Pierce Background Eliminator

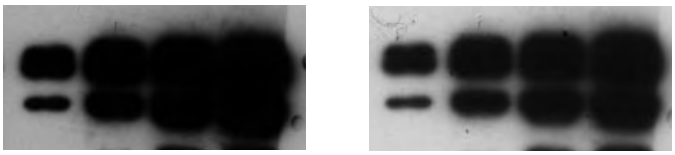


Figure 5. Thermo Scientific Pierce Background Eliminator lightens overexposed bands. Recombinant human wild-type p53 baculovirus lysate was separated on a 12% SDS-polyacrylamide gel. The proteins were transferred to a nitrocellulose membrane and blocked with SuperBlock Blocking Buffer in PBS (Product # 37515). The protein was detected with mouse anti-p53 followed by Goat anti-Mouse-HRP (Product # 31434) and SuperSignal West Pico Substrate (Product # 34080). The membrane was exposed to film for 1 minute (A). The film had overexposed bands and was treated with Pierce Background Eliminator for 6 minutes. The resulting image provided better visualization of the different p53 protein bands (B).

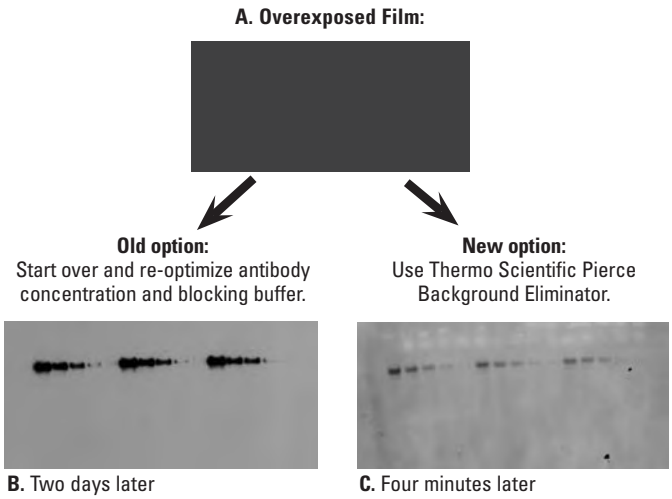


Figure 6. Thermo Scientific Pierce Background Eliminator lightens the entire film evenly in four minutes vs. the two days traditional methods require to start over and reoptimize experiment conditions. A431 cell lysate was electrophoresed on a 4-12% NuPage Gel (Novex) and transferred overnight to nitrocellulose. The membrane was blocked with SuperBlock Blocking Buffer in PBS (Product # 37515) for 1 hour and incubated with 1.25 ng/ml of HRP-labeled mouse anti-phosphotyrosine (PY20) for 1 hour. After the membrane was washed for 30 minutes, SuperSignal West Dura Substrate was added. The blot was exposed to film for 10 seconds and resulted in a completely black image caused by the antibody cross-reacting with the blocking buffer (A). Using the old option, another gel was prepared to optimize assay conditions. The proteins were transferred overnight and then the membrane was blocked with a 5% dry milk solution for 1 hour. The blot was detected with 2.5 ng/ml of anti-phosphotyrosine (PY20)-HRP and SuperSignal West Dura Substrate. The blot was exposed to film for 10 seconds. This optimization required a two-day procedure (B). Using the new option, the initial dark film (A) was treated with Pierce Background Eliminator to allow the band images to appear in 4 minutes (C).

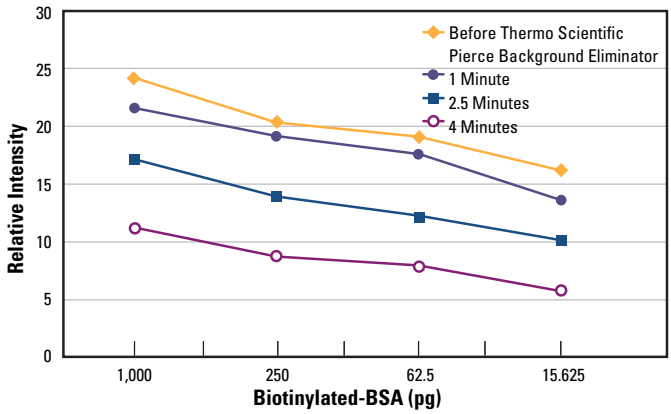


Figure 7. Densitometry data on dot blot comparing before and after use of the Thermo Scientific Pierce Background Eliminator. Dot blots were prepared on nitrocellulose (Product # 77010) using Biotinylated-BSA (Product # 29130) at 1,000, 250, 62.5 and 15.6 pg. The blot was blocked with SuperBlock Blocking Buffer in PBS (Product # 37515) and incubated with a 1/50,000 dilution of SA-HRP (Product # 21126). The blot was then washed for 30 minutes, incubated in SuperSignal West Pico Substrate (Product # 34080) and exposed to film (Product # 34092) for 5 minutes. The resulting film had high background that was cut into four strips each containing three replicates per concentration. The Background Eliminator Working Solution was used on separate film strips at 1, 2.5 and 4 minutes, leaving a control strip for comparison. After scanning on a densitometer, the relative signal intensity was compared. The signal intensity decreased evenly with time when treated with the Background Eliminator Solution maintaining similar slopes on a dose response curve.

Optimize the Signal-to-Noise Ratio

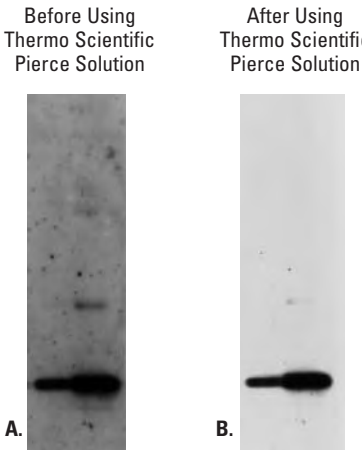


Figure 8. Thermo Scientific Pierce Background Eliminator erases speckling. Recombinant Human TNFα was electrophoresed on a 4-20% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane. The membrane was blocked and detected with Mouse anti-Human TNFα followed by Goat anti-Mouse-HRP (Product # 31434) and SuperSignal West Dura Substrate (Product # 34075). 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 eliminate the background speckling (B).

Highlights:

- Reduces signal evenly over the film – no "altering" of results
- Fast, easy background elimination from overexposed, speckled or shaded films
- Works with any X-ray film, new or old
- No need for time-consuming re-exposures to find the optimal image
- No need to re-optimize assay reagents to obtain the optimal image

Remove background from any application that uses X-ray film exposures including:

- Western, Northern and Southern blots that use SuperSignal Substrates and the Thermo Scientific Pierce Chemiluminescent Hybridization and Detection Kit
- In-gel detection systems
- Gel-shift assays
- Ribonuclease protection assays (RPA)

Ordering Information

Product #	Description	Pkg. Size
21065	Pierce Background Eliminator *Sufficient reagent to prepare 3 L of working solution.* Includes: Pierce Reagent A Pierce Reagent B	Kit 100 ml 100 ml

Blotting with Chemiluminescence

Troubleshooting Guide

Most of the time, troubleshooting a problem with any given Western blot system involves optimization of the amount of enzyme (normally an HRP-conjugated secondary antibody) in the system. The amount of enzyme is affected by a variety of factors, the most important of which are the amount of primary and secondary antibody used. Optimization of the antibody concentration is discussed on pages 37-38.

The most important aspect to remember when using a chemiluminescent substrate is that too much enzyme is detrimental to signal development. This is counter-intuitive to many people, especially to those accustomed to blotting with colorimetric systems, in which increasing the amount of enzyme increases the amount of color generated. In a colorimetric system, the enzyme permanently converts a non-colored substrate into a precipitated colored byproduct, but this is not what happens in chemiluminescent systems.

In chemiluminescent systems, the enzyme (HRP) converts the substrate (luminol) into a product that temporarily emits light. How much light is generated and how long the signal lasts depends on the ratio of the enzyme to the substrate. The amount of substrate is relatively constant, but the amount of enzyme changes depending on how much someone adds.

If not enough enzyme is added, then no signal is generated. If too much enzyme is added, the reaction between the enzyme and the substrate occurs so rapidly that there is a flash of light that can last mere seconds (Figure 1). The signal terminates before a picture can be taken. Too much enzyme is by far the primary cause of problems with a chemiluminescent Western blot. It is essential that you adhere to the substrate manufacturer's dilution instructions – not the antibody manufacturer's instructions – when determining antibody titer because most substrates require different concentration levels.

The following is a list of several indicators of too much enzyme:

1. Inconsistent signal length
   a. No signal (signal fades before it can be detected by an imaging system)
   b. Signal terminates quickly
   c. System gives inconsistent signal length from day to day; i.e., "it worked great yesterday, but not very well today" syndrome.

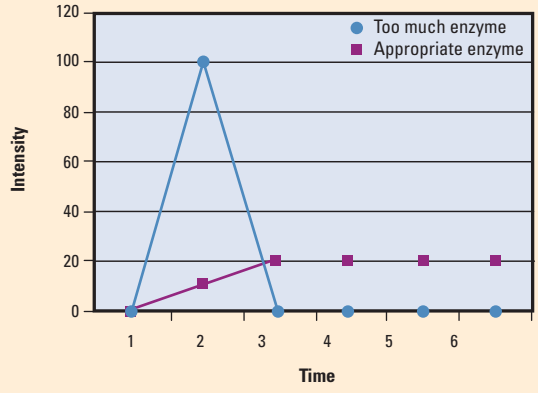


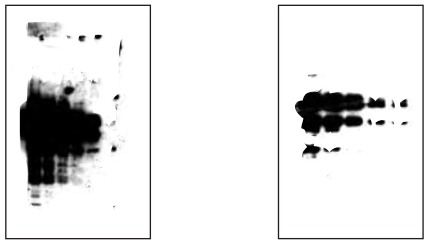
Figure 1. A signal that peaks and terminates quickly is usually caused by the use of too much enzyme.

2. Reverse image on the **film** (dark background with clear or "ghost" band where the protein of interest is expected)

Figure 2. A "ghosting" of bands where the protein of interest is expected could be caused by using too much enzyme.

3. Brown bands on the **membrane** where the protein of interest is expected

Figure 3. The appearance of brown bands where the protein of interest is expected could be caused by the use of too much enzyme.



Primary Antibody 1:500
Secondary Antibody 1:5,000

Primary Antibody 1:5,000
Secondary Antibody 1:50,000

4. High background and/or unwanted bands

Figure 4. High background and/or unwanted bands are often caused by using too much enzyme.

5. Bands glow visibly directly on the membrane – this should never occur and will certainly over-expose sensitive X-ray film

High Background that is Uniformly Distributed

Possible Causes	Precautions/Solutions
Antibody concentrations are too high	• The primary and/or secondary antibody can cause high background if the concentrations used are too high. • Decrease antibody concentrations.
Incompatible blocking buffer was used	• Compare different blocking buffers.
Insufficient blocking of nonspecific sites	• Optimize blocking buffer. The best blocking buffer is system-dependent. • Increase the concentration of protein in the blocking buffer. • Optimize blocking time and/or temperature. Block for at least 1 hour at RT or overnight at 4°C. • Add Tween-20 Detergent to blocking buffer. Use a final concentration of 0.05% Tween-20 Detergent. Skip this step if you use StartingBlock T20 Blocking Buffer in PBS (Product # 37539) or TBS (Product # 37543) or SuperBlock T20 Blocking Buffer in PBS (Product # 37516) or TBS (Product # 37536). These buffers already contain Tween-20 Detergent at optimized concentrations. • Prepare antibody dilutions in blocking buffer that contains 0.05% Tween-20 Detergent.
Cross-reactivity of antibody with other proteins in blocking buffer	• Use a different blocking buffer. • Do not use milk with avidin-biotin systems. Milk contains biotin. • Test for cross-reactivity. Block a clean piece of membrane, incubate with antibodies and then detect with SuperSignal Chemiluminescent Substrate. • Reduce the concentration of the HRP conjugate.
Insufficient washing	• Increase number of washes and the volume of buffer used. • Add Tween-20 Detergent to wash buffer if it's not already included. Use a final concentration of 0.05% Tween-20 Detergent. *(Caution: If the concentration of Tween-20 is too high, it can strip proteins off the membrane.)* Skip this step if you use StartingBlock T20 Blocking Buffer in PBS (Product # 37539) or TBS (Product # 37543) or SuperBlock T20 Blocking Buffer in PBS (Product # 37516) or TBS (Product # 37536). These buffers already contain Tween-20 Detergent at optimized concentrations.
Exposure time is too long	• Reduce the time the blot is exposed to film.
Membrane problems	• Make sure membranes are wetted thoroughly and according to the manufacturer's instructions. • Use new membranes. • Ensure the membrane is adequately covered with liquid at all times to prevent it from drying. • Use agitation during all incubations. • Handle membranes carefully – damage to the membrane can cause nonspecific binding. • Do not handle membrane with bare hands. Always wear clean gloves or use forceps.
Contamination or growth in buffers	• Prepare new buffers.

High Background that is Blotchy or Speckled

Possible Causes	Precautions/Solutions
Antibody concentrations are too high	• The primary and/or secondary antibody can cause high background if the concentrations used are too high. • Decrease antibody concentrations.
Aggregate formation in the HRP conjugate can cause speckling	• Filter the conjugate through a 0.2 µm filter. • Use a new, high-quality conjugate.
Incompatable blocking buffer was used	• Compare different blocking buffers.
Insufficient blocking of nonspecific sites	• Optimize blocking buffer. The best blocking buffer is system-dependent. • Increase concentration of protein in the blocking buffer. • Optimize blocking time and/or temperature. Block for at least 1 hour at RT or overnight at 4°C. • Add Tween-20 Detergent to blocking buffer. Use a final concentration of 0.05% Tween-20 Detergent. Skip this step if you use StartingBlock T20 Blocking Buffer in PBS (Product # 37539) or TBS (Product # 37543) or SuperBlock T20 Blocking Buffer in PBS (Product # 37516) or TBS (Product # 37536). These buffers already contain Tween-20 Detergent at optimized concentrations. • Make up antibody dilutions in blocking buffer with 0.05% Tween-20 Detergent.
Cross-reactivity of antibody with other proteins in blocking buffer	• Use a different blocking buffer. • Do not use milk with avidin-biotin systems. Milk contains biotin. • Test for cross-reactivity. Block a clean piece of membrane, incubate with antibodies and then detect with SuperSignal Chemiluminescent Substrate. • Reduce the concentration of the HRP conjugate.
Membrane was not wetted properly	• Wet membrane according to the manufacturer's instructions. • Do not handle membrane with bare hands. Always wear clean gloves or use forceps. • Use a new membrane. • Make sure the membrane is covered with a sufficient amount of liquid at all times to prevent it from drying. • Use agitation during all incubations. • Incubate membranes separately to ensure that membrane strips are not covering one another during incubations. • Handle membranes carefully – damage to the membrane can cause nonspecific binding.
Contamination in buffers	• Use new buffers. • Filter buffers before use.
Contaminated equipment	• Make sure electrophoresis equipment, blotting equipment and incubation trays are clean and free of foreign contaminants. • Make sure there are no pieces of gel left on the membrane after transfer. Proteins can stick to the pieces of gel and cause background.

Weak Signal or No Signal

Possible Causes	Precautions/Solutions
Proteins did not transfer properly to the membrane	• After transfer is complete, stain the gel with a total protein stain to determine transfer efficiency. *(Note: Total protein stains may not be able to detect low quantities of antigen.)* • Use Thermo Scientific Pierce Reversible Membrane Stain to check membrane for transfer efficiency. • Make sure there is sufficient contact between the gel and membrane during transfer. • Make sure the transfer sandwich is assembled correctly. • Be sure to follow the membrane manufacturer's instructions for wetting the membrane. • Make sure transfer unit does not overheat during electroblotting procedure. • Use positive control and/or molecular weight markers. • Optimize transfer time and current. • Use Pierce Lane Marker Sample Buffer. The tracking dye transfers to the membrane. • Make sure sample preparation conditions prior to blotting of the protein have not destroyed antigenicity of the sample. *(Caution: Some proteins cannot be run under reducing conditions.)*
Insufficient binding to membrane	• Adding 20% methanol to the transfer buffer helps binding. Low MW antigen may pass through the membrane. Use a membrane with a smaller pore size.
Insufficient amount of antibodies	• Increase antibody concentrations. Antibody may have poor affinity for the protein of interest. • Antibody may have lost activity. Perform a dot blot to determine activity.
Antibody concentrations are too high.	• Using too much primary or secondary antibodies can cause the signal to fade quickly, which appears as a weak signal.
Insufficient amount of antigen present	• Load more protein onto the gel.
The antigen is masked by the blocking buffer	• Try different blocking buffers. • Optimize blocking buffer protein concentration.
Buffers contain sodium azide	• Sodium azide is an inhibitor of HRP; do not use sodium azide as a preservative in buffers.
Exposure time is too short	• Lengthen the film exposure time. *(Note: SuperSignal Chemiluminescent Substrates will continue to glow for at least six hours.)*
Substrate incubation is too short	• A five-minute substrate incubation is required when using SuperSignal Substrates.
Inactive substrate	• SuperSignal West Pico Chemiluminescent Substrate and SuperSignal West Dura Chemiluminescent Substrate are stable for up to 12 months at RT. SuperSignal West Femto Chemiluminescent Substrate is stable for at least six months at RT. • To evaluate the substrate activity, prepare a small amount of working solution. In a darkroom, add a small amount of HRP conjugate. A blue light should be observed. If no glow is observed, either the substrate or the HRP conjugate is inactive. • Ensure that there is no cross-contamination between the two bottles of substrate. Contamination between the two substrate reagents can cause a decline in activity.
Membrane has been stripped and reprobed	• There may be some antigen loss or denaturation during membrane stripping procedures. Optimize stripping procedure. • Reprobe only when necessary. • Avoid repeated reprobing of the same membrane.
Digestion of antigen on the membrane	• Blocking substance may have proteolytic activity (e.g., gelatin).
Protein degradation from blot storage	• Prepare a new blot.

Nonspecific Bands

Possible Causes	Precautions/Solutions
Antibody concentrations are too high	• Reduce antibody concentrations.
SDS caused nonspecific binding to immobilized protein bands	• Wash blots after transfer. • Do not use SDS during immunoassay procedure.

Diffuse Bands

Possible Causes	Precautions/Solutions
Antibody concentrations are too high	• Reduce antibody concentrations.
Too much protein is loaded onto the gel	• Reduce the amount of protein loaded onto the gel.

Black Blots with White Bands or Signal That Decreases Quickly

Possible Causes	Precautions/Solutions
Antibody concentrations are too high	• Reduce antibody concentrations, especially the HRP conjugate. Signal that decreases quickly and the appearance of white bands are indications that there is too much HRP in the system.

Partly Developed Area or Blank Areas

Possible Causes	Precautions/Solutions
Incomplete transfer of proteins from the gel	• Make sure there are no air bubbles between the gel and membrane during transfer. • Wet membrane according to the manufacturer's instructions. • Do not handle the membrane with bare hands. Always wear clean gloves or use forceps. • Use a new membrane. • Incubate membranes separately to ensure that membrane strips are not covering one another during incubations.

Full-Length Western Blotting Protocol

Using Chemiluminescent Substrates

1. Make the protein solution of interest in a sample buffer and heat it to boiling for 5 minutes. The sample buffer should contain the following:
   - 0.03 M Tris•HCl
   - 5% SDS to denature the protein and to generate a constant anionic charge-to-mass ratio for the denatured protein chains
   - 50% glycerol to give the sample a higher density than the running buffer, allowing the sample to "sink" to the bottom of the well
   - A low-MW dye for dye-front determination
   - As needed, a reducing agent such as 100 mM 2-mercaptoethanol, dithiothreitol or TCEP that will reduce the disulfide bonds present in the protein sample

   Adjust solution to pH 6.8.
2. Add the protein solution in the sample buffer to an SDS-polyacrylamide gel.
3. Separate the proteins electrophoretically by MW.
4. Transfer the protein from the gel to a membrane.

Thermo Scientific Substrate	Recommended Membrane
Pierce ECL Substrate	Nitrocellulose or PVDF
SuperSignal West Pico Substrate	Nitrocellulose or PVDF
SuperSignal West Femto Substrate	Nitrocellulose or PVDF
SuperSignal West Dura Substrate	Nitrocellulose or PVDF
Lumi-Phos WB Substrate	Nitrocellulose

5. Remove the membrane blot and block the nonspecific sites with a blocking buffer for 20-60 minutes at RT with shaking. For best results, block for 1 hour at RT. Optimization of blocking buffer may be required to achieve best results. **Please see the Blocking Buffer Optimization section, page 11.**
6. Incubate the blot with the primary antibody with shaking for 1 hour. For recommended antibody dilutions, see the table below. If desired, blots can be incubated with primary antibody overnight at 2°C-8°C. The necessary dilution will vary depending on the primary antibody used and the amount of antigen that was transferred. **Please see the Optimize Antibody Concentration section, page 7.**

Thermo Scientific Substrate	Recommended Primary Antibody Dilutions (from 1 mg/ml stock)
Pierce ECL Substrate	1:100-1:5,000 or 0.2-10 µg/ml
SuperSignal West Pico Substrate	1:1,000-1:5,000 or 0.2-1.0 µg/ml
SuperSignal West Femto Substrate	1:5,000-1:100,000 or 0.01-0.2 µg/ml
SuperSignal West Dura Substrate	1:1,000-1:50,000 or 0.02-1.0 µg/ml
Lumi-Phos WB Substrate	1:200-1:2,000 or 0.5-5.0 µg/ml

7. Wash the membrane with wash buffer. Use at least four to six changes of the wash buffer and as large a volume as possible. For each wash, suspend the membrane in wash buffer and agitate for at least 5 minutes. Increasing the wash buffer volume and/or the number of washes might reduce background. Tris-buffered saline (TBS), phosphate-buffered saline (PBS) or another suitable wash buffer can be used. Including a final concentration of 0.05% Tween-20 to the wash buffer may also help reduce background.

Note 1: Briefly rinsing the membrane in wash buffer before incubation will increase the efficiency of the wash step.

Note 2: If using an enzyme-conjugated primary antibody, proceed directly to Step 10.

8. Incubate the blot with enzyme-conjugated secondary antibody or avidin for 1 hour with shaking at RT. For recommended antibody- or avidin-conjugate dilutions, see the table below. The necessary dilution will vary depending on the enzyme conjugate used, the primary antibody used in Step 6 and the amount of antigen that was transferred.

Thermo Scientific Substrate	Recommended Secondary Antibody Dilutions (from 1 mg/ml stock)
Pierce ECL Substrate	1:1,000-1:15,000 or 1-0.067 µg/ml
SuperSignal West Pico Substrate	1:20,000-1:100,000 or 10-50 ng/ml
SuperSignal West Femto Substrate	1:100,000-1:500,000 or 2.0-10 ng/ml
SuperSignal West Dura Substrate	1:50,000-1:250,000 or 4.0-20 ng/ml
Lumi-Phos WB Substrate	1:5,000-1:25,000 or 40-200 ng/ml

9. Repeat Step 7 to wash away any unbound enzyme-conjugated secondary antibody. It is crucial to thoroughly wash the membrane after the incubation with the enzyme conjugate.

10. If the working solution has not been prepared, prepare it now. For SuperSignal West Substrates, mix equal volumes of the Luminol/ Enhancer Solution and the Stable Peroxide Solution. Prepare a sufficient volume to ensure that the blot is completely wetted with substrate and the blot does not dry out. Lumi-Phos WB Substrate is provided in a ready-to-use format, but it should be brought to room temperature. **Recommended volume: 0.1 ml/cm^2 of blot surface.**

11. Incubate the blot with SuperSignal Substrate Working Solution for 5 minutes or with Lumi-Phos WB Substrate Working Solution for 3 minutes.

12. Remove the blot from the substrate working solution and place it in a plastic membrane protector. (A plastic sheet protector works very well, although plastic wrap may also be used.) Remove all air bubbles between the blot and the surface of the membrane protector.

13. Place the wetted blot against the film and expose. Standard autoradiographic film can be used. A recommended first exposure time is 60 seconds. Vary exposure time to obtain optimum results. The use of enhanced or pre-flashed autoradiographic film is unnecessary.

Note: If a cooled CCD Camera (e.g., Alpha Innotech Corporation's ChemiImager Camera) is used, longer exposure times may be necessary.

14. Develop the film using appropriate developing solution and fixative for the type of film used.

15. On an optimized blot, the light generated should last a minimum of six hours. The blot can be re-exposed to film, as needed, to obtain the optimal results. Longer exposure times may be necessary as the blot ages.

Recommended Reading

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Thermo Scientific SwellGel® Technology is protected by U.S. patent # 6,709,743.
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