do more. see more.

learn more.

Efficient, convenient and reliable products
for the entire Western blot workflow

Fisher Scientific

Thermo SCIENTIFIC

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Western blotting overview

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.

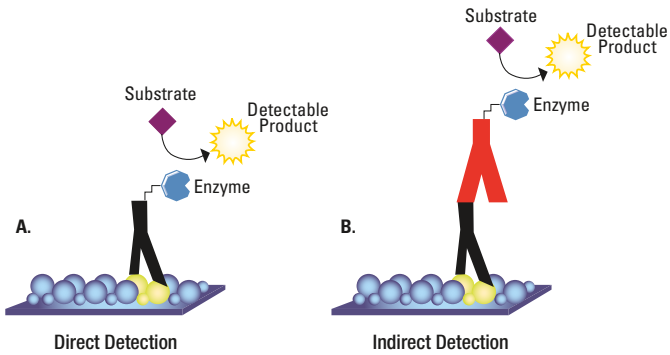


Figure 1.

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.

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 (HRP) or alkaline phosphatase (AP). The indirect method offers many advantages over the direct method (Table 2).

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 epitopes 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	

10 steps to do more. see more. learn more.

step 1

step 2

Gel Electrophoresis

The first step to a successful Western blot is to separate your proteins in your sample.

- Tris-Hepes-SDS Running Buffer (# PI28398)
- Lane Marker Reducing Sample Buffer (5X) (# PI39000)
- Lane Marker Non-Reducing Sample Buffer (5X) (# PI39001)
- LDS Sample Buffer, Non-reducing (4X) (# PI84788)
- Pierce Protein Ladders (many available, see pages 7–12)
- SuperSignal Molecular Weight Protein Ladders (#s PI84785 and PI84786)

Electro-Transfer

After electrophoresis transfer protein from the gel to a membrane using electrophoretic transfer.

- Pierce G2 Fast Blotter (# PI62288)
- Pierce 1-Step Transfer Buffer (#s PI84731 and PI84742)
- Methanol-Free Transfer Buffer (# PI35040)
- Fast Semi-Dry Transfer Buffer (# PI35035)
- Tris-Glycine Transfer Buffer (# PI28380)
- Pierce Reversible Protein Stain Kit for Nitrocellulose Membranes (# PI24580) and for PVDF Membranes (# PI24585)
- SuperSignal Western Blot Enhancer (#s PI46640 and PI46641)
- Pierce Western Blot Signal Enhancer (# PI21050)
- Pierce Antibody Extender NC (#s PI32110 and PI32105)
- Nitrocellulose Membrane, 0.2µm (#s PI77012, PI88013 and PI88024)
- Nitrocellulose Membrane, 0.45µm (#s PI77010, PI88014 and PI88025)
- PVDF Membrane, 0.45µm (#s PI88585 and PI88518)
- Low-fluorescence PVDF Membrane, 0.2µm (# PI22860)
- Western Blotting Filter Paper (# PI88600)
- Extra Thick Blotting Filter Paper (#s PI88605, PI88610, PI88615, PI88620)

step 3

step 4

Blocking

Next, block the unreacted sites on the membrane to reduce the amount of nonspecific binding.

- Protein-free Blocking Buffer (#s PI37570, PI37571, PI37572 and PI37573)
- StartingBlock Blocking Buffer in PBS (# PI37538) and in TBS (# PI37542)
- StartingBlock T20 Blocking Buffer (Contains 0.05% Tween-20) in PBS (# PI37539) or TBS (# PI37543)
- SuperBlock Buffer in PBS (#s PI37515 and PI37518) and in TBS (# PI37535)
- SuperBlock T20 Blocking Buffer (Contains 0.05% Tween-20) in PBS (# PI37516) or TBS (# PI37536)
- SuperBlock Blocking Buffer – Blotting in PBS (# PI37517) and in TBS (# PI37537)
- Pierce Fast Blocking Buffer (#s PI37575 and PI37576)
- Pierce Clear Milk Blocking Buffer (10X) (# PI37587)
- Casein in PBS (# PI37528) and in TBS (# PI37532)
- BSA in PBS (# PI37525) and in TBS (# PI37520)
- SEA BLOCK Buffer (# PI37527)
- BLOTTO in TBS (# PI37530)

Primary Incubation

Incubate the membrane with primary antibody.

Our antibodies are fully validated, eliminating the need to screen numerous antibodies to find the correct one.

Thermo Scientific™ Pierce™ Antibodies are developed for a wide variety of application needs. We offer over 40,000 antibodies for over 50 research areas and all of our antibodies are validated and guaranteed to perform in the stated application and species. We also offer Antibody conjugates available with Thermo Scientific™ DyLight™ Dyes, biotin, HRP, alkaline phosphatase and more. Our website enables you to easily search by protein target and then filter by the specific assays that interest you.

Our custom antibody services leverages our experience and proprietary antigen design tools to produce more robust antibodies.

Visit **thermoscientific.com/pierce-abs** to find your antibody.

Wash

Remove unbound primary reagents and reduce background.

Buffered Saline Solutions

- BupH Phosphate Buffered Saline Packs (# PI28372)
- Pierce 20X Phosphate Buffered Saline (# PI28348)
- BupH Tris Buffered Saline (#s PI28376, PI28358)
- Modified Dubecco's PBS Buffer (#s PI28344, PI28374)

Surfact-Amps Detergents

- Tween-20 Detergent (# PI28320)
- Tween-80 Detergent (# PI28328)
- Triton X-100 Detergent (# PI28314) NP-40 (# PI28324)

Skip this step if you use Thermo Scientific™ StartingBlock™ T20 Blocking Buffer in PBS or TBS or Thermo Scientific™ SuperBlock™ T20 Blocking Buffer in PBS or TBS. These buffers already contain Tween-20 Detergent at optimized concentrations.

Secondary Incubation

Incubate the membrane with secondary antibody.

Choose an appropriate secondary detection probe for your Western blot. Our secondary antibodies and detection reagents are available in a variety of formats and conjugated types including HRP, AP, DyLight Dyes and others. Check out our secondary antibody selection guide to find the secondary antibody or detection reagent that is right for you.

We also offer a complete line of Biotin-binding Proteins and Conjugates (Avidin, Streptavidin, etc.), Antibody-binding Proteins (Protein A, G, etc.) and Specialized Detection Probes and Kits. Visit **thermoscientific.com/Western** for a complete list.

thermoscientific.com/pierce-abs

Wash

Remove unbound secondary reagents and reduce background.

Buffered Saline Solutions

- BupH Phosphate Buffered Saline Packs (# PI28372)
- Pierce 20X Phosphate Buffered Saline (# PI28348)
- BupH Tris Buffered Saline (#s PI28376, PI28358)
- Modified Dubecco's PBS Buffer (#s PI28344, PI28374)

Surfact-Amps Detergents

- Tween™-20 Detergent (# PI28320)
- Tween™-80 Detergent (# PI28328)
- Triton™ X-100 Detergent (# PI28314) NP-40 (# PI28324)

Incubation with Substrate

Add the detection reagent to your secondary HRP or AP.

Chemiluminescent Substrates:

- Pierce ECL Substrate (#s PI32106, PI32209 and PI32109)
- Pierce Fast Western Blot Kits: SuperSignal West Pico Substrate (#s PI35065, PI35060, PI35066 and PI35061); SuperSignal West Dura Substrate (#s PI35075, PI35070, PI35076 and PI35071); SuperSignal West Femto Substrate (#s PI35080 and PI35081); ECL Substrate (#s PI35050 and PI35055)
- SuperSignal West Pico Chemiluminescent Substrate (#s PI34077 and PI34080); also available in an economical 1-L package (# PI34078)
- SuperSignal West Femto Maximum Sensitivity Substrate (#s PI34096 and PI34095)
- SuperSignal West Dura Extended Duration Substrate (#s PI34076 and PI34075)
- Lumi-Phos WB Substrate (# PI34150)
- Pierce ECL Plus Substrate (#s PI32132 and PI32134)

Colorimetric Substrates:

- Pierce Chloronaphthol (# PI34012)
- TMB-Blotting (# PI34018)
- NBT/BCIP (# PI34042)
- Metal Enhanced DAB (# PI34065)

Target Detection

Capture and analyze your image.

- CL-XPosure Film (#s PI34089, PI34090, PI34091)
- MyImage Analysis Software (# PI62237)
- MyECL Imager (# PI62236)
- Pierce Background Eliminator Kit (# PI32065)

Stripping (if necessary)

Reprobe the blot if needed.

- Restore Western Blot Stripping Buffer (# PI21059)
- Restore PLUS Western Blot Stripping Buffer (# PI46430)
- Restore Fluorescent Western Blot Stripping Buffer (#s PI62299 and PI62300)

Troubleshooting

Tips for common Western blotting issues

Refer to this section for tips to obtain the best Western blot data. Learn how to avoid and rectify common issues like low signal, high background, ghost bands, brown bands or glowing bands. Our troubleshooting tips and quick fixes help you optimize so you get the best signal-to-noise ratio.

Electrophoresis

SDS-PAGE is a commonly used technique to separate proteins in the sample based on size. The gel is typically cast in buffer containing sodium dodecyl sulfate (SDS) and protein samples are heated with SDS before electrophoresis so that the charge-density of all proteins is made roughly equal. Heating in SDS, an anionic detergent, denatures proteins in the sample and SDS binds tightly to the uncoiled protein molecule. Usually, a reducing agent such as dithiothreitol (DTT) is also added to cleave protein disulfide bonds and to ensure that no quaternary or tertiary protein structure remains. Consequently, when these samples are electrophoresed, proteins separate according to mass alone, with very little effect from compositional differences.

We offer ready-to-use pre-cast uniform and gradient polyacrylamide mini gels for SDS-PAGE that provide long shelf-life, fast run-time, and excellent resolution. Go to thermoscientific.com/pierce to select the best option for your target protein.

When a set of proteins of known molecular weight are run alongside samples in the same gel, they provide a reference by which the mass of sample proteins can be determined. These sets of reference proteins are called molecular weight markers (MW markers) or standards, and they are available commercially in several forms. SDS-PAGE is also used for routine separation and analysis of proteins because of its speed, simplicity and resolving capability.

Protein Gel Accessories

Tris-HEPES-SDS Running Buffer

Required running buffer for use with Precise Gels.

Both Thermo Scientific™ Pierce™ and Thermo Scientific™ Precise™ Protein Gels use a unique Tris-HEPES-SDS running buffer to improve band resolution and reduce run-time. The buffer can be made according to the recipe provided in either the Pierce or Precise Gel product instructions or purchased in preformulated dry blend form (Product # PI28398).

LDS Sample Buffer

The Thermo Scientific LDS Sample Buffer, Non-Reducing (4X) is specifically formulated and recommended for use with Pierce Protein Gels. The solution is a convenient sample buffer for use in SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The buffer contains coomassie dye, enabling visualization of the electrophoresis progress by the location of the dye front. The LDS Sample Buffer, Non-Reducing (4X) may be used in denaturing gels and is compatible with coomassie dye and silver staining, and Western blotting procedures.

Lane Marker Sample Buffers

The 5X concentration allows you to load more sample!

Highlights:

- Bright pink hydrophobic tracking dye (5X) for SDS-PAGE that transfers to nitrocellulose membranes
- Transfer of the dye front is an indicator of protein transfer efficiency
- Dye front is visible on both the gel and nitrocellulose membrane for determination of molecular weight (Rf values)

Note: *These products are not compatible with fluorescent detection systems. (The pink tracking dye fluoresces strongly.)*

Ordering Information

Product #	Description	Pkg. Size
PI28398	BupH Tris-HEPES-SDS Running Buffer Each pack yields 500mL of 100mM Tris, 100mM HEPES, 3mM SDS, pH 8 ± 0.25 when dissolved in 500mL distilled water (5L total).	10 pack
PI28368	20X Tris/HEPES/SDS Buffer	0.5mL
PI28362	10X Tris-Glycine SDS Buffer	1L
PI84788	LDS Sample Buffer, Non-Reducing (4X)	5mL
PI39000	Lane Marker Reducing Sample Buffer (5X) 0.3 M Tris•HCl, pH 6.8, 5% SDS, 50% Glycerol, 100mM Dithiothreitol, Lane Marker Tracking Dye	5mL
PI39001	Lane Marker Non-Reducing Sample Buffer (5X) 0.3 M Tris•HCl, pH 6.8, 5% SDS, 50% Glycerol, Lane Marker Tracking Dye	5mL

Protein Ladders

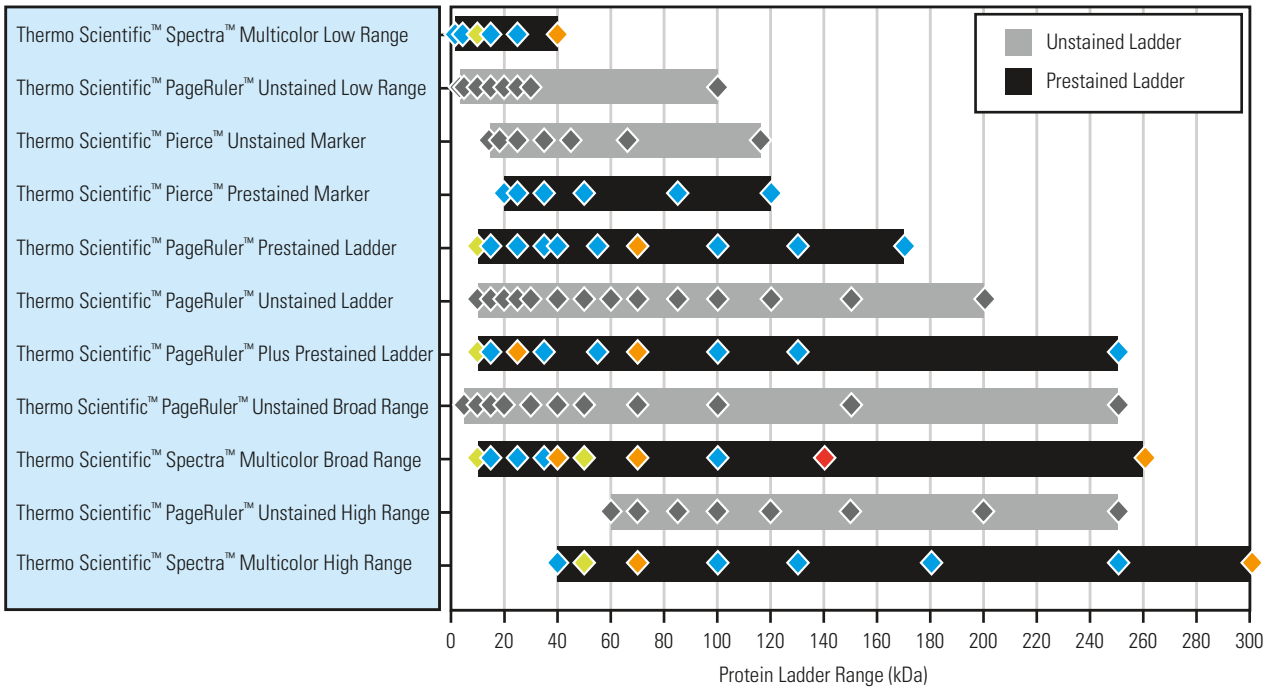
The Thermo Scientific™ Protein Ladder Product Portfolio contains a broad range of products for most any need. The Prestained Protein Ladders are recommended for approximate determination of molecular weight, monitoring the progress of the electrophoresis run and estimating the efficiency of the protein transfer to the membrane during Western blotting procedures.

For precise determination of molecular weights of target proteins in any buffer system, we recommend using our unstained protein ladders.

Highlights:

- **Cost effective** – high quality without the high price
- **Performance** – sharp protein band resolution and consistent migration patterns provide easy molecular weight determination
- **Convenient** – protein ladders are ready to load with no heating or boiling required
- **Reliable** – exceptional lot-to-lot consistency and reproducibility

Thermo Scientific™ Pierce™ Prestained and Unstained Molecular Weight Markers and Protein Ladders. Bars on the following infographic indicate the ranges of each product listed at the left side. Diamonds indicate the location and color of protein bands in each protein ladder.



Gel Electrophoresis

Choosing the right protein ladder. Use this table as a quick reference guide to select the right protein ladder for your application.

		Thermo Scientific Pierce Protein Ladders					Application: SDS PAGE					Application: Western blotting	
		Product Name	Product #	# Proteins	MW Range	Reference Bands	Protein MW determination	Protein band visualization	Monitoring electrophoresis run	Coomassie/silver/ fluorescent staining	NIR detection	Monitoring protein transfer	Chemiluminescent band visualization
Colorimetric	Unstained	PageRuler Unstained Broad Range Protein Ladder	**PI26630**	11	5-250kDa	20, 50 and 100kDa	**Best**	—	—	**Best**	—	—	Good†
		PageRuler Unstained Protein Ladder	**PI26614**	14	10-200kDa	50kDa	Good	—	—	Good	—	—	Good†
		PageRuler Unstained Low Range Protein Ladder	**PI26632**	8	3.4-100kDa	25kDa	**Best**	—	—	**Best**	—	—	Good†
		PageRuler Unstained High Range Protein Ladder	**PI26637**	8	60-250kDa	150kDa	**Best**	—	—	**Best**	—	—	Good†
		Pierce Unstained Protein Molecular Weight Marker	**PI26610**	7	14.4-116kDa	—	Good	—	—	Good	—	—	—
	Prestained	PageRuler Prestained Protein Ladder	**PI26616, PI26617**	10	10-170kDa	Green 10kDa; Orange 70kDa	Good	Good	Good	—	Good‡	Good	—
		PageRuler Plus Prestained Protein Ladder	**PI26619, PI26620**	9	10-250kDa	Green 10kDa; Orange 25 and 70kDa	Good	Good	Good	—	Good‡	Good	—
		Spectra Multicolor Broad Range Protein Ladder	**PI26634, PI26623**	10	10-260kDa	Green 10 and 50kDa; Orange 40, 70 and 260kDa; Pink 140kDa	Good	**Best**	**Best**	—	Good‡	**Best**	—
		Spectra Multicolor Low Range Protein Ladder	**PI26628**	6	1.7-40kDa	Green 10kDa; Orange 40kDa	Good	**Best**	**Best**	—	Good‡	**Best**	—
		Spectra Multicolor High Range Protein Ladder	**PI26625**	8	40-300kDa	Green 50kDa; Orange 70 and 300kDa	Good	**Best**	**Best**	—	Good‡	**Best**	—
		Pierce Prestained Protein Molecular Weight Marker	**PI26612**	6	20-120kDa	—	Good	Good	Good	—	Good	Good	—
Chemi. Lum.		SuperSignal Molecular Weight Protein Ladder	**PI84785, PI84786**	8	20-150kDa	—	Good	—	—	Good	—	Good	**Best**
NIR		PageRuler Prestained NIR Protein Ladder	**PI26635**	10	11-250kDa	55kDa	Good	Good	Good	—	**Best**	Good	—

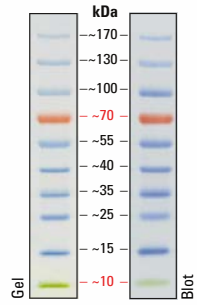
† with Strep-Tactin™ HRP or AP Conjugates

‡ Blue and green prestained bands fluoresce in NIR region

Prestained

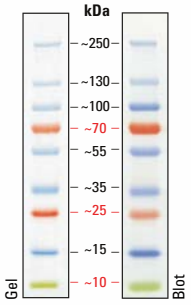
The **PageRuler** and **PageRuler Plus Prestained Protein Ladders** are comprised of highly purified recombinant proteins bound to three different chromophores for easy protein molecular weight determination. The green and orange reference bands in the PageRuler Protein Ladders provide easy orientation of the protein bands.

PageRuler Prestained Protein Ladder



4-20% Tris-glycine SDS-PAGE
Product #s PI26616, PI26617

PageRuler Plus Prestained Protein Ladder

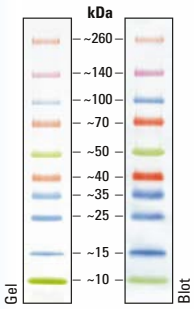


4-20% Tris-glycine SDS-PAGE
Product #s PI26619, PI26620

Multicolor

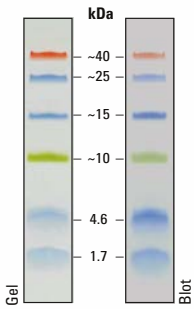
Four different chromophores bound to proteins in **Spectra Multicolor Broad Range Protein Ladders** produce a brightly colored ladder with an easy-to-remember pattern. The Spectra Multicolor High Range and Low Range Protein Ladders are designed for large and small protein analysis, respectively.

Spectra Multicolor Broad Range Protein Ladder



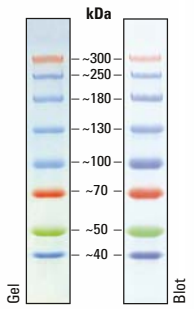
4-20% Tris-glycine SDS-PAGE
Product #s PI26623, PI26634

Spectra Multicolor Low Range Protein Ladder



18% SP Tris-tricine SDS-PAGE
SP - Small Peptide
SDS - polyacrylamide gel
with higher crosslinking, C=5%
Product # PI26628

Spectra Multicolor High Range Protein Ladder

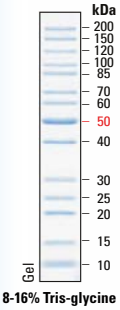


4-12% Tris-glycine SDS-PAGE
Product # PI26625

Unstained

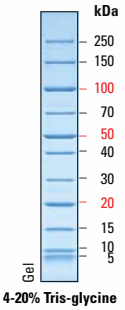
PageRuler Unstained Protein Ladders are mixtures of recombinant, highly purified proteins designed for accurate sizing of proteins by SDS-PAGE. The ladders resolve into clearly identifiable sharp bands when analyzed by SDS-PAGE and stained with Coomassie Blue or silver stain. Each ladder contains protein bands of greater intensity that serve as reference bands. Proteins can be detected on Western blots by staining with Ponceau S or Coomassie Blue (except for 3.4kDa and 5kDa polypeptides) or by using Strep-Tactin conjugates.

PageRuler Unstained Protein Ladder



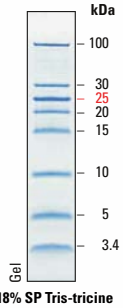
8-16% Tris-glycine SDS-PAGE
Product # PI26614

PageRuler Unstained Broad Range Protein Ladder



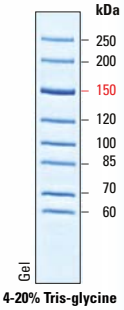
4-20% Tris-glycine SDS-PAGE
Product # PI26630

PageRuler Unstained Low Range Protein Ladder



18% SP Tris-tricine SDS-PAGE
Product # PI26632

PageRuler Unstained High Range Protein Ladder

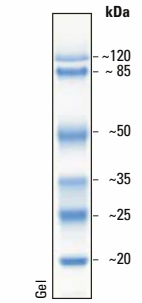


4-20% Tris-glycine SDS-PAGE
Product # PI26637

Cost effective

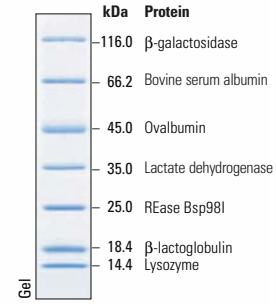
The **Pierce Prestained Protein Molecular Weight Marker** is designed to monitor protein migration during SDS-polyacrylamide gel electrophoresis and monitor protein transfer onto membranes. **Pierce Unstained Protein Molecular Weight Marker** produces sharp bands on SDS-polyacrylamide gel following staining with Coomassie-based or silver stains and is recommended for accurate sizing of proteins on SDS-PAGE and Western blots.

Pierce Prestained Protein MW Marker



8-16% Tris-glycine SDS-PAGE
Product # PI26612

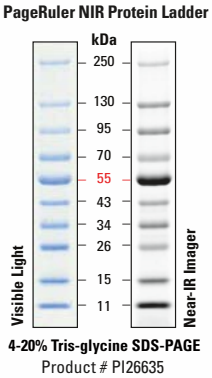
Pierce Unstained Protein MW Marker



8-16% Tris-glycine SDS-PAGE
Product # PI26610

Prestained NIR

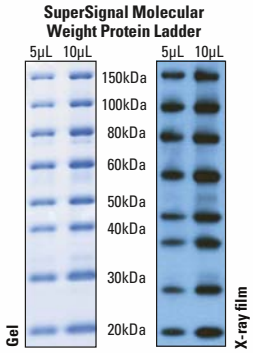
The **Thermo Scientific™ PageRuler™ Prestained NIR Protein Ladder** is a mixture of 10 proteins (11 to 250kDa) that are blue-stained and fluor-labeled for near-IR fluorescent visualization and protein sizing. The protein MW markers in this ladder resolve into sharp bands when analyzed by SDS-PAGE and are labeled with a fluorescent dye for visualization with instruments equipped with near-infrared (NIR) fluorescence detection. The bands are also directly visible because the proteins are prestained blue.



Product # PI26635

Chemiluminescent

Thermo Scientific™ SuperSignal™ Molecular Weight Protein Ladders contain a ready-to-use stabilized mixture of eight recombinant proteins ranging in size from 20 to 150kDa. These recombinant proteins bind antibodies used in the Western blot through an IgG binding site. The protein markers can then be visualized either using appropriate substrates for enzyme-labeled antibodies or via fluorescent dye-labeled antibodies. Use SuperSignal Enhanced Molecular Weight Protein Ladders with mouse monoclonals.



Product #s PI84785, PI84786

Ordering Information

Product #	Description	Pkg. Size
PI26610	Pierce Unstained Protein MW Marker	2 x 1mL
PI26612	Pierce Prestained Protein MW Marker	500µL
PI26614	PageRuler™ Unstained Protein Ladder	2 x 250µL
PI26616	PageRuler Prestained Protein Ladder	2 x 250µL
PI26617	PageRuler Prestained Protein Ladder	10 x 250µL
PI26619	PageRuler Plus Prestained Protein Ladder	2 x 250µL
PI26620	PageRuler Plus Prestained Protein Ladder	10 x 250µL
PI26634	Spectra Multicolor Broad Range Protein Ladder	2 x 250µL
PI26623	Spectra Multicolor Broad Range Protein Ladder	10 x 250µL

Product #	Description	Pkg. Size
PI26625	Spectra Multicolor High Range Protein Ladder	2 x 250µL
PI26628	Spectra Multicolor Low Range Protein Ladder	250µL
PI26630	PageRuler Unstained Broad Range Protein Ladder	2 x 250µL
PI26632	PageRuler Unstained Low Range Protein Ladder	2 x 250µL
PI26635	PageRuler Prestained NIR Protein Ladder	2 x 250µL
PI84785	SuperSignal Molecular Weight Protein Ladder	250µL
PI84786	SuperSignal Enhanced Molecular Weight Protein Ladder	250µL

Electro-Transfer

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.

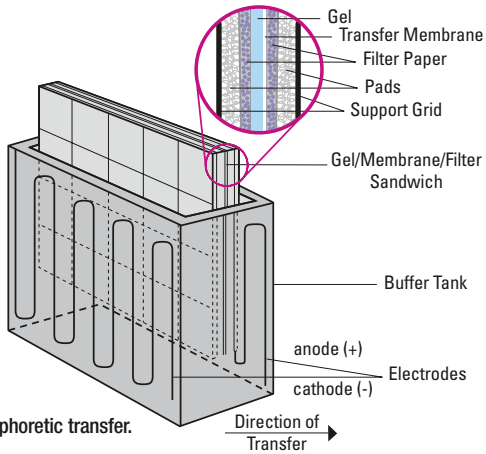
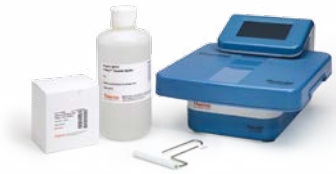


Figure 1. Electrophoretic transfer.

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.

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 # PI24580) or PVDF (Product # PI24585) 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.

Western Transfer System



Thermo Scientific™ Pierce™ G2 Fast Blotter

Combining high-performance protein transfer with speed, the Pierce G2 Fast Blotter allows for the fast and efficient transfer of proteins ranging in sizes of 10 to 300kDa from polyacrylamide gel to blotting membrane.

Traditional blotting techniques often require transfer times of one hour to overnight to achieve good transfer efficiency. When combined with the Thermo Scientific™ Pierce™ 1-Step Transfer Buffer, the Pierce G2 Fast Blotter delivers similar transfer performance in as few as 5-10 minutes without gel pre-equilibration. The system has been tested using pre-cast SDS-PAGE gels from several sources as well as some commonly used homemade gel formulations. The Pierce G2 Fast Blotter can also be used for standard semi-dry transfer protocols with Towbin Transfer buffer.

The Pierce G2 Fast Blotter has an easy-to-use color LCD menu touchscreen interface for quick programming of the transfer time based on the number and size of gels. Or create your own custom transfer settings.

Pierce G2 Fast Transfer Blotter Highlights:

- **Fast** – transfer proteins in 5-10 minutes
- **Excellent transfer efficiency** – transfer low-, medium- and high-MW proteins (10-300kDa) equivalent to or better than conventional semi-dry or wet transfer methods
- **Integrated power supply** – allows consistent high-efficiency protein transfer
- **Easy-touch programming** – access pre-programmed transfer methods based on the gel number, gel size and molecular weight range of proteins using color LCD menu touchscreen; easily create, run and save custom transfer methods
- **Convenient** – simultaneously transfer up to four mini-sized gels or two midi-sized gels
- **Versatile** – use Pierce 1-Step Transfer Buffer for fast blotting programs or Towbin transfer buffer for conventional semi-dry transfer methods

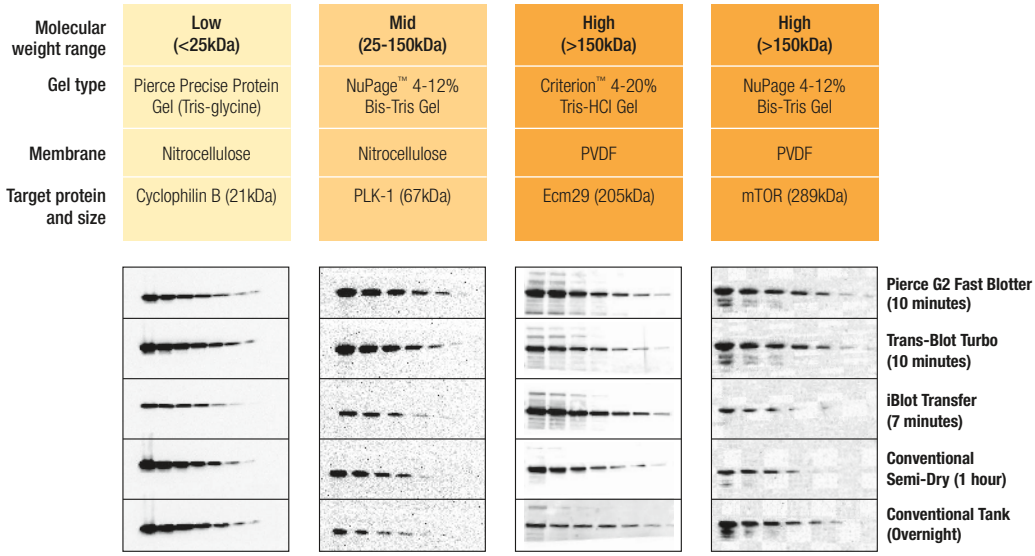


Figure 2. The Thermo Scientific Pierce G2 Fast Blotter allows rapid transfers of low-, medium-, and high-molecular weight proteins. HeLa lysate was serially diluted, prepared for SDS-PAGE, and electrophoresed according to the gel suppliers' recommendations. The proteins were then transferred from gel to nitrocellulose or PVDF membrane using the following techniques: Trans-Blot™ Turbo™ Transfer device with appropriate Trans-Blot Turbo Transfer Pack for 10 minutes; Pierce G2 Fast Blotter with Pierce 1-Step Transfer Buffer, filter paper and membrane for 10 minutes; iBlot™ transfer with appropriate transfer stack for 7 minutes; classical semi-dry using Towbin transfer buffer (25 mM Tris, 139 mM glycine, 20% methanol), appropriate filter paper and membrane for 1 hour (25V constant); or classical overnight tank transfer using Towbin transfer buffer (30V constant, 16 hours). Membranes were probed with Thermo Scientific™ anti-Cyclophilin B (Product # PA1-027A), anti-PLK-1 (Product # MA1-848), anti-Ecm29 (Product # PA3-035) and anti-mTOR Antibody (Product # PA1-518). The resulting blots were simultaneously imaged using the Thermo Scientific™ MyECL™ Imager and densitometry was determined using Thermo Scientific™ MyImageAnalysis™ Software.

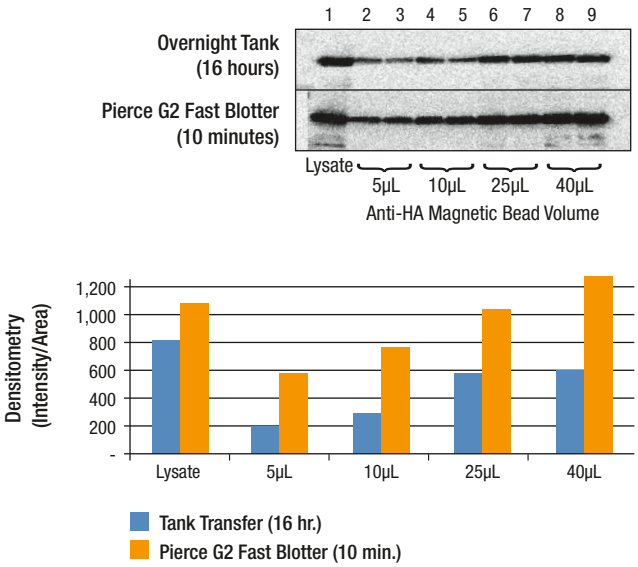


Figure 3. Thermo Scientific Pierce G2 Fast Blotter provides efficient protein transfer in 10 minutes. GST-PI3K-SH2-HA (37kDa) was expressed in *E. coli* and purified with varying volumes of Thermo Scientific™ Anti-HA Magnetic Beads (Product # PI88836). Resulting samples were prepared for SDS-PAGE and electrophoresed. The proteins were then transferred from gel to nitrocellulose membrane using either classical overnight tank transfer with Towbin transfer buffer (25mM Tris, 139 mM glycine, 20% methanol) or Pierce 1-Step Transfer Buffer and the Pierce G2 Fast Blotter (10 minutes). Membranes were probed with anti-HA antibody for 1 hour, washed 5 times, probed with goat anti-mouse HRP for 30 minutes, washed 5 times and incubated in Thermo Scientific™ SuperSignal™ West Femto Substrate (Product # PI34096). The resulting blots were simultaneously imaged using the MyECL Imager and densitometry determined using MyImageAnalysis Software.

Ordering Information

Product #	Description	Pkg. Size
PI62288	**Pierce G2 Fast Blotter** *Sufficient for semi-dry transfer of proteins from polyacrylamide gels to nitrocellulose or PVDF membranes.* Includes: Pierce G2 Fast Blotter Control Unit, 1 device Pierce G2 Fast Blotter Cassette, 1 device Power Cord with C/13 Connector, 1 device Western Blot Roller, 1 device Quick Start Guide	1 unit
PI62289*	**Pierce G2 Fast Blotter Cassette**	1 unit
PI62291*	**Pierce G2 Fast Blotter Control Unit**	1 unit
PI84747*	**Western Blot Roller**	1 unit
PI84731*	**1-Step Transfer Buffer, 1L** *Sufficient for 20 mini blots at 50mL each*	1L
PI84783	**Western Blotting Filter Paper, 7cm x 8.4cm** Thickness: 0.83mm	100 sheets
PI84784	**Western Blotting Filter Paper, 8cm x 13.5cm** Thickness: 0.83mm	100 sheets

**Replacement for G2 Blotter components that come with the unit.*

Transfer Buffers

Thermo Scientific™ BupH™ Tris-Glycine Buffer Packs

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

Thermo Scientific™ BupH™ Tris Buffered Saline Packs

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

Thermo Scientific™ Pierce™ Methanol-Free Transfer Buffer, 10X

Our Methanol-Free Tank Transfer Buffer does not require cooling. Simply dilute the 10X solution with water and use directly.

Thermo Scientific™ Pierce™ 1-Step Transfer Buffer

The Pierce 1-Step Transfer Buffer is a high ionic strength formulation designed for rapid semi-dry transfer of 10-300kDa proteins from polyacrylamide gels (SDS-PAGE) to nitrocellulose or PVDF membranes using the Pierce G2 Fast Blotter. It comes as a ready-to-use 1X solution.

Highlights:

- Safe and environmentally friendly – Pierce 1-Step Transfer Buffer contains no alcohol
- Economical – transfer up to 20 mini-sized gels or 10 midi-sized gels with one 1L bottle. No special consumables required; simply use Western blotting filter paper and membrane of choice

Ordering Information

Product #	Description	Pkg. Size
PI28380	BupH Tris-Glycine Buffer Packs	40 pack
PI28376	BupH Tris Buffered Saline Packs	40 pack
PI28379	BupH Tris Buffered Saline Packs	10 pack
PI35040	Pierce Methanol-Free Transfer Buffer, 10X	5L
PI84731*	1-Step Transfer Buffer, 1L *Sufficient for 20 mini blots at 50mL each.*	1L

Transfer Membranes

Thermo Scientific™ Pierce™ Nitrocellulose and PVDF Membranes are high-quality, high-capacity transfer membranes especially for Western blot analysis. Both membranes are available in rolls and precut sheets, and in a variety of pore sizes for colorimetric, chemiluminescent and fluorescent detection.

Ordering Information

Product #	Description	Pkg. Size
Nitrocellulose Membranes		
PI88013	Nitrocellulose Membrane, 0.2µm 7.9cm x 10.5cm	15/pkg.
PI88018	Nitrocellulose Membrane, 0.45µm 33cm x 3m	1 roll
PI88014	Nitrocellulose Membrane, 0.45µm 7.9cm x 10.5cm Minimum 87 sheets when cut to 7.9cm x 10.5cm; minimum 52 sheets when cut to 11.5cm x 12.5cm.	15/pkg.
PI88024	Nitrocellulose Membrane, 0.2µm 8cm x 8cm	15/pkg.
PI77012	Nitrocellulose Membrane, 0.2µm 8cm x 12cm	25/pkg.
PI88025	Nitrocellulose Membrane, 0.45µm 8cm x 8cm	15/pkg.
PI77010	Nitrocellulose Membrane, 0.45µm 8cm x 12cm	25/pkg.
Polyvinylidene Difluoride (PVDF) Membranes		
PI22860	Low-Fluorescence PVDF Transfer Membrane, 0.2µm 7cm x 8.4cm	10/pkg.
PI88585	PVDF Transfer Membrane, 0.45µm 10cm x 10cm	10 sheets
PI88518	PVDF Transfer Membrane, 0.45µm 26.5cm x 3.75m	1 roll
Western Blotting Filter Paper		
PI88600	Western Blotting Filter Paper	100 sheets
PI88605	Extra Thick Western Blotting Filter Paper 7cm x 10.5cm	50 sheets per pkg
PI88610	Extra Thick Western Blotting Filter Paper 8.5cm x 9cm	50 sheets per pkg
PI88615	Extra Thick Western Blotting Filter Paper 8cm x 13.5cm	50 sheets per pkg
PI88620	Extra Thick Western Blotting Filter Paper 20cm x 20cm	50 sheets per pkg
PI84783	Western Blotting Filter Paper, 7cm x 8.4cm Thickness: 0.83mm	100 sheets
PI84784	Western Blotting Filter Paper, 8cm x 13.5cm Thickness: 0.83mm	100 sheets

Protein Stain Kits

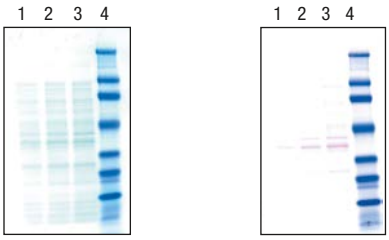
Thermo Scientific™ Pierce™ Reversible Protein Stain for Nitrocellulose and PVDF Membranes

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 4-6). Try these reversible protein stains for nitrocellulose and PVDF membranes and you will never use Ponceau S again.

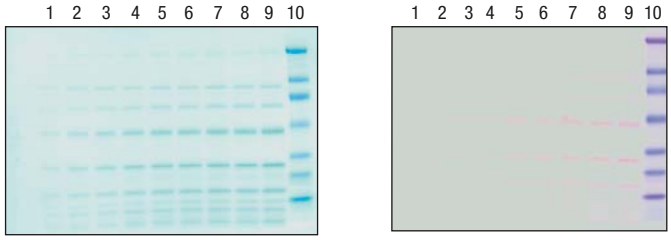
Highlights:

- 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



A. Thermo Scientific Pierce Reversible Stain **B. Ponceau S Stain**

Figure 4. 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 seconds and destained according to the protocol. **Blot B** was stained with 0.1% Ponceau S stain for 5 minutes and destained. The blot stained with Pierce Reversible Stain demonstrates superior visual detection of bands. GST lysate loading volumes (**Lanes 1-3**). **Lane 1.** 5µL, **Lane 2.** 10µL, **Lane 3.** 15µL and **Lane 4.** Pierce Prestained Protein MW Marker (Product # PI26681), 10µL.



A. Thermo Scientific Pierce Reversible Stain **B. Ponceau S Stain**

Figure 5. Comparison of Thermo Scientific Pierce Reversible Protein Stain with Ponceau S stain on PVDF membrane. Pierce Unstained Protein MW Markers (Product # PI26671) 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 # PI26681).

A. Control **B. Thermo Scientific Pierce Reversible Stain**

Figure 6. 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 Thermo Scientific™ SuperSignal™ West Dura Substrate (Product # PI34075). **Lane 1:**125pg, **Lane 2:** 250pg, **Lane 3:** 500pg and **Lane 4:** 1ng.

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: 250ng	• Detection limit: 25-50ng
• 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

Ordering Information

Product #	Description	Pkg. Size
PI24580	**Pierce Reversible Protein Stain Kit for Nitrocellulose Membranes** *Sufficient material to stain protein and reverse the stain from 10 (8cm x 8cm) 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 250mL 1,000mL 500mL

Product #	Description	Pkg. Size
PI24585	**Pierce Reversible Protein Stain Kit for Polyvinylidene Difluoride Membrane** *Sufficient material to stain protein and reverse the stain from 10 (8cm x 8cm) 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 250mL 250mL 1,000mL 500mL

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

Signal Enhancement

Thermo Scientific™ Pierce™ Antibody Extender NC

Get the most out of your primary antibody.

A simple 10-minute, post-transfer treatment of the target protein on nitrocellulose can reduce the amount of primary antibody used by 3-, 10-, 25- and even 100-fold, while maintaining equivalent signal compared to an untreated control.

Highlights:

- **Achieves equivalent signal while using less antibody** – uses 3- to 100-fold less primary antibody [average Primary Antibody Reduction Factor (PAR) is 28.2-fold]
- **Inexpensive** – costs approximately US$6 to treat an 8 x 10cm blot
- **Conserves antibody, regardless of detection system** – works with colorimetric, chemiluminescent, HRP and AP systems
- **Simple and ready to use** – fast 10-minute protocol

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.

Ordering Information

Product #	Description	Pkg. Size
PI32110	**Pierce Antibody Extender Solution NC** *Sufficient reagent for up to 20 nitrocellulose membranes (1,600cm²).*	500mL

Primary antibody comparison.

Primary Antibody Cost: US $230		Primary Antibody Volume: 200µg†		Minimum Savings: US $184
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	$153.35	$306.65	$184.65
12X	$1.91	$38.35	$421.65	$299.65
25X	$0.92	$18.40	$441.60	$319.60

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

Thermo Scientific™ SuperSignal™ Western Blot Enhancer

Increase signal-to-noise ratio and band development for better sensitivity.

The SuperSignal Western Blot Enhancer contains a membrane treatment reagent and a primary antibody diluent that increase both signal intensity and sensitivity 3- to 10-fold compared to detection performed using conventional Western blotting.

When a protein or antigen is difficult to detect because of low abundance or poor immunoreactivity, the SuperSignal Western Blot Enhancer can significantly reduce background and enhance detection of low-abundance and weakly immunoreactive antigens. This versatile kit works with PVDF and nitrocellulose membranes and is compatible with fluorescence, chromogenic and chemiluminescent detection.

Highlights:

- **Increase sensitivity** – achieve 3- to 10-fold increase in signal intensity and sensitivity
- **Improve specificity** – improves signal-to-noise ratio for poor quality and low affinity antibodies
- **Better clarity** – reduces background for cleaner Western blots
- **Membrane compatibility** – provides effective signal enhancement with PVDF and nitrocellulose membranes
- **Substrate compatibility** – validated for use with chromogenic, chemiluminescent and fluorescent detection methods

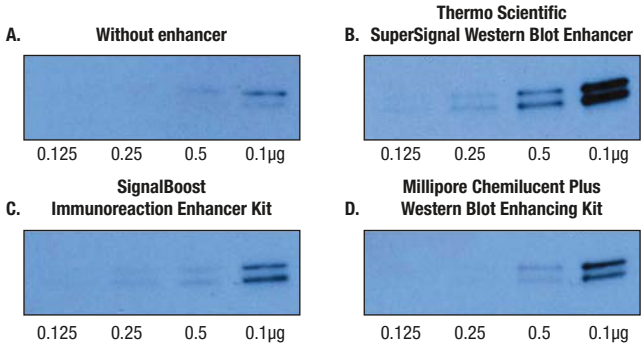


Figure 7. Comparison of Thermo Scientific SuperSignal Western Blot Enhancer with other commercially available enhancers. HeLa cell lysate was separated by electrophoresis, transferred to nitrocellulose (Product # PI88013) and Western blotting was performed using the conventional method (**Panel A**), SuperSignal Western Blot Enhancer (**Panel B**), EMD Biosciences SignalBoost™ Immunoreaction Enhancer Kit (**Panel C**) Millipore Chemilucent™ Plus Western Blot Enhancing Kit or (**Panel D**) according to manufacturers' directions. Blots were probed with rabbit anti-MAP Kinase (Millipore Product # 06-182) at 1µg/mL followed by HRP-conjugated goat anti-rabbit IgG (Product # PI31460) at 0.1µg/mL. Detection was performed with Pierce ECL Substrate (Product # PI32209) and exposed to film for 10 minutes.

Ordering Information

Product #	Description	Pkg. Size
PI46640	**SuperSignal Western Blot Enhancer** *Sufficient for 25 mini blots or 2,000cm² membrane.* Includes: Antigen Pretreatment Solution Primary Antibody Diluent	25-blot Kit 250mL 250mL
PI46641	**SuperSignal Western Blot Enhancer** *Sufficient for 2 mini blots or 160cm² membrane.* Includes: Antigen Pretreatment Solution Primary Antibody Diluent	2-blot Kit 25mL 25mL

SuperSignal Western Blot Enhancer and Pierce Western Blot Signal Enhancer

How do I choose one over the other?

Comparison of Thermo Scientific Western Blot Enhancers

Product	Benefit	Mode of action	When to use
SuperSignal Western Blot Enhancer	Enhances sensitivity and decreases background	Increases antigen specificity by decreasing weak antibody-antigen interactions	More sensitivity is needed and when primary antibody is "dirty" yielding nonspecific bands
Pierce Western Blot Signal Enhancer	Enhances sensitivity	Unraveling targets for increased epitope accessibility	More sensitivity is needed

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 8-9).

The Pierce Western Blot Signal Enhancer membrane treatment is a simple, 15-minute procedure (Figure 10) 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

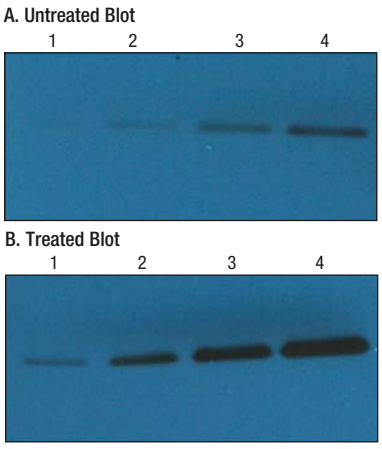


Figure 8. Enhanced chemiluminescent detection of identical serial dilutions of IL-6. **Panel A:** before and **Panel B:** after treatment with Pierce Western Blot Signal Enhancer. **Lane 1:** 250pg, **Lane 2:** 500pg, **Lane 3:** 1,000pg and **Lane 4:** 2,000pg.

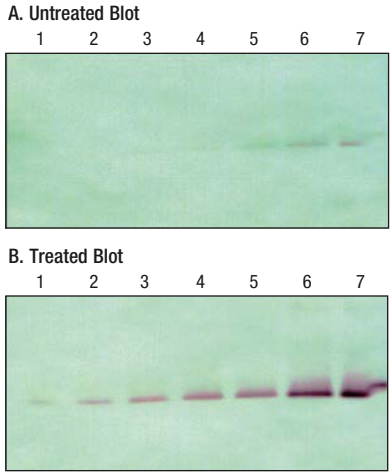


Figure 9. Enhanced chromogenic detection of identical serial dilutions of IL-6. Panel A: before and **Panel B:** after treatment with Pierce Western Blot Signal Enhancer. **Lane 1.** 100pg, **Lane 2:** 200pg, **Lane 3:** 300pg, **Lane 4**: 400pg, **Lane 5:** 500pg, **Lane 6:** 1,000pg and **Lane 7:** 5,000pg.

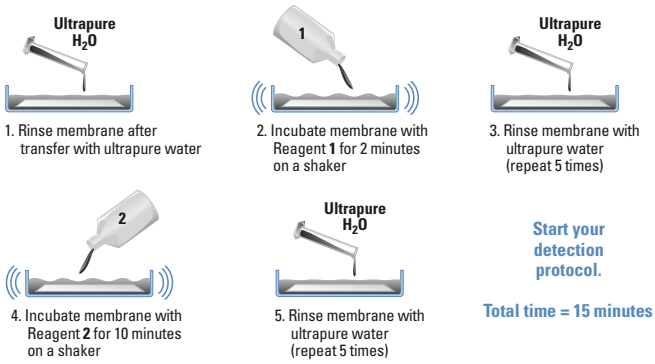


Figure 10. Thermo Scientific Pierce Western Blot Signal Enhancer Protocol performed after transfer and before blocking.

Ordering Information

Product #	Description	Pkg. Size
PI21050	Pierce Western Blot Signal Enhancer*	Kit
	Sufficient reagent for ten (10cm x 10cm) blots.	
	Includes: Enhancer Reagent 1	250mL
	Enhancer Reagent 2	250mL

** 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.*

Blocking

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.

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™, StartingBlock™ and Protein-free Blocking Buffer Ingredients:
A single protein or protein alternative, PBS or TBS buffer, and a preservative.

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 # PI37515) 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.

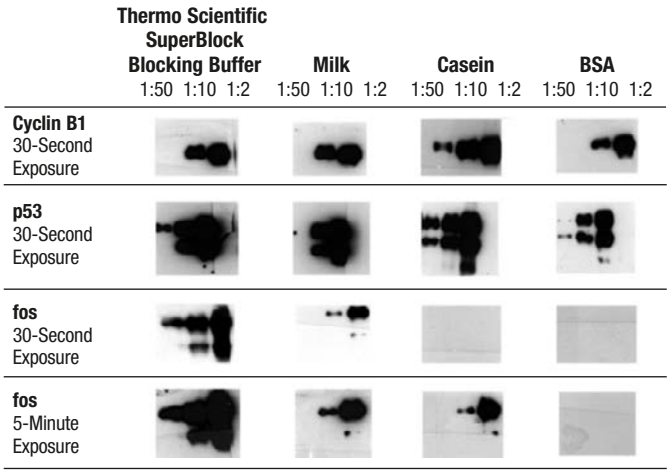


Figure 1. Blocking buffer optimization. Recombinant human cyclin B1,wild-type p53 and mouse fos baculovirus lysates were diluted in Lane Marker Reducing Sample Buffer (1:50, 1:10 or 1:2) and separated electrophoretically on a 12% SDS-polyacrylamide gel. The proteins were transferred to nitrocellulose membrane and cut into strips. The membrane strips were blocked for 1 hour at RT with shaking in Thermo Scientific 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 0.5µg/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 25ng/mL. The membranes were washed with TBS. A working solution of Thermo Scientific 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
PI37538	StartingBlock (PBS) Blocking Buffer	✔	✔	✔	✔	
PI37542	StartingBlock (TBS) Blocking Buffer	✔	✔	✔	✔	
PI37539	StartingBlock T20 (PBS) Blocking Buffer	✔	✔	✔	✔	
PI37543	StartingBlock T20 (TBS) Blocking Buffer	✔	✔	✔	✔	
PI37515	SuperBlock Blocking Buffer in PBS	✔	✔	✔	✔	✔
PI37535	SuperBlock Blocking Buffer in TBS	✔	✔	✔	✔	✔
PI37517	SuperBlock Blocking Buffer – Blotting in PBS		✔	✔	✔	
PI37537	SuperBlock Blocking Buffer – Blotting in TBS		✔	✔	✔	
PI37516	SuperBlock T20 PBS Blocking Buffer	✔	✔	✔	✔	✔
PI37536	SuperBlock T20 TBS Blocking Buffer	✔	✔	✔	✔	✔
PI37527	SEA BLOCK Blocking Buffer	✔	✔	✔		
PI37520	Blocker BSA in TBS	✔	✔	✔	✔	✔
PI37525	Blocker BSA in PBS	✔	✔	✔	✔	✔
PI37532	Blocker Casein in TBS	✔	✔	✔	✔	✔
PI37528	Blocker Casein in PBS	✔	✔	✔	✔	✔
PI37530	Blocker BLOTTO in TBS	✔	✔	✔	✔	✔
PI37570	Protein-Free (TBS) Blocking Buffer	✔	✔	✔	✔	
PI37571	Protein-Free T20 (TBS) Blocking Buffer	✔	✔	✔	✔	
PI37572	Protein-Free (PBS) Blocking Buffer	✔	✔	✔	✔	
PI37573	Protein-Free T20 (PBS) Blocking Buffer	✔	✔	✔	✔	
PI37576	Pierce Fast Blocking Buffer		✔			
PI37587	Pierce Clear Milk Blocking Buffer		✔			

Blocking Buffers

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 (Figure 2). Thermo Scientific 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

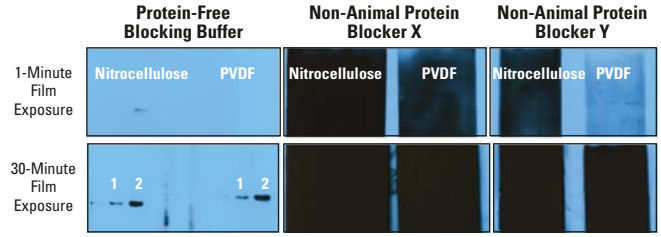


Figure 2. Thermo Scientific Protein-Free Blocking Buffer efficiently blocks Western blotting membranes. Jurkat apoptotic lysate (**Lane 1.** 0.25µg, **Lane 2.** 0.50µg) was separated in 4-20% Tris-glycine gels and transferred to nitrocellulose or PVDF membranes. The membranes were blocked for 1 hour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

Ordering Information

Product #	Description	Pkg. Size
PI37570	**Protein-Free (TBS) Blocking Buffer** Proprietary formulation in Tris-buffered saline at pH 7.4 with Kathon™ Antimicrobial Agent.	1L
PI37571	**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.	1L
PI37572	**Protein-Free (PBS) Blocking Buffer** Proprietary formulation in phosphate-buffered saline at pH 7.4 with Kathon Antimicrobial Agent.	1L
PI37573	**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.	1L

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 3). They may be the only blockers you ever use.

Figure	3A	3B	3C	3D
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.*

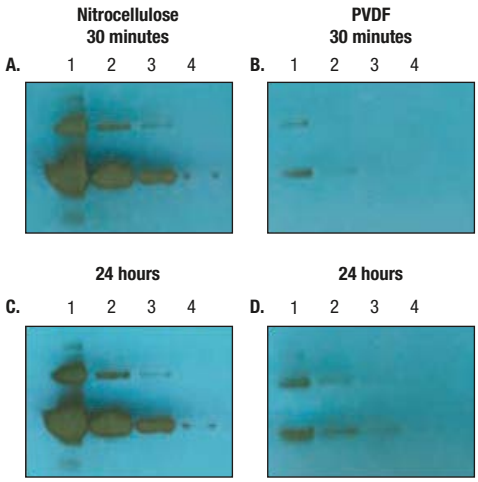


Figure 3A-D. Thermo Scientific StartingBlock Blocking Buffer performance after stripping and reprobing. Nitrocellulose and PVDF membranes that had been blocked with StartingBlock Blocking Buffer and used to detect a different primary target were then stripped with Restore Western Blot Stripping Buffer (Product # PI21059) and probed for the transferrin receptor (CD71). Detection was with SuperSignal West Dura Chemiluminescent Substrate (Product # PI34075) and exposed to film for 30 minutes and then again 24 hours. Very little background occurs with either membrane or exposure time, indicating exceptional blocking performance.

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 Thermo Scientific Restore Stripping Buffer (Product # PI21059) 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
PI37538	**StartingBlock (PBS) Blocking Buffer** A protein-based blocker formulation in phosphate-buffered saline (pH 7.5) for use in Western blotting and ELISA applications.	1L
PI37542	**StartingBlock (TBS) Blocking Buffer** A protein-based blocker formulation in Tris-buffered saline (pH 7.5) for use in Western blotting and ELISA applications. StartingBlock Blocking Buffers are also available with an optimized amount of Tween-20 Detergent to provide the lowest background.	1L
PI37539	**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.	1L
PI37543	**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.	1L

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

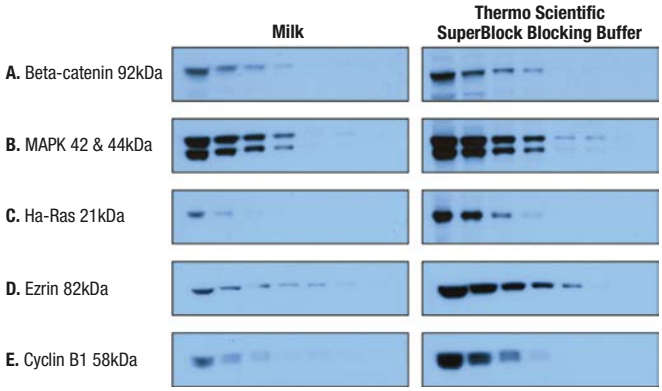


Figure 4. Thermo Scientific SuperBlock Blocking Buffer in PBS is better than milk for sensitive detection of target proteins. HeLa cell lysate (20, 10, 5, 2.5, 1.25, 0.625 and 0.3125µg) was separated by SDS-PAGE, and transferred to nitrocellulose (**Panels A-C**, Product # PI88014) or PVDF (**Panels D-E**, Product # PI88585) membrane. Membranes were blocked for one hour using 5% nonfat milk in Tris-buffered saline with 0.05% Tween-20 Detergent or SuperBlock Blocking Buffer in phosphate-buffered saline with 0.05% Tween-20 Detergent. Membranes were probed for the indicated targets. Blots were incubated in SuperSignal West Pico Chemiluminescent Substrate (Product # PI34080) for 5 minutes and exposed to film (Product # PI34091).

Thermo Scientific™ 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 200mL 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
PI37515	**SuperBlock (PBS) Blocking Buffer**	1L
PI37516	**SuperBlock T20 (PBS) Blocking Buffer** (Contains 0.05% Tween-20 Detergent)	1L
PI37518	**SuperBlock (PBS) Blocking Buffer**	5L
PI37535	**SuperBlock (TBS) Blocking Buffer**	1L
PI37536	**SuperBlock T20 (TBS) Blocking Buffer** (Contains 0.05% Tween-20 Detergent)	1L
PI37517	**SuperBlock (PBS) Blocking Buffer – Blotting***	1L
PI37537	**SuperBlock (TBS) Blocking Buffer – Blotting***	1L
PI37545	**SuperBlock (TBS) Blocking Buffer Dry Blend Blocking Buffer** Each pouch yields 200mL when reconstituted.	5 pouches

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

Thermo Scientific™ SEA BLOCK™ Blocking Buffer

No mammalian proteins, reducing the risk of nonspecific interaction.

Highlights:

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

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
PI37527	SEA BLOCK Blocking Buffer	500mL

Thermo Scientific™ Pierce™ Clear Milk Blocking Buffer

Long shelf-life replacement for homemade milk blocking buffers.

Highlights:

- **Excellent stability** – stable for 1 year stored at 4°C, unlike typical homemade milk buffers
- **Convenient** – concentrated formulation saves storage space and can be diluted easily to obtain optimal blocking results for specific applications
- **Easy to use** – no waiting for powdered milk to dissolve with this ready-to-dilute solution
- **Popular** – nonfat milk has been used for many years in a variety of protein methods, although it is not recommended for avidin-based techniques because it contains some endogenous biotin

Ordering Information

Product #	Description	Pkg. Size
PI37587	Pierce Clear Milk Blocking Buffer (10X)	100mL

Thermo Scientific™ Pierce™ Fast Blocker

Shorten the typical Western blot process by over 2 hours.

Highlights:

- **Fast** – use both buffers to shorten the typical Western blot development by over 2 hours
- **Simple** – optimized protocol makes Western blot analysis easier than ever
- **Low background** – provides results comparable to classic Western blotting buffers

Ordering Information

Product #	Description	Pkg. Size
PI37575	Pierce Fast Blocking Buffer *Sufficient for 15 mini blots at 30mL each.*	500mL
PI37576	Pierce Fast Blocking Buffer *Sufficient for 3 mini blots at 30mL each.*	100mL

Thermo Scientific™ 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
PI37532	Blocker Casein in TBS 1% (w/v) Casein Hammersten Grade in TBS; Contains Kathon Antimicrobial Reagent as preservative, pH 7.4.	1L
PI37528	Blocker Casein in PBS 1% (w/v) Casein Hammersten Grade in PBS; Contains Kathon Antimicrobial Reagent as preservative, pH 7.4.	1L

Thermo Scientific™ Blocker BLOTTO

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

Highlights:

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

Ordering Information

Product #	Description	Pkg. Size
PI37530	**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.	1L

Thermo Scientific™ 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
PI37525	**Blocker BSA in PBS (10X)**	200mL
PI37520	**Blocker BSA in TBS (10X)**	125mL

Thermo Scientific™ 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
PI28320	**Surfact-Amps 20 Purified Detergent Solution**	6 x 10mL

Primary Incubation

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 **antibodyresource.com** or **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.

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.

for more product information ▶ thermoscientific.com/pierce-abs

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 1mg/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.

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 32–37.

Primary Antibodies

Over 40,000 antibodies.

With over 40,000 antibodies, our portfolio includes antibodies that are developed for a wide variety of application needs. Pierce Antibodies are validated and guaranteed to perform in the stated application and species and are highly validated in areas such as epigenetics, cancer, stem cell research and many more. Our website enables you to easily search by protein target and then filter by the specific assays you are interested in. All of our antibodies are validated in the stated applications and are guaranteed to perform.

Applications

- Agglutination
- Competition Assay
- ChIP Assay
- Cytotoxicity Assay
- Control
- ELISA
- Electron Microscopy
- FACS
- Functional Assay
- Gel Shift
- Hemagglutination Assay
- Inhibition Assay
- Immunocytochemistry
- Immunodiffusion
- Immunofluorescence
- Immunohistochemistry
- Immunohistochemistry (Frozen)
- Immunohistochemistry (Paraffin)
- Immunohistochemistry (Paraffin, Frozen)
- Infection
- Immunoprecipitation
- Immunoradiometric Assay
- Radioimmune Assay
- Western Blot

Custom Antibody Services

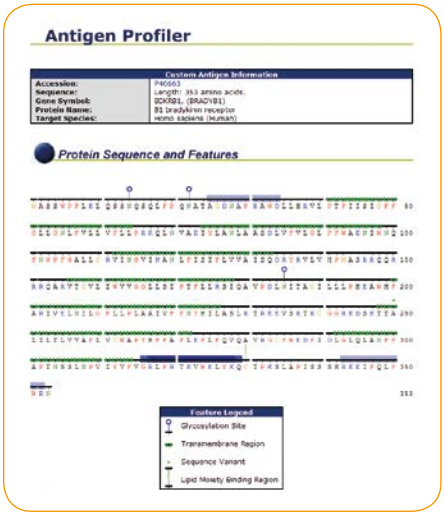
Design and produce better custom antibodies.

The Thermo Scientific™ Custom Antibody Development Service leverages our experience in making more than 18,500 antibodies to peptides and recombinant proteins. Our proprietary antigen design tools, including the Thermo Scientific™ Antigen Profiler Software, and targeted antigen display produces more robust antibodies that perform better in your targeted assays.

When you initiate a custom antibody project with us we provide you access to our online project management tool. This secure account gives you easy access to project information and allows you to provide specific instructions for your projects.

For more information on Thermo Scientific Custom Antibody Services, please visit **thermoscientific.com/custom-abs**

A.



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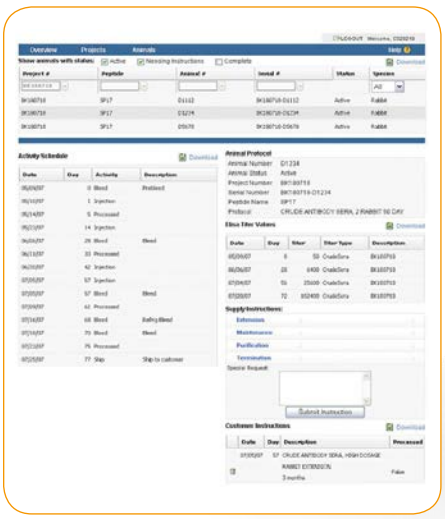


Figure 1. Thermo Scientific Antigen Profiler Software provides the tools to develop better antibodies. The online software allows you to examine comparative antigenicity based on *in vivo* titer data from over 13,000 peptide antibodies and to identify important structural motifs to produce high specificity antibodies. The software also includes a peptide tutorial to aid in synthesizing soluble peptides and curated BLAST analysis for specificity determination. A sample report **(A.)** and online project tool **(B.)** are shown here.

Epitope Tag and Loading Control Antibodies

Better control yields better protein information.

Thermo Scientific™ Pierce™ Epitope Tag Antibodies include monoclonal and polyclonal epitope tag antibodies to the majority of tags used by researchers. Epitope tags are frequently used with recombinant protein purification from *E. coli*, yeast, insect or mammalian cell cultures to allow researchers to selectively extract a target protein from the endogenous proteins. Pierce Epitope Tag Antibodies can also serve as a universal detection method by using tag-specific antibodies to these sequences in applications such as ELISA, immunofluorescence, immunoprecipitation, and Western blotting. We now offer a wide selection of epitope tag antibodies, including conjugates to biotin, HRP and DyLight Dyes for the most popular tags.

Thermo Scientific™ Pierce™ Loading Control Antibodies include antibodies against the most common loading and expression control proteins. Loading Control Antibodies are essential in the proper assessment of Western blots and are used to compare the amount of protein loaded in each well across the gel. These controls help determine whether sample to sample expression level differences of the protein of interest is due to actual protein levels in a given cell lysate or from loading variance in sample preparations. Pierce Loading Control Antibodies can also be used as a complementary antibody stain in immunofluorescence studies with your protein of interest. We now offer loading control antibodies to GAPDH, β-Actin, and β-Actin conjugated to biotin, HRP and our DyLight Dyes.

Conjugated/Unconjugated Thermo Scientific Pierce Epitope Tag Antibodies.

Epitope Tag	Target	Unconjugated	Biotin	HRP	DyLight 488	DyLight 550	DyLight 650	DyLight 680	DyLight 800 4× PEG
FLAG™ Epitope Tag Antibody (FG4R)	DYKDDDDK	MA1-91878	MA1-91878-BITN	MA1-91878-HRP	MA1-91878-D488	MA1-91878-D550	MA1-91878-D650	MA1-91878-D680	MA1-91878-D800
His Epitope Tag Antibody (HIS.H8)	6x-His	MA1-21315	MA1-21315-BITN	MA1-21315-HRP	MA1-21315-D488	MA1-21315-D550	MA1-21315-D650	MA1-21315-D680	MA1-21315-D800
HA Epitope Tag Antibody (2-2.2.14)	YPYDVPDYA	PI26183	26183-BITN	26183-HRP	26183-D488	26183-D550	26183-D650	26183-D680	26183-D800
Myc Epitope Tag Antibody (Myc.A7)	EQKLISEEDL	MA1-21316	MA1-21316-BITN	MA1-21316-HRP	MA1-21316-D488	MA1-21316-D550	MA1-21316-D650	MA1-21316-D680	MA1-21316-D800
GST Tag Antibody (8-326)	Glutathione-S-Transferase	MA4-004	MA4-004-BITN	MA4-004-HRP	MA4-004-D488			MA4-004-D680	MA4-004-D800
GFP Tag Antibody (GF28R)	Green Fluorescent Protein N-terminal Peptide	MA5-15256	MA5-15256-BITN	MA5-15256-HRP			MA5-15256-D650	MA5-15256-D680	MA5-15256-D800
V5 Epitope Tag Antibody (E10/V4RR)	GKPIPNPLLGLDST	MA5-15253	MA5-15253-BITN	MA5-15253-HRP	MA5-15253-D488		MA5-15253-D650	MA5-15253-D680	MA5-15253-D800

Thermo Scientific Pierce Conjugated/Unconjugated Loading Control Antibodies.

Loading Control	Target	Unconjugated	Biotin	HRP	DyLight 488	DyLight 550	DyLight 650	DyLight 680	DyLight 800 4× PEG
GAPDH Loading Control Antibody (GA1R)	Full-length GAPDH	MA5-15738	MA5-15738-BITN	MA5-15738-HRP	MA5-15738-D488	MA5-15738-D550	MA5-15738-D650	MA5-15738-D680	MA5-15738-D800
β-Actin Loading Control Antibody (BA3R)	β-Actin N-terminal Peptide	MA5-15739	MA5-15739-BITN	MA5-15739-HRP	MA5-15739-D488			MA5-15739-D680	MA5-15739-D800
β-Tubulin Loading Control Antibody (BT7R)	β-Tubulin N-terminal Peptide	MA5-16308	MA5-16308-BITN	MA5-16308-HRP	MA5-16308-D488	MA5-16308-D550	MA5-16308-D650	MA5-16308-D680	MA5-16308-D800

Thermo Scientific Pierce Unconjugated Epitope Tag Antibodies.

Epitope Tag	Target	Unconjugated
Biotin Antibody (BTN.4)	Biotin	MA5-11251
Horseradish peroxidase (HRP) Antibody (HP-03)	HRP	MA1-10371
FITC Antibody (#9)	FITC	MA5-14696
Phycoerythrin B Antibody	Phycoerythrin B	PA1-28741
RFP Tag Antibody (RF5R)	Red Fluorescent Protein N-terminal Peptide	MA5-15257
GFP Epitope Tag Antibody	Enhanced Green Fluorescent Protein	MA1-952
Turbo GFP Antibody	Full-length recombinant TurboGFP	PA5-22688
VSV-G Tag Antibody	YTDIEMNRLGK	PA1-29903
TAMRA Antibody (5G5)	TAMRA Molecule	MA1-041
Maltose Binding Protein (MBP) Antibody	Recombinant maltose binding protein	PA1-989
TAP Tag Antibody	C-terminus of the TAP construct	CAB1001
TEV Cleavage Site Antibody	TEV Cleavage Site	PA1-119
T7 Antibody	MASMTGGQQMG	PA1-32386
BSA Antibody	BSA	PA5-23403
Streptavidin Antibody (S10D4)	Streptavidin	MA1-20010
Beta-Gal Antibody (S.394.9)	Recombinant β-galactosidase protein	MA5-15222
cAMP Antibody (9H4C4)	cAMP Molecule	MA5-15393
E7 Antibody (3D6F1)	E7 Fragment	MA5-15439
Pan SUMO Antibody	Pan SUMO	PA5-11373
AU1 Tag Antibody	DTYRYI	PA1-26547
AU5 Tag Antibody	TDFYLK	PA1-26552
Glu-Glu Tag Antibody	Synthetic Peptide	PA5-17335

Thermo Scientific Pierce Unconjugated Loading Control Antibodies.

Loading Control	Target	Unconjugated
γ- Tubulin Loading Control Antibody (4D11)	γ-Tubulin C-terminal Peptide	MA1-850
Lamin A/C Loading Control Antibody (mab636)	Lamin A/C	MA3-1000
Cyclophilin B Loading Control Antibody	Cyclophilin B C-terminal Peptide	PA1-027A
Cyclophilin D Loading Control Antibody	Cyclophilin D C-terminal Peptide	PA3-022
COX IV Loading Control Antibody (K.473.4)	COX IV N-terminal Peptide	MA5-15078
Histone H3 Loading Control Antibody (E.960.2)	Histone H3 C-terminal Peptide	MA5-15150
Profilin 1 Loading Control Antibody	Profilin 1 Synthetic Peptide	PA5-17444
VDAC1 Loading Control Antibody	VDAC1 N-terminal Peptide	PA5-17460

Wash

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 # PI28320) 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.

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.

Thermo Scientific™ BupH™ Phosphate Buffered Saline Packs (PBS)

Great wash buffer for Western blots!

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

Ordering Information

Product #	Description	Pkg. Size
PI28372	BupH Phosphate Buffered Saline Packs	40 pack
PI28348	20X Phosphate Buffered Saline	500mL
PI28352	20X PBS Tween-20	500mL

Thermo Scientific™ BupH™ Tris Buffered Saline (TBS)

Great wash buffer for Western blots!

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

Ordering Information

Product #	Description	Pkg. Size
PI28380	BupH Tris-Glycine Buffer Packs	40 pack
PI28376	BupH Tris Buffered Saline Packs	40 pack
PI28379	BupH Tris Buffered Saline Packs	10 pack

Thermo Scientific™ 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

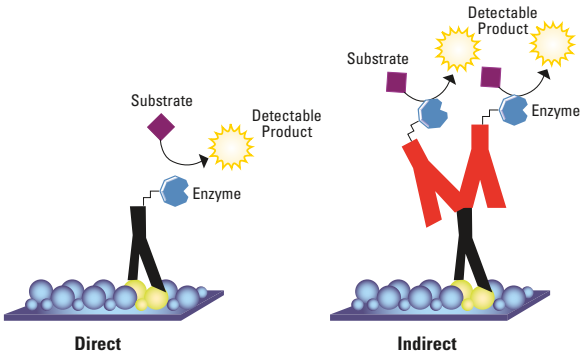
Ordering Information

Product #	Description	Pkg. Size
PI28320	Surfact-Amps 20 Solution	6 x 10mL

Secondary Incubation

A secondary antibody aids in the detection, sorting or purification of target antigens by binding to a primary antibody, which directly binds to the target antigen. The use of secondary antibodies to indirectly detect target antigens requires more steps than using the primary antibody alone but can also offer significant advantages over primary antibodies that are directly labeled. Secondary antibodies offer increased sensitivity through the signal amplification that occurs as multiple secondary antibodies bind to a single primary antibody. In addition, a given secondary antibody can be used with any primary antibody of the same isotype and target species, making it a more versatile reagent than individually labeled primary antibodies.

Because the vast majority of primary antibodies are produced in just a few host animal species and most are of the IgG class, it is economical to produce and supply ready-to-use secondary antibodies for many methods and detection systems. From a relatively small number of secondary antibodies, many options are available for purity level, specificity and label type for a given application.



Specificity of Secondary Antibodies

Secondary antibodies are generated by immunizing a host animal with an antibody from a different species. For example, anti-mouse antibodies are raised by injecting specific purified mouse antibody into an animal other than a mouse. Goat, donkey, sheep, chicken and rabbit are the most commonly used host species for raising secondary antibodies, though others are available.

The most common types of secondary antibodies are those generated against a pooled population of immunoglobulins from a target species. For example, immunizing a goat with purified mouse IgG will generate goat anti-mouse IgG antibodies that will bind to all classes, heavy and light chains (H&L) and fragments of mouse IgG, as well as any other molecules sharing the same conserved domains (e.g., IgM share the same kappa light chains as IgG). In contrast, immunizing a goat with only mouse IgG_1 antibodies will only generate antibodies specific for mouse IgG_1 antibodies and molecules sharing the same conserved domains.

Because of the high degree of conservation in the structure of many immunoglobulin domains, class-specific secondary antibodies must be affinity purified and cross-absorbed to achieve minimal cross-reaction with other immunoglobulins. Using the example described above, immobilized mouse IgG_1 antibodies would be used to affinity purify all goat antibodies that bind to mouse IgG_1. These anti-mouse IgG_1 antibodies would then be further purified by passage through a chromatography column(s) containing mouse IgG_{2a}, IgG_{2b}, IgG_3, IgM, etc., to remove any antibodies that cross-react with non-IgG_1 isotypes.

Additionally, secondary antibodies can be further purified by passage through columns containing the immobilized serum proteins from species other than those used to immunize the host. This method of cross-absorption (referred to as "Cross-Absorbed") is an additional purification step recommended for applications where primary antibodies from multiple species will be used and when immunoglobulins or other serum proteins may be present in the samples being probed.

Commonly used abbreviations for target species.

Target Species	Abbreviation
Bovine	Bv
Canine	Ca
Chicken	Ck
Donkey	Do
Feline	Fe
Goat	Gt
Guinea Pig	GP
Hamster	Hm
Human	Hu
Horse	Eq
Monkey	Nhp
Mouse	Ms
Rabbit	Rb
Rat	Rt
Sheep	Ov
Pig	Po

Secondary Antibody Fragments

Secondary antibodies may be provided in several formats: whole IgG, divalent F(ab')$_2$ fragments and monovalent Fab fragments.

Whole IgG

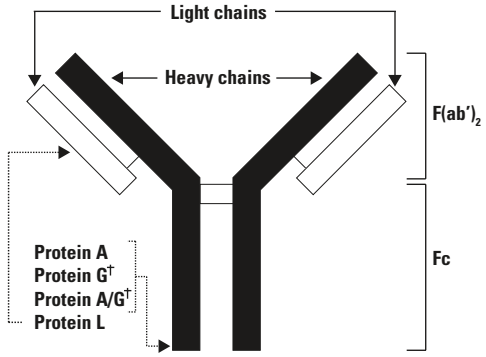
The most widely used secondary antibodies are whole IgG molecules. They are typically affinity purified from the pooled serum from a host immunized with the desired purified immunoglobulin from a different species. Isolated IgGs can then be further purified and depleted against unwanted cross-reactive proteins or immunoglobulins from other species to further enhance specificity.

F(ab')$_2$ antibody fragments

While whole immunoglobulins are compatible with most assays, certain methods benefit from removing the Fc portion of the antibody in order to reduce the mass of the antibody, due to cross-reactivity of other antibodies with host Fc, or because the probed samples contain active Fc-binding proteins (e.g., Fc receptors, IgG-like domains, Protein A, Protein G). The Fc portion can be removed from several species of IgG by digestion with pepsin, leaving the divalent F(ab')$_2$ fragment (~100 kDa) of the antibody intact.

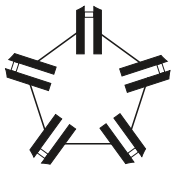
Fab antibody fragments

Most species of IgG can be enzymatically digested with papain to cleave the antibody between the antigen binding domain and the hinge region to produce two Fab fragments and an Fc fragment. The monovalent Fab antibody fragments are useful in blocking applications and other special circumstances where controlled binding ratios and/or the elimination of Fc interactions is required. The small size (~50 kDa) of Fab fragments may improve antigen detection by penetrating deeper than whole IgG into tissue sections and other complex samples. Fab fragments can also be useful in targeting intracellular antigens as they are small enough to pass through the membrane in living cells.



Whole IgM

Whole IgM is comprised of five Y-shaped units connected through their Fc domains by a J-chain. The pentamer has 10 heavy chains, 10 light chains, and 10 antigen-binding sites. Anti-IgM secondary antibodies are generated by injecting a host with purified whole IgM. The use of secondary antibodies that recognize whole IgM frequently may result in unacceptable background and lower specificity for two reasons. First, light chains are shared by all immunoglobulin types. Second, IgG tends to be the predominant species in serum and other samples. Consequently, an antibody to whole IgM tends to cross-react with IgG light chains. Quite often a better choice is a secondary antibody that reacts specifically with the unique IgM mu (μ) heavy chain or the IgM pentameric Fc5μ fragment.

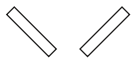


IgM Fc5μ Fragment

The Fc5μ fragment consists of the interconnected bases of the five Y-shaped units. The upper portions of the heavy chains (μ chains) and the entire light chains are absent. Thermo Scientific™ Pierce™ Anti-Fc5μ secondary antibodies are generated by injecting a host with the fragment. Because these secondary antibodies recognize the IgM fragment but neither immunoglobulin light chains nor non-IgM heavy chains, their use typically results in less nonspecific binding and background signal than a secondary antibody that recognizes whole IgM molecule.

Mu Chain

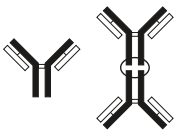
Mu (μ) chains are the heavy chains that define the IgM class of immunoglobulins. Individual μ chains are univalent with only a portion of a single antigen-binding site instead of 10 antigen-binding sites, as on the whole pentamer. The light chains and "J" chain are absent. Mu-chain specific secondary antibodies are produced by injecting a host with whole IgM and then absorbing the anti-serum to remove antibodies against light chains. These secondary antibodies can detect whole IgM, Fab, F(ab')$_2$, Fc5μ, and Fcμ fragments, as well as the μ chain itself. Because they only recognize epitopes found on the μ chain, cross-reactivity with other immunoglobulin light chains and non-IgM heavy chains is eliminated. Mu-chain specific secondary antibodies typically produce less nonspecific binding and background signal than antibodies produced against whole IgM.



Immunoglobulin Light Chains

Secondary antibodies are available to specifically recognize either the kappa (κ) or lambda (λ) light chains of human immunoglobulins. Light chains consist of constant (CL) and variable (VL) domains, each containing about 110 amino acids. The proximal constant domain of light chains is shared by all immunoglobulins (IgG, IgM, IgA, IgE, and IgD) within a species. The terminal variable domain is involved in antigen recognition. The two types of constant regions give the light chains their designation as either kappa or lambda. Whether kappa or lambda, all light chains are bound to heavy chains through disulfide bonds and hydrophobic interactions. Knowing the type of light chain that predominates in a sample can be critical. For example, lambda chains (and some kappa subgroups) do not bind well to Protein L. Consequently, Protein L is a poor choice for the immunoaffinity purification of immunoglobulins whose light chains are of the lambda variety.

Whole IgA



Whole IgA is made of two Y-shaped units connected through their Fc domains by a J-chain. The dimer has four heavy chains, four light chains, and four antigen-binding sites. Injecting a host with whole IgA produces anti-IgA secondary antibodies. However, using secondary antibodies that recognize whole IgA often results in unacceptable background signal and lower specificity. This is because light chains are shared by all immunoglobulin types, and IgG tends to be the predominant species in serum and other samples. Therefore, antibodies to whole IgA can cross-react with IgG light chains. To increase specificity and decrease background signal, it may be necessary to choose a secondary antibody that reacts specifically with the IgA (alpha-Heavy Chain).

IgA (alpha-Heavy Chain) Fragments

Alpha chains are the heavy chains that define the IgA class of antibodies. Individual alpha chains are univalent with only a portion of a single antigen-binding site instead of four antigen-binding sites, as on the whole dimer. The light chains and "J" chain are absent. Alpha-chain specific secondary antibodies are generated by injecting a host with whole IgA and then absorbing the anti-serum to remove antibodies against light chains. These secondary antibodies can detect both IgA_1 and IgA_2 isotypes. Because they only recognize epitopes found on the alpha chain, IgA (alpha-Heavy Chain) fragments do not cross-react with IgG, IgM, IgE, or IgD heavy chains, T-cells, monocytes, granulocytes, or erythrocytes. Consequently, alpha-chain specific secondary antibodies typically produce less nonspecific binding and background signal than antibodies produced against whole IgA.

Whole IgD and Whole IgE

Secondary antibodies against whole IgD and whole IgE are less common, but are available. IgD and IgE are monomeric antibodies with two Ig light chains and two class-specific heavy chains, delta (δ) for IgD and epsilon (ε) for IgE. Injecting a host with whole IgD produces anti-IgD secondary antibodies, while injection with IgE produces anti-IgE secondary antibodies.

Secondary Antibody Common Targets

Secondary antibodies are commonly generated with specificity against whole immunoglobulins (IgG, IgM, IgA, IgD, IgE) and against specific antibody fragments (e.g., Fc, mu, alpha-Heavy) as outlined in the table. IgG is the most common primary antibody type. However, the other immunoglobulin isotypes and their fragments are important secondary antibody targets.

Commonly used abbreviations for secondary antibody specificity. In addition to class and species specificity, secondary antibodies can be generated against specific antibody fragments [$F(ab')_2$, Fab] or individual antibody chains (mu, gamma, kappa) and domains. The following is a list of commonly used notations that indicate the specificity of secondary antibodies:

H+L	(heavy and light chains) whole immunoglobulin (Ig) and any molecule containing those chains or domains. This generally means that antibodies produced using this method will recognize the predominant isotypes IgG, IgM, and IgA due to their conserved regions.
Fc	(Fragment, crystallizable region) heavy chain regions forming the hinge and binding sites for Fc receptors, Protein A and Protein G
Fab	(Fragment, antigen binding) heavy and light chain regions forming the antigen binding domain
$F(ab')_2$	heavy and light chain regions forming the antigen-binding domains as well as the hinge region
IgM	whole immunoglobulin IgM
Fc5μ	5 connected Fc regions of IgM including the lower portion of the mu heavy chain
M	mu heavy chain (IgM class)
γ	gamma heavy chain (IgG class)
κ	kappa light chain
λ	lambda light chain
IgA	whole immunoglobulin A
alpha-heavy	alpha heavy chain (IgA class)
IgD	whole immunoglobulin IgD
IgE	whole immunoglobulin IgE

Choosing a Secondary Antibody

In particular methods, typical secondary antibodies are either too specific (e.g., recognize only one host species of primary antibody) or too general (e.g., recognize whole IgG and any fragments thereof). In most cases, these limitations can be overcome by carefully designing the experimental system and choosing the appropriate secondary probe. The considerations in the graphic below are useful to help choose a secondary antibody.

Once these considerations have been taken into account, it may be helpful to understand how Pierce Antibodies are presented including both what the secondary antibody is and what it targets. This example may be helpful in distinguishing the structure and specificity of a particular secondary antibody as found on our website.

$F(ab')_2$ Goat anti-Mouse IgG-$F(ab')_2$ Cross-Absorbed Antibody, DyLight 550 Conjugate

This name describes a goat IgG that has been purified to the $F(ab')_2$ fragment. It targets mouse IgG and is specific to the $F(ab')_2$ region. It is also cross-absorbed against serum proteins of other species and is conjugated to DyLight Dyes.

Choosing a secondary antibody.

1 Determine origin of the primary antibody.

- Mouse monoclonal
- Rat monoclonal
- Rabbit poly/monoclonal
- etc.

2 Select an appropriate host species for the secondary antibody.

- Goat Anti-Mouse IgG
- Anti-Rabbit IgG
- etc.

3 Consider cross-reactivity or specificity issues of the secondary:

- Cross-absorbed – for multiple-labeling applications or when using samples with endogenous antibodies
- Specificity – binds to unique fragments, classes or chains of the primary antibody

4 Detection or purification method.

- Label – appropriately conjugated to the correct enzyme, tag or fluorophore for the chosen detection method
- Ability to bind to Protein A, Protein G or Protein L – make sure the secondary antibody chosen has sufficient affinity for the molecules used upstream or downstream (i.e., Protein A-coated microplates.)

5 Consider requirements of the supplied secondary:

- Supplied state – sterile liquid or lyophilized, suspended in PBS or Tris buffer
- Contains carrier proteins such as gelatin or albumin, or the addition of stabilizers such as sucrose or microbial inhibitors

6 Order your product from our list to the right, or visit **thermoscientific/pierce-abs.com** for a complete list

Incubation

Target	Specificity	Host	Conjugate												
			Unconju-gated	AP	Biotin	HRP	DyLight Dyes								Additional Fluors
							350	488	550	594	650	680	775	800	
Bovine	Bovine IgG	Chicken			SA172014	SA172015									SA172013
	Bovine IgG	Goat	PA128695		PA128699	PA128700									PA128696
	Bovine IgG	Mouse	MA119384			MA516733									
	Bovine IgG	Rabbit	PA128546	PA130375	PA128693	PA128694									PA128690
	Bovine IgG	Sheep				PA184664									
	Bovine IgG_1	Mouse	MA516735			MA516736									
	Bovine IgG_1	Sheep				PA18 4658									
	Bovine IgG_2	Mouse				MA516737									
	Bovine IgG_2	Sheep				PA184661									
	Bovine IgM	Mouse	MA190180												
	Bovine IgM	Sheep				PA184688									
	Bovine IgG/ IgM/IgA	Rabbit	SA136042												SA136043
	Bovine IgG/ IgM/IgA	Mouse	SA110639												
Cat	Feline IgG	Goat			PA184673										
	Feline IgG	Mouse	SA110641												
Chicken	Chicken IgG (IgY)	Bovine	SA19500		SA19505										
	Chicken IgG (IgY)	Donkey	SA172002		SA172003	SA172004									SA172000
	Chicken IgG (IgY)	Goat	SA114012	PA130369	PA128657	PA128658	SA510069+	SA510070+	SA510071+	SA510072+	SA510073+	SA510074+	SA510075+	SA510076+	
	Chicken IgG (IgY)	Mouse	SA135320			SA135325									
	Chicken IgG (IgY)	Rabbit	PI31104	SA19514	PI31720	PI31401									PI31501
	Chicken IgG (IgY), Fab specific	Goat	SA172005												SA172006
	Chicken IgG (IgY), Fab specific	Goat	SA172009			SA172012									
	Chicken IgA	Goat				PA184679									
	Chicken IgM	Goat				PA184676									
	Chicken IgM	Mouse	SA135322												
	Chicken IgG/ IgM/IgA	Mouse	SA135321												
Dog	Canine IgG/ IgM/IgA	Mouse	MA516757												
	Canine IgG	Goat				PA129738									
	Canine IgG	Sheep				PA184644									
	Canine IgM	Goat				PA184638									PA184637
	Canine IgA	Mouse	MA516752												
Donkey	Donkey IgG	Goat	PA128733	PA130377	PA128737	PA128738									PA128734
	Donkey IgG	Rabbit	SA126816	PA130343	PA128591	PA128592	SA510061	SA510062	SA510063	SA510064	SA510065	SA510066	SA510067	SA510068	PA128588
Goat	Goat IgG	Donkey	PA128659	PA130370	PA128663	PA128664 PA186326+	SA510085+	SA510086+	SA510087+	SA510088+	SA510089+	SA510090+	SA510091+	SA510092+	PA128660
	Goat IgG, Fab specific	Donkey			PA129616	PA129617									
	Goat IgG	Mouse	PI31107		PI31730	PI31400									PI31512
	Goat IgG	Rabbit	PI31105	PI31300	PI31732	PI31402									PI31509
	Goat IgG	Rabbit					SA510077	SA510078	SA510079	SA510080	SA510081	SA510082	SA510083	SA510084	
	Goat IgG, Fab specific	Rabbit			PI31753	PI31403									PI31553

✚ *Indicates cross-adsorbed antibody*

Target	Specificity	Host	Conjugate												
			Unconjugated	AP	Biotin	HRP	DyLight Dyes								Additional Fluors
							350	488	550	594	650	680	775	800	
Goat (cont.)	Goat IgG, Fc specific	Rabbit	PI31133	PI31337		PI31433									PI31533
	Goat IgG	$F(ab')_2$-Rabbit	PI31109+	PI31302+	PA129560+										
	Goat IgM	Rabbit				PA129384									
Guinea Pig	Guinea Pig IgG	Goat	PA128674	PA130372	PA128678	PA128679	SA5-10093+	SA5-10094+	SA5-10095+	SA5-10096+	SA5-10097+	SA5-10098+	SA5-10099+	SA5-10100+	PA128675
	Guinea Pig IgG	Rabbit	PA128549	PA130344	PA128596	PA128597									PA128593
	Guinea Pig IgG	Mouse	MA180575												
Hamster	Hamster IgG	Goat	PI31115 PA533248+	PA129558	PI31750 PA533284+	PA129626 PA533286+									PA128666 PA533285+
	Hamster IgG	Rabbit	SA126817	PA130348	PA130346	PA130347									PI31587
Human	Human IgG	Bovine	SA19501		SA19506										
	Human IgG	Chicken	SA172036		SA172038	SA172039									SA172037
	Human IgG	Donkey					SA5-10125+	SA5-10126+	SA5-10127+	SA5-10128+	SA5-10129+	SA5-10130+	SA5-10131+	SA5-10132+	
	Human IgG	Duck	D01062B												
	Human IgG	Goat	PI31130 PI31119+	PI31310+	PI31770 PI31774+	PI31410 PI31774+	SA5-10117+	SA5-10118+	SA5-10119+	SA5-10120+	SA5-10121+	SA5-10122+	SA5-10123+	SA5-10124+	PI31529 PI31531+
	Human IgG	$F(ab')_2$-Goat	PI31165												
	Human IgG	Mouse	SA135468 PI31137+		PI31784+	SA135470 PI31420+									SA135469
	Human IgG	Rabbit	PI31143	PA130342	PI31786	PA128587	SA5-10109+	SA5-10110+	SA5-10111+	SA5-10112+	SA5-10113+	SA5-10114+	SA5-10115+	SA5-10116+	SA136099
	Human IgG	Sheep		PA130368		PA128652									PA516924
	Human IgG Fab specific	Chicken	SA172043		SA172044	SA172045									
	Human IgG Fab specific	Goat	PI31122 PI31132+	PI31312		PI31482 PA533290+									PI31628
	Human IgG Fab specific	Mouse	SA119255												MA110377
	Human IgG Fc specific	Chicken	SA172046		SA172048	SA172049									SA172047
	Human IgG Fc specific	Goat	PI31125+			PI31413+	SA5-10133+	SA5-10134+	SA5-10135+	SA5-10136+	SA5-10137+	SA5-10138+	SA5-10139+	SA5-10140+	
	Human IgG Fc specific	$F(ab')_2$-Goat	PI31163												
	Human IgG Fc specific	Mouse	MA516858			MA516859									MA110379
	Human IgG Fc specific	Rabbit	PI31142	PI31318	PI31789	PI31423									PI31535
	Human IgM	Chicken	SA172050			SA172053									
	Human IgM	Goat	PI31124		PI31778	PI31415	SA5-10101+	SA5-10102+	SA5-10103+	SA5-10104+	SA5-10105+	SA5-10106+	SA5-10107+	SA5-10108+	PI31575
	Human IgM	Mouse	SA126391			SA135473									SA119598
	Human IgM	Rabbit	SA1400												PA516598
	Human IgA	Chicken	PY10126												
	Human IgA	Goat	PI31140	PI31314	PA186082	PI31417									PI31577
	Human IgA	Mouse	SA119258	MA183582		SA135467									SA135466
	Human IgA	Rabbit	PA516388												SA136096
	Human IgA	Sheep	PA516923												
	Human IgA, Fc specific	Mouse	MA511208												
	Human IgG/IgM/IgA	Goat	PI31128	PI31316	PI31782	PI31418									
	Human IgG/IgM/IgA	$F(ab')_2$-Goat	PI31539												

+ *Indicates cross-adsorbed antibody*

Incubation

Target	Specificity	Host	Conjugate												
			Unconjugated	AP	Biotin	HRP	DyLight Dyes								Additional Fluors
							350	488	550	594	650	680	775	800	
Human (cont.)	Human IgE	Chicken	PY10111												
	Human IgE	Mouse	MA182854	MA191884											MA110374
	Human IgE	Rabbit	PA516396												
	Human IgD	Mouse	MA190969												
	Human IgD	Rabbit	PA516400												
	Human Gamma Chain	Goat	PI31118												
	Human Kappa Chain	Goat	PI31129		PI31780	PA174407									PA174405
	Human Kappa Chain	Mouse	MA135486												
	Human Lambda Chain	Goat	PI31131												
	Human Lambda Chain	Mouse	SA119254												MA180937
	Human IgG_1	Mouse	MA134581												
	Human IgG_1	Sheep	PA533139												
	Human IgG_1, Fc Specific	Mouse	MA183240												
	Human IgG_2	Mouse	MA183241												
	Human IgG_2 , Fab specific	Mouse	MA516715			MA134754									
	Human IgG_{2a}	Rat	MA190900												
	Human IgG_3	Sheep	PA533246			PA186429									
	Human IgG_3	Mouse	MA183242												
	Human IgG_3, Hinge Region	Mouse	MA516717			MA516718									
	Human IgG_4	Mouse	MA516716			MA134437									
	Human IgG_4, Fc Specific	Mouse				MA182201									
Horse	Equine IgG	Goat			PI31760										
	Equine IgM	Goat				PA184647									
	Equine IgG/ IgM/IgA	Rabbit	SA136091												SA136092
Monkey	Monkey IgG	Goat	PA184629			PA184631									
	Monkey IgG	Mouse	SA110653												
	Monkey IgA	Mouse	MA516729		MA516730										
Mouse	Mouse IgG	Bovine	SA19502		SA19507										
	Mouse IgG	Chicken	SA172018		SA172020	SA172021									
	Mouse IgG	Donkey	PA129956	PA128749	PA128627	PA128748 SA1100+	SA5-10165+	SA5-10166+	SA5-10167+	SA5-10168+	SA5-10169+	SA5-10170+	SA5-10171+	SA5-10172+	PA129772
	Mouse IgG	Goat	PI31160 PI31164+	PI31320 PI31322+	PI31800 PI31802+	PI31430 PI31432+	PI62271 PI62273+	PI35502 PI35503+	PI84540 SA5-10173+	PI35510 PI35511+	PI84545 SA5-10174+	PI35518 PI35519+	SA5-10175+	PI35521 SA5-10176+	PI31569 PI31541+
	Mouse IgG	F(ab')$_2$-Goat	PI31185+			PI31438+									PI31565+
	Mouse IgG	Horse	PI31181		PI31806										
	Mouse IgG	Rabbit	PI31188 PI31190+	PI31329 PI31334+	PA128567+	PI31450 PI31452+	SA5-10157+	SA5-10158+	SA5-10159+	SA5-10160+	SA5-10161+	SA5-10162+	SA5-10163+	SA5-10164+	PI31561+
	Mouse IgG	Rat	SA135633		SA135634	SA135636									SA135635
	Mouse IgG	Sheep			PA128622										PA128620
	Mouse IgG, Fab specific	Chicken	SA172022		SA172024	SA172025									SA172023

✚ *Indicates cross-adsorbed antibody*

Target	Specificity	Host	Conjugate												
			Unconjugated	AP	Biotin	HRP	DyLight Dyes								Additional Fluors
							350	488	550	594	650	680	775	800	
Mouse (cont.)	Mouse IgG, Fab specific	Goat	PI31166	PI31324	PI31803	PI31436									PI31543
	Mouse IgG, Fab specific	Rabbit	PI31192 PA533287+	PI31331		PI31451									PI31559
	Mouse IgG, Fc specific	Chicken	SA172026		SA172028	SA172029									SA172027
	Mouse IgG, Fc specific	Goat	PI31168 PI31170+	PI31325 PI31327+	PI31805	PI31437 PI31439+									PI31547 PI31630+
	Mouse IgG, Fc specific	Rabbit	PI31194	PI31332	PI31813	PI31455									PI31555
	Mouse IgG, Fc specific	Rat	MA180696		SA125198										
	Mouse IgM	Goat	PI31172	PI31326	PI31804	PI31440 PA184383+	SA5-10149+	SA5-10150+	SA5-10151+	SA5-10152+	SA5-10153+	SA5-10154+	SA5-10155+	SA5-10156+	PI31992
	Mouse IgM	$F(ab')_2$-Goat	PI31178 PI31186+	PA129923	PA128499	PA129581									
	Mouse IgM	Rabbit	PI31196	PI31333		PI31456									PI31557
	Mouse IgM	Rat	MA516793		MA516795										MA516796
	Mouse IgG + IgM	Goat	PI31182	PI31328 PI31330+	PI31807	PI31444 PI31446+									
	Mouse IgG + IgM	$F(ab')_2$-Goat				PI31448+									
	Mouse IgG + IgM	Rabbit	PI31198	PI31335		PI31457									
	Mouse IgA	Goat	PA129741			PA174397									
	Mouse IgA	Rabbit	PA130826												
	Mouse IgA	Rat	SA135626		SA135627										
	Mouse IgG/IgM/IgA	Goat	PA185986			PA184388									
	Mouse IgE	Goat	PA129273			PA184764									
	Mouse IgE	Rat	MA516777		MA516779	MA516781									MA516780
	Mouse IgG_1	Goat	PI31236+			PA174421									
	Mouse IgG_1	Rabbit				PA186329									
	Mouse IgG_1	Rat	MA516782		MA516784	MA516786									MA516785
	Mouse IgG_{2a}	Goat	PA128885 PI31237+												PI31634+
	Mouse IgG_{2a}	Rat	SA135642		MA516788	MA516790									MA516789
	Mouse IgG_{2b}	Rat	SA135647		SA135648	SA135650									SA135649
	Mouse IgG_{2c}	Goat	PA128887			PA129288									
	Mouse Ig_{G3}	Rat	SA135651		SA135652	SA135654									SA135653
	Mouse Kappa	Goat				PA186015									
	Mouse Kappa	Rat	SA125258			MA134732									
	Mouse Lambda	Goat		PA186022	PA186025										
Pig	Porcine IgG	Goat	PA184097			PA128685									PA184850
	Porcine IgG, Fc Specific	Goat				PA184628									
	Porcine IgG	Mouse	MA180544												
	Porcine IgG_1	Mouse	MA180545												
	Porcine IgG_2	Mouse	MA180546												
	Porcine IgG	Rabbit				PA128602									
	Porcine IgM	Goat				PA184622									
	Porcine IgA	Goat				PA184625									PA184624
	Porcine IgA	Mouse	MA180548												

✚ *Indicates cross-adsorbed antibody*

Incubation

Target	Specificity	Host	Conjugate													
			Unconjugated	AP	Biotin	HRP	DyLight Dyes								Additional Fluors
							350	488	550	594	650	680	775	800	
Rabbit	Rabbit IgG	Donkey	PI31238+	PI31345+	PI31821+	PI31458+	SA510037+	SA510038+	SA510039+	SA510040+	SA510041+	SA510042+	SA510043+	SA510044+	PI31568+
	Rabbit IgG	Goat	PI31210 PI31212+	PI31340 PI31342+	PI31820 PI31822+	PI31460 PI31462+	PI62270 PI62272+	PI35552 PI35553+	PI84541	PI35560 PI35561+	PI84546	PI35568 PI35569+		PI35571	PI31635 PI31583+
	Rabbit IgG	Fab'$_2$-Goat	PI31239+												PI31579 PI31636+
	Rabbit IgG	Mouse	PI31213+		PI31824+	PI31464+									PI31584+
	Rabbit IgG Fc Specific	Goat	PI31216	PI31341		PI31463									
	Rabbit IgG Fab Specific	Goat	PI31234	PI31343	PI31823	PI31461									PI31573
	Rabbit IgA	Goat				PA174362									
Rat	Rat IgG	Chicken	PA128630	PA130741	PA130361	PA130362									PA129948
	Rat IgG	Donkey					SA510025+	SA510026+	SA510027+	SA510028+	SA510029+	SA510030+	SA510031+	SA510032+	PA533282+
	Rat IgG	Goat	PI31220	PI31350	PI31830	PI31470									
	Rat IgG	Goat					SA510017	SA510018	SA510019	SA510020	SA510021	SA510022	SA510023	SA510024	
	Rat IgG, Fab specific	Goat		PA129930	PA129634	PI31474									PI31629
	Rat IgG, Fc specific	Goat	PI31226			PI31475									PI31621
	Rat IgG	Mouse	SA110652												
	Rat IgG	Rabbit	PI31218 PI31219+	PA130338	PI31834	PA128573									PA128569
	Rat IgG, Fab specific	Rabbit		PA129928	PA129926	PA533281									PA533280
	Rat IgG	Sheep	PA128637	PA130363	PA128641	PA128642									PA128638
	Rat IgG$_1$	Goat				PA184708									
	Rat IgG$_1$	Mouse	SA125263		SA125262										
	Rat IgG$_1$, Fc specific	Mouse	MA516814		MA516815										
	Rat IgG$_{2a}$	Goat	PA184755			PA184709									PA184761
	Rat IgG$_{2a}$	Mouse													SA125265
	Rat IgG$_{2a}$, Fc specific	Mouse	MA516816		MA516817	MA516818									
	Rat IgG$_{2b}$	Goat				PA184710									
	Rat IgG$_{2b}$	Mouse			SA125268										
	Rat IgG$_{2b}$, Fc specific	Mouse	MA516819												SA125267
	Rat IgG$_{2c}$	Goat				PA184711									
	Rat IgG$_{2c}$	Mouse	SA125269												SA125270
	Rat IgM	Goat			PI31832	PI31476	SA510009+	SA510010+	SA510011+	SA510012+	SA510013+	SA510014+	SA510015+	SA510016+	
	Rat IgM	Mouse			SA125271										SA125272
	Rat IgM, Heavy Chain	Mouse			MA516820										MA516821
	Rat IgE	Goat	PA129379												
	Rat IgE	Mouse	MA516810			MA134725									MA516812
	Rat IgE	Sheep	PA184247		MA516811										
	Rat IgA	Goat				PA184707									
	Rat IgD	Mouse	MA180788		MA516809										
	Rat IgG/IgM/IgA	Rabbit	SA136147												SA136148
Sheep	Sheep IgG	Donkey			SA174000	SA174002									SA174001
	Sheep IgG	Rabbit	PI31240	PI31360	PI31840	PI31480	SA510053+	SA510054+	SA510055+	SA510056+	SA510057+	SA510058+	SA510059+	SA510060+	PI31627
Protein	NeutrAvidin	Protein	PI31000	PI31002		PI31001		PI21832	PI84606	PI21842	PI84607	PI21848		PI21851	PI31006
	Streptavidin	Protein	PI21122	PI21324		PI21126		PI22832	PI84542	PI22842	PI84547	PI22848		PI22851	PI21124

✚ *Indicates cross-adsorbed antibody*

HRP Conjugates

Thermo Scientific™ Goat Anti-Mouse HRP and Goat Anti-Rabbit HRP Conjugates

The recommended choice for routine ELISA and Western blotting applications.

These affinity-purified polyclonal antibodies have well-characterized specificity for mouse immunoglobulin G (IgG) or rabbit IgG. The antibodies are affinity-purified using their target mouse IgG and rabbit IgG antigens immobilized on agarose supports. The purified antibodies are conjugated to HRP and lyophilized in the presence of bovine serum albumin as the stabilizer. Make Goat Anti-Mouse and Goat Anti-Rabbit HRP Conjugates the workhorses for your typical ELISA and Western blotting applications.

Ordering Information

Product #	Description	Pkg. Size
PI31430	Goat Anti-Mouse IgG (H+L), Peroxidase Conjugated	2.0mL
PI31460	Goat Anti-Rabbit IgG (H+L), Peroxidase Conjugated	2.0mL

Both are supplied lyophilized from PBS with BSA; results in 0.8mg/mL upon reconstitution in 2mL of water.

Thermo Scientific™ Pierce™ Stabilized HRP Conjugates

Pre-diluted horseradish peroxidase conjugates for hassle-free solution prep.

Pierce Stabilized HRP Conjugates are stabilized in pre-diluted form for greater accuracy and convenience in preparing probing solutions for ELISA, Western blotting and other detection procedures. The conjugates are accurately prepared, dispensed and supplied at 10µg/mL (100X more dilute than typical 1mg/mL preparations), eliminating the inaccuracies associated with two-stage dilution schemes required with traditional conjugate preparations for use with chemiluminescent substrates and other high-sensitivity detection methods. The liquid formulation of each pre-diluted HRP-conjugate is stable at 4°C, eliminating the need to freeze stock solutions for storage.

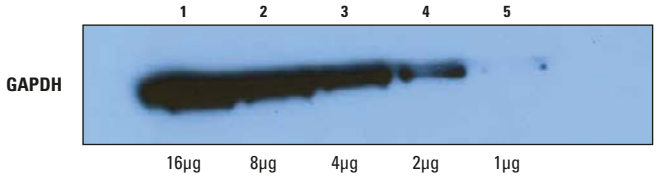


Figure 1. Superb Western blot results with Thermo Scientific Pierce Stabilized HRP Conjugates. Western blot of HeLa lysate samples probed with a mouse anti-GAPDH antibody (1:2,000 dilution of 1mg/mL stock solution) and then Stabilized Goat Anti-Mouse-HRP (4ng/mL, 1:2,500 dilution of 10µg/mL stock solution). HRP activity was detected with SuperSignal West Femto Chemiluminescent Substrate (Product # PI34095). If a typical 1mg/mL stock solution of secondary antibody had been used, it would have required a 250,000-fold dilution, which is both tedious, prone to pipetting error and usually wasteful.

Ordering Information

Product #	Description	Pkg. Size
PI32430	Stabilized Goat Anti-Mouse IgG (H+L), Peroxidase Conjugated (10µg/mL)	2mL
PI32460	Stabilized Goat Anti-Rabbit IgG (H+L), Peroxidase Conjugated (10µg/mL)	2mL

Thermo Scientific™ Pierce™ High Sensitivity HRP Conjugates

High-performance horseradish peroxidase conjugates for the most demanding immunoassays.

Pierce High Sensitivity HRP Conjugates meet the demands of today's scientists for utmost sensitivity in ELISA and Western blotting applications. Our Thermo Scientific™ Streptavidin and NeutrAvidin™ Protein Conjugates are ideal for immunohistochemistry (IHC), Western blotting and ELISA applications using chemiluminescence, chemifluorescence or colorimetric substrates. Each Pierce High Sensitivity HRP Conjugate is packaged in an easy-to-use stabilized solution that enables convenient storage at 4°C for up to 1 year.

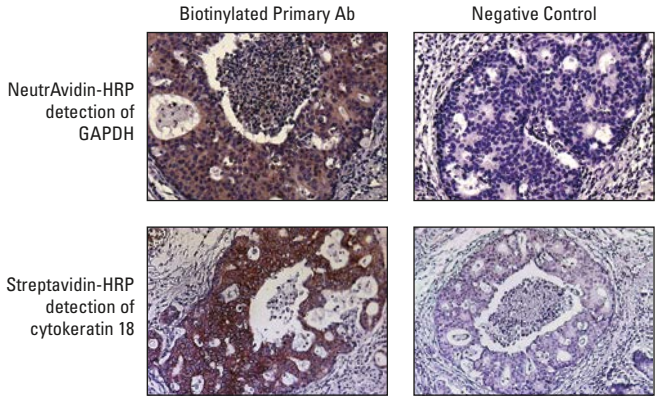


Figure 2. Excellent IHC staining of GAPDH and cytokeratin 18 in human colon carcinoma with Thermo Scientific High Sensitivity HRP Conjugates. Formalin-fixed paraffin embedded human colon carcinoma tissues were stained using Metal Enhanced DAB Substrate (Product # PI36000). The tissues were incubated with biotinylated mouse anti-GAPDH at 4.6µg/mL or rabbit anti-cytokeratin 18 at 0.1µg/mL or blocking buffer alone (**Right Panels**). The sections were incubated with High Sensitivity Neutravidin HRP (4µg/mL, **Top Panels**) or High Sensitivity Streptavidin HRP (4µg/mL **Bottom Panels**). Tissues were counterstained using Harris-modified hematoxylin solution (blue staining in **All Panels**).

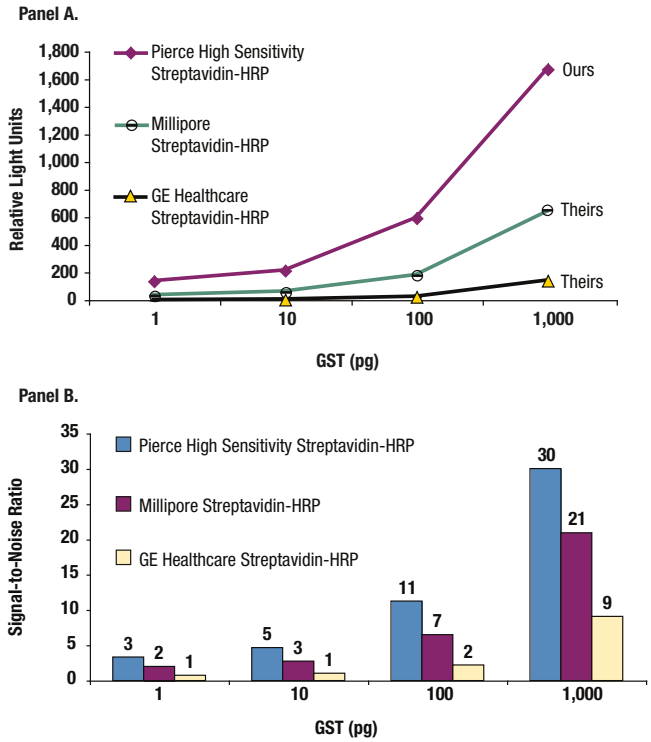


Figure 3. Thermo Scientific Pierce High Sensitivity Streptavidin-HRP enables low-level target detection (Panel A) with high signal-to-noise ratios (Panel B). Recombinant GST (Product # PI20237) was serially diluted (0-10,000pg/mL) with StartingBlock (PBS) Blocking Buffer (Product # PI37538). Each dilution (100µL) was added to a Thermo Scientific™ Pierce™ 96-well Glutathione-coated Plate (Product # PI15340) in four replicates, including a negative control. The plate was incubated for 60 minutes at room temperature and washed three times with PBS Tween-20 Detergent (Product # PI28348). Biotinylated anti-GST (0.25ng; Santa Cruz) was added to all wells. The plate was incubated for 30-60 minutes at room temperature and washed three times with wash buffer. Streptavidin-HRP conjugates were diluted in blocking buffer as per manufacturer's directions. Pierce High Sensitivity Streptavidin-HRP was diluted 1:10,000 and conjugates from other suppliers were diluted to 1:1,000. The conjugate solutions (100µL) were added to the plate and incubated for 60 minutes at room temperature. The plate was washed five times with wash buffer and 150µL/well of Thermo Scientific™ SuperSignal™ ELISA Pico Substrate was added. Signal intensity was measured using a luminometer. Similar results are obtained with the Pierce High Sensitivity NeutrAvidin-HRP Conjugate.

Ordering Information

Product #	Description	Pkg. Size
PI21130	Pierce High Sensitivity Streptavidin-HRP (1mg/mL)	0.5mL
PI21132	Pierce High Sensitivity Streptavidin-HRP 1mg/mL)	5mL
PI21134	Pierce High Sensitivity Streptavidin-HRP, pre-diluted (10µg/mL)	2mL
PI31030	Pierce High Sensitivity NeutrAvidin-HRP (1mg/mL)	0.5mL
PI31032	Pierce High Sensitivity NeutrAvidin-HRP (1mg/mL)	5mL

Thermo Scientific™ Pierce™ Poly-HRP Conjugates

Get the ultimate sensitivity in immunodetection techniques with Pierce Poly-HRP Conjugates.

Pierce Poly-HRP Conjugates deliver the highest sensitivity and low background in immunoassays where sample volume is limited or when the target molecule is present at low levels. Pierce Poly-HRP Conjugates are purified to remove unconjugated probe molecules that reduce signal intensity by competing for binding sites with HRP-conjugated molecules. In addition, these conjugates are free of HRP monomers that may lead to increased background signal. Together, these features provide consistent and reliable sensitivity and deliver higher sensitivity than conventional HRP and poly-HRP conjugates without the need for additional signal amplification steps. These conjugates are compatible with chromogenic, fluorogenic and chemiluminescent HRP substrates used in ELISA, Western blotting, IHC and nucleic acid hybridization assays.

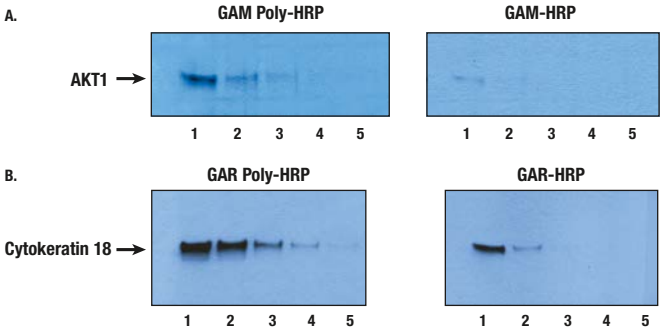


Figure 4. Thermo Scientific Pierce Poly-HRP Secondary Antibodies enable low-level target detection in Western blotting applications. Panel A: Blots were probed with 2,000ng/mL of mouse anti-AKT 1 antibody and detected with 80ng/mL of either goat anti-mouse (GAM) HRP or Pierce Goat Anti-Mouse Poly-HRP. **Panel B:** Blots were probed with 2,000ng/mL of rabbit anti-cytokeratin 18 antibody and detected with 80ng/mL of either goat anti-rabbit (GAR) HRP or Pierce Goat Anti-Rabbit Poly-HRP. **Lanes 1-5** contained 25, 12.5, 6.25, 3.1, 1.6ng of HeLa cell lysate.

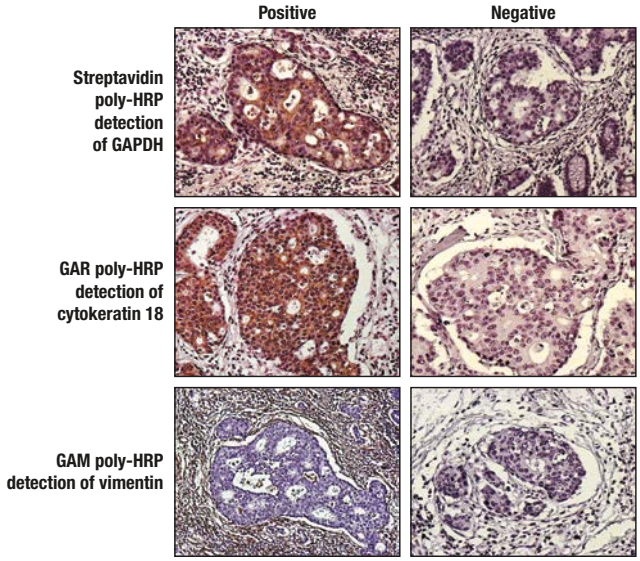


Figure 5. Obtain immunohistochemical images with outstanding clarity using Thermo Scientific Pierce Poly-HRP Conjugates. Formalin-fixed paraffin embedded human colon carcinoma tissues were stained using Thermo Scientific™ Metal Enhanced DAB Substrate (Product # PI36000). The tissues were incubated with biotinylated mouse anti-GAPDH at 4.6µg/mL or rabbit anti-cytokeratin 18 at 1µg/mL or mouse anti-vimentin at 2µg/mL or blocking buffer alone (**Right Panels**). The sections were incubated with Streptavidin Poly-HRP (4µg/mL, **Top Panels**) or Goat anti-Rabbit Poly-HRP (2µg/mL, **Middle Panels**) or Goat anti-Mouse Poly-HRP (2µg/mL, **Bottom Panels**). Tissues were counterstained using Harris-modified hematoxylin solution (blue staining in **All Panels**).

Ordering Information

Product #	Description	Pkg Size
PI32230	Pierce Goat Anti-Mouse Poly-HRP Concentration: 0.5mg/mL	0.5mL
PI32260	Pierce Goat Anti-Rabbit Poly-HRP Concentration: 0.5mg/mL	0.5mL

Antibody Stabilizers and Storage Solutions

Polyclonal secondary antibodies are typically stable when stored frozen as concentrated stock solutions in simple phosphate or Tris buffers containing sodium azide or other antimicrobial agents. Most uses of secondary antibodies require more than 1,000-fold dilution using only a few microliters of stock, and the daily need for Western blotting or ELISA experiments inevitably leads to repeated freeze-thaw cycles that are damaging to the antibody and conjugated enzyme.

Freeze-thaw cycles can be avoided by storing a concentrated antibody stock as a mixture containing 20-50% of an anti-freeze compound such as glycerol or ethylene glycol. Glycerol is most frequently used for this purpose but commonly contains impurities that can adversely affect protein function. High-quality ethylene glycol is superior to glycerol for this purpose. Pierce Peroxidase Stabilizer is an anti-freeze solution that provides the highest purity and performance for antibodies conjugated with horseradish peroxidase (HRP).

The most convenient option is to store antibodies at the working concentration so that dilutions do not have to be repeated for each use. This is rarely possible with typical buffers, but Thermo Scientific™ Guardian™ Peroxidase Conjugate Stabilizer/Diluent provides this sort of protection for antibodies conjugated with HRP. Antibodies can be stored at 1-1,000ng/mL for more than six months at room temperature without losing activity.

For pure enzymes and other non-antibody proteins, use the Thermo Scientific™ Protein Stabilizing Cocktail for storage and preservation.

Storing Enzyme Conjugates

We provide a variety of reagents to help preserve enzyme conjugate activity. Typically, conjugates are aliquoted in 50-100µL increments using purified ethylene glycol (Product # PI29810) 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 # PI31503), diluting the conjugate 1:1 in the stabilizer and storing at -20°C for up to one year as a stock solution. Guardian Peroxidase Conjugate Stabilizer/Diluent (Product #s PI37548 and PI37552) allows peroxidase conjugates to be reconstituted and stored at 4°C as a 1:1,000 or a 1:100,000 dilution.

Ordering Information

Product #	Description	Pkg Size
PI29810	**Ethylene Glycol (50% solution), 200mL** *Sufficient for Mixture 1:1 (v/v) with 200mL of protein samples for freezer storage.*	200mL
PI31503	**Pierce Peroxidase Conjugate Stabilizer, 25mL**	25mL
PI37548	**Guardian Peroxidase Conjugate Stabilizer/Diluent, 200mL**	200mL
PI37552	**Guardian Peroxidase Conjugate Stabilizer/Diluent, 1L**	1L
PI89806	**Protein Stabilizing Cocktail** *Sufficient for 40mL of storage solution.*	10mL

Thermo Scientific™ Clean-Blot™ IP Detection Reagents

Clearly better Western blots.

Antibody bands often mask target proteins when performing Western blots on immunoprecipitated samples. Clean-Blot IP Detection Reagents are unique HRP conjugates that reveal your target protein, allowing clear, specific Western blot detection from immunoprecipitation (IP) experiments and tissue extracts without any interference from denatured IgG (Figure 6). Whereas conventional secondary antibodies recognize both denatured and native IgG, our new reagents bind to only native IgG (Figure 7). So unmask your results by simply substituting the secondary antibody with Clean-Blot IP Detection Reagents for clear Western blots (Figure 8).

Highlights:

- **Versatile** – recognize most native antibodies independent of the host species (Table 5)
- **Compatible** – clear results with IPs performed using Protein A, Protein G or anti-IgG agarose beads and any blocking buffer (e.g., milk, BSA or SuperBlock or StartingBlock Blocking Buffers)
- **Cost effective** – eliminates the need to immobilize IgG and purchase separate kits specific for the primary antibody species; membranes can be stripped and reprobed when chemiluminescent substrate is used
- **Flexible** – use any HRP or AP substrate, including chemiluminescent, fluorescent or colorimetric substrates
- **Easy to use** – simply replace the conventional secondary antibody with the Clean-Blot IP Detection Reagents in your Western blotting protocol
- **Unobstructed detection** – clear IP/Western blot results without interference from denatured IgG bands

Our Clean-Blot IP Detection Reagents are the perfect substitute for traditional secondary antibody conjugates. These unique conjugates recognize most primary antibodies, independent of the host species (Table 1), and can be used with IPs performed using Protein A or G agarose resins. This versatility eliminates the need to buy separate detection kits based on primary antibody species.

Our conjugates are conveniently stored at 2-8°C and are compatible with any HRP or AP substrate, including Thermo Scientific™ Pierce ECL, SuperSignal Chemiluminescent Substrates (Table 2). For added convenience, the HRP conjugate is available in a kit that contains StartingBlock T20 Blocking Buffer and Pierce ECL Chemiluminescent Substrate.

Table 1. Thermo Scientific Clean-Blot IP Detection Reagents recognize the various polyclonal antibodies and the specific monoclonal antibodies listed.
To determine specific antibody compatibility, perform a dot-blot analysis.

Species	Monoclonal Isotype(s)
Bovine	IgG_2
Goat	IgG_2
Human	IgG_1, IgG_2, IgG_4
Mouse	IgG_{2a}, IgG_{2b}, IgG_3
Rat	IgG_{2c}
Sheep	IgG_2

Table 2. Recommended dilution ranges for the Thermo Scientific Clean-Blot IP Detection Reagents when using our chemiluminescent substrates.

Product #	Chemiluminescent Substrate	Recommended Dilution Range
PI34095	SuperSignal West Femto Chemiluminescent Substrate	1:200 to 1:4,000
PI34075	SuperSignal West Dura Chemiluminescent Substrate	1:200 to 1:2,000
PI34080	SuperSignal West Pico Chemiluminescent Substrate	1:40 to 1:1,000
PI32209	Pierce ECL Western Blotting Substrate	1:40 to 1:400

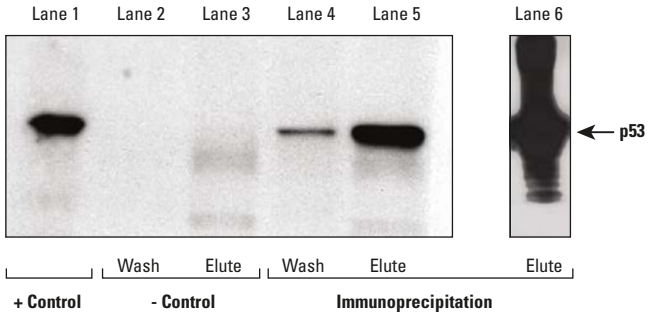


Figure 6. Immunoprecipitation (IP) and Western blot experiments demonstrate specificity of the Thermo Scientific Clean-Blot IP Detection Reagent (HRP). Lane 1. A431 total cell extract expressing p53 **(positive control)**, **Lanes 2 and 3.** No-lysate negative control of IP wash **(Lane 2)** and elution **(Lane 3)** fractions, **Lanes 4-6**. Complete IP experiment of wash **(Lane 4)** and elution **(Lanes 5 and 6)** fractions. **Lanes 1-5** were probed with Clean-Blot IP Detection Reagent (HRP) and **Lane 6** was detected with GAM-HRP.

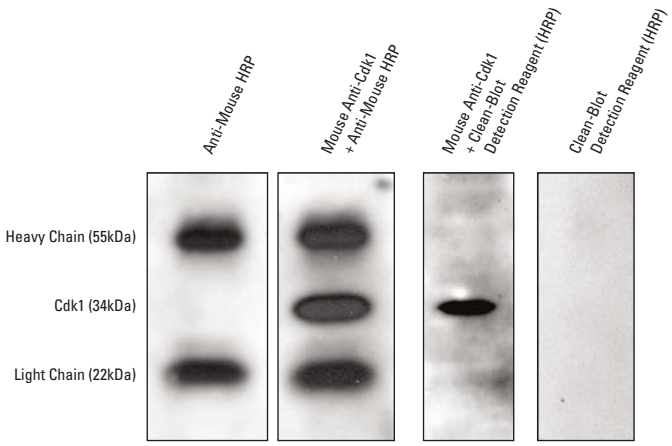


Figure 7. Easily distinguish your target protein on a Western blot with Thermo Scientific Clean-Blot Detection Reagent (HRP). Mouse liver extract (50µg) total protein was separated on a Bio-Rad Criterion™ Gel, transferred to PVDF membrane and blocked with 5% milk in TBST. The membrane was probed with mouse monoclonal anti-Cdk1 (LabVision) (0.2µg/mL) and goat anti-mouse HRP (0.16µg/mL) or Clean-Blot Detection Reagent (HRP) (0.2µg/mL). SuperSignal West Pico Substrate (Product # PI34080) was used for detection of Cdk1 protein.

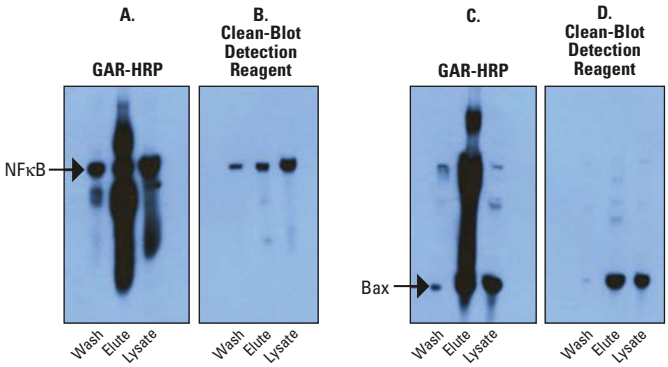


Figure 8. Reveal your target protein with Thermo Scientific Clean-Blot Detection Reagent (HRP). To demonstrate unmasking of the target protein, we performed IP and Western blot experiments. NFκB and Bax were immunoprecipitated from A549 lysate using Protein A/G Agarose Resin and rabbit anti-NFκB **(Panels A and B)** and rabbit anti-Bax **(Panels C and D). Panels A and C** were detected with goat anti-rabbit HRP, which masked the target. **Panels B and D** were detected with the Clean-Blot Detection Reagent (HRP), revealing the target protein.

Ordering Information

Product #	Description	Pkg. Size
PI21230	**Clean-Blot IP Detection Reagent (HRP)** *Sufficient reagent for approximately 100 Western blots.*	2.5mL
PI21232	**Clean-Blot IP Detection Kit (HRP)** *Sufficient reagent for approximately 2,000cm² of membrane.*	Kit
	Clean-Blot Detection Reagent (HRP)	2.5mL
	StartingBlock T20 (TBS) Blocking Buffer	1L
	Pierce ECL Detection Reagent 1, Peroxide Solution	125mL
	Pierce ECL Detection Reagent 2 Luminol Enhancer Solution	125mL

To view data on our Clean-Blot Detection Reagents, visit thermoscientific.com/pierce.

Antibody Binding Proteins

Thermo Scientific™ Protein A

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

Highlights:

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

Ordering Information

Product #	Description	Pkg. Size
PI21181	Protein A	5mg
PI29989	Biotinylated Protein A	1mg

Thermo Scientific™ Pierce™ 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 ~ 44.6kDa) recombinant Protein A

Ordering Information

Product #	Description	Pkg. Size
PI21184	Pierce Recombinant Protein A	5mg
PI77673	Pierce Recombinant Protein A	50mg
PI77674	Pierce Recombinant Protein A	500mg
PI32400	Pierce Purified Recombinant Protein A, Peroxidase Conjugated	1mg

Thermo Scientific™ Pierce™ 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 = 22kDa)
- 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
PI21193	Pierce Recombinant Protein G	5mg
PI77675	Pierce Recombinant Protein G	50mg
PI77676	Pierce Recombinant Protein G	500mg
PI29988	Biotinylated Protein G	0.5mg
PI31499	Protein G, Peroxidase Conjugated	0.5mg

Thermo Scientific™ Pierce™ 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
PI21186	Pierce Recombinant Protein A/G	5mg
PI77677	Pierce Recombinant Protein A/G	50mg
PI77678	Pierce Recombinant Protein A/G	500mg
PI32391	Protein A/G, Alkaline Phosphatase Conjugated	0.5mg
PI32490	Protein A/G, Peroxidase Conjugated	0.5mg

Thermo Scientific™ Pierce™ 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
PI21189	Pierce Purified Recombinant Protein L.	1mg
PI77679	Pierce Recombinant Protein L	50mg
PI77680	Pierce Recombinant Protein L	500mg
PI32420	Protein L, Peroxidase Conjugated	0.5mg
PI29997	Biotinylated Protein L	0.5mg

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
NeutrAvidin Biotin-Binding Protein	60kDa	6.3	No
Streptavidin	53kDa	6.8-7.5	No
Avidin	67kDa	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 14µg/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

1. Hiller, Y., *et al.* (1987). *Biochem. J.* **248**, 167-171.
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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		Pkg. Size
PI22831	NeutrAvidin, DyLight 405 Conjugated	• Excellent photostability • Intense emission provides superior sensitivity and requires less conjugate	1mg
PI22832	NeutrAvidin, DyLight 488 Conjugated		1mg
PI22842	NeutrAvidin, DyLight 594 Conjugated		1mg
PI22844	NeutrAvidin, DyLight 633 Conjugated		1mg
PI22848	NeutrAvidin, DyLight 680 Conjugated		1mg
PI22853	NeutrAvidin, DyLight 800 Conjugated		1mg
PI31000	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 • 11-17µg biotin bound/mg NeutrAvidin Protein	10mg
PI31050	NeutrAvidin Biotin-Binding Protein		100mg
PI31001	NeutrAvidin Horseradish Peroxidase Conjugated	• Better signal-to-noise ratio in assay systems • 1-2 moles HRP/mole NeutrAvidin Protein • 3-8µg biotin bound/mg conjugate	2mg
PI31002	NeutrAvidin Alkaline Phosphatase Conjugated	• Lower nonspecific binding than streptavidin conjugates • Better signal-to-noise ratio in assay systems • 3-8µg biotin bound/mg conjugate	2mg
PI31006	NeutrAvidin Fluorescein Conjugated	• Fluorescent-labeled NeutrAvidin Biotin-Binding Protein • Absorption: 490nm; Emission 520nm • ≥ 2 moles fluorescein/mole NeutrAvidin Protein	5mg
PI31007	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	5mg

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 53kDa) and has a more neutral isoelectric point (pI 6.8-7.5). Our 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
PI21122	Streptavidin	• Lyophilized, stable powder • No carbohydrate	• Immunoassay reagent when bound to biotinylated enzymes or when conjugated to enzymes	1mg
PI21125	Streptavidin	• Much less soluble in water than avidin • 13-22µg biotin bound/mg of protein	• Blocking protein for biotin-rich tissue sections (use at 0.1% for inhibition of endogenous biotin)	5mg
PI21135	Streptavidin	• Recombinant	• Can be used with biotinylated enzymes (Product # PI29339 or PI29139)	100mg
PI21126 PI21124 PI21127	Horseradish Peroxidase Conjugated Horseradish Peroxidase Conjugated Horseradish Peroxidase Conjugated	• 1-2 moles HRP/mole streptavidin • ≥ 100 peroxidase units/mg conjugate • Lyophilized, stable powder • 6-9µg biotin bound/mg conjugate	• Histochemistry • Western blotting • Conti, L.R., *et al.* (2001). *J. Biol. Chem.* **276**, 41270-41278.	1mg 2mg 5mg
PI21324 PI21323	Alkaline Phosphatase Conjugated Alkaline Phosphatase Conjugated	• ≥ 3µg biotin bound/mg conjugate • ≥ 100 phosphatase units/mg conjugate	• Histochemistry • 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.	1mg 3mg
PI21224	Fluorescein (FITC) Conjugated	• Fluorescently labeled streptavidin • Ex/Em: 490nm and 520nm • 3-5 moles FITC/mole streptavidin	• Histochemical staining • Fluorescence-activated cell sorting (FACS)	1mg
PI21724	Rhodamine (TRITC) Conjugated	• Fluorescently labeled streptavidin • Excitation: 515-520nm and 550-555nm • Emission: 575nm • 1-3 moles TRITC/mole streptavidin	• Histochemical staining • Fluorescence-activated cell sorting (FACS)	1mg
PI21627	R-Phycoerythrin Conjugated	• Fluorescently labeled streptavidin • Ex/Em: 480, 545 and 565nm and 578nm	• Histochemical staining • Fluorescence-activated cell sorting (FACS)	1mL
PI21629	Allophycocyanin Conjugated	• Fluorescently labeled streptavidin • Ex/Em: 650nm and 660nm	• Histochemical staining • Fluorescence-activated cell sorting (FACS)	0.5mL
PI21120	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	2mg
PI21831 PI21832 PI21842 PI21844 PI21848 PI21851	Streptavidin, DyLight 405 Conjugated Streptavidin, DyLight 488 Conjugated Streptavidin, DyLight 594 Conjugated Streptavidin, DyLight 633 Conjugated Streptavidin, DyLight 680 Conjugated Streptavidin, DyLight 800 Conjugated	• Ex/Em 400/420 • Ex/Em 493/518 • Ex/Em 593/618 • Ex/Em 638/658 • Ex/Em 692/712 • Ex/Em 777/790	• Excellent photostability • Fluorescence microscopy • Flow cytometry • ELISA • High content screening and other array platforms • Intense emission sensitivity and requires less conjugate from pH 4-9	1mg 1mg 1mg 1mg 1mg 1mg

Thermo Scientific™ Avidin Products

Convenient conjugates for assay detection.

Avidin is a tetrameric glycoprotein (MW 67kDa) 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. Our 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.
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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
PI21121	**Avidin**	• Hen egg white glycoprotein, affinity- purified, salt-free, lyophilized powder • 11-14µg 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)	10mg
PI21128	**Avidin**			20mg
PI21123	**Horseradish Peroxidase Conjugated**	• Purified using special affinity techniques to eliminate nucleic acids • 1-2 moles HRP/mole avidin • 5-10µg biotin bound/mg protein • ≥ 80 peroxidase units/mg protein	• Use in immunohistochemistry where endogenous phosphatase is a problem • Western blotting	2mg
PI29994	**Horseradish Peroxidase Conjugated**			5mg
PI21221	**Fluorescein (FITC) Conjugated**	• Fluorescent-labeled avidin • Ex/Em: 490nm and 520nm • No free fluorescein • ~3.5 moles fluorescein/mole avidin	• Fluorescence-activated cell sorting (FACS) • Histochemical staining	5mg

Wash

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 # PI28320) 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.

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.

Thermo Scientific™ BupH™ Phosphate Buffered Saline Packs (PBS)

Great wash buffer for Western blots!

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

Ordering Information

Product #	Description	Pkg. Size
PI28372	BupH Phosphate Buffered Saline Packs	40 pack
PI28348	20X Phosphate Buffered Saline	500mL
PI28352	20X PBS Tween-20 Solution	500mL

Thermo Scientific™ BupH™ Tris Buffered Saline (TBS)

Great wash buffer for Western blots!

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

Ordering Information

Product #	Description	Pkg. Size
PI28380	BupH Tris-Glycine Buffer Packs	40 pack
PI28376	BupH Tris Buffered Saline Packs	40 pack
PI28379	BupH Tris Buffered Saline Packs	10 pack

Thermo Scientific™ 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
PI28320	Surfact-Amps 20 Solution	6 x 10mL

Incubation with Substrate

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.

Chromogenic Substrates for HRP

TMB Substrates

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. Thermo Scientific™ Pierce™ TMB-Blotting Solution (Product # PI34018) 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 Solution is well suited to applications that require a high signal-to-noise ratio. Thermo Scientific™ 1-Step Ultra TMB-Blotting Solution (Product # PI37574) is an enhanced single-component HRP substrate for Western blotting that allows bands to be visualized in under one minute.

Ordering Information

Product #	Description	Pkg. Size
PI34018	Pierce 1-Step TMB-Blotting Solution	250mL
PI37574	1-Step Ultra TMB-Blotting Solution *Sufficient for 25 mini blot experiments at 10mL each.*	250mL

4-CN Substrates

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
PI34010	Pierce 4-Chloro-1-Napthol Powder	25g powder
PI34012	1-Step Chloronaphthol Solution *Sufficient for 25 uses at 10mL each.*	250mL

Thermo Scientific Colorimetric Substrates guide.

Substrate	Product #	Measurement / Color	Dilution Range of Antibody (From 1mg/mL stock)	Approximate Sensitivity*	Enzyme
Pierce TMB-Blotting Substrate	PI34018	Dark blue PPT	1° 1:500 2° 1:2K-20K	1ng	HRP
Pierce 4-CN Substrate	PI34012	Blue-purple PPT	1° 1:500 2° 1:2K-20K	1ng	HRP
Pierce CN/DAB Substrate	PI34000	Black PPT	1° 1:500 2° 1:2K-20K	1ng	HRP
Pierce DAB Substrate	PI34001	Brown PPT	1° 1:500 2° 1:2K-20K	1ng	HRP
Pierce Metal Enhanced DAB Substrate	PI34065	Brown-black PPT	1° 1:500 2° 1:2K-20K	20pg	HRP
Pierce BCIP Substrate	PI34040	Blue-purple PPT	1° 1:250 2° 1:2.5K	100pg	AP
Pierce NBT Substrate	PI34035	Blue-purple PPT	1° 1:250 2° 1:2.5K	100pg	AP
Pierce NBT/BCIP Substrate	PI34042	Black-purple PPT	1° 1:500 2° 1:2.5K	30pg	AP
Pierce NBT/BCIP + Suppressor Substrate	PI34070	Black-Purple PPT	1° 1:500 2° 1:2.5K	30pg	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

DAB 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
PI34002	Pierce DAB Substrate Kit	275mL
	Includes: DAB (10X)	25mL
	Stable Peroxide Buffer	250mL
PI34065	Pierce Metal Enhanced DAB Substrate Kit	275mL
	Includes: 10X Metal Enhanced DAB	25mL
	Stable Peroxide Buffer	250mL

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
PI34000	Pierce CN/DAB Substrate Kit	275mL
	Includes: CN/DAB (10X)	25mL
	Stable Peroxide Buffer	250mL

Substrate Buffer

Thermo Scientific™ Pierce™ Stable Peroxide Substrate Buffer

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, Pierce Stable Peroxide Substrate Buffer (Product # PI34062). 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
PI34062	Pierce Stable Peroxide Buffer (10X)	100mL

Chromogenic Substrates for Alkaline Phosphatase

Thermo Scientific™ Pierce™ NBT

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
PI34035	Pierce Nitro-Blue Tetrazolium Chloride	1g powder

Thermo Scientific™ Pierce™ BCIP

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 immuno-histochemical studies.

Ordering Information

Product #	Description	Pkg. Size
PI34040	Pierce 5-Bromo-4-chloro-3'-indolyphosphate *p*-toluidine Salt	1g powder

Thermo Scientific™ Pierce™ NBT/BCIP

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.

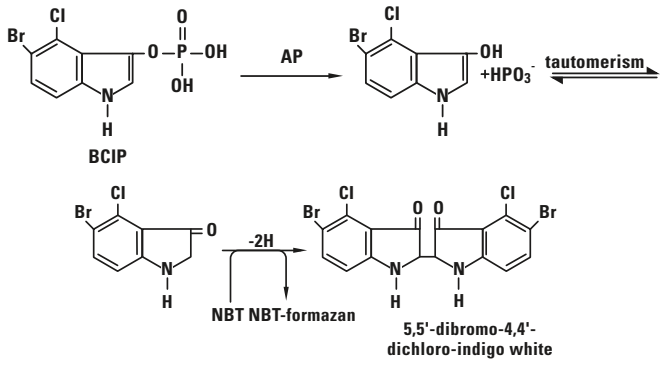


Figure 1. Reaction of AP with BCIP and NBT.

Ordering Information

Product #	Description	Pkg. Size
PI34042	Pierce NBT/BCIP	250mL
PI34070	Pierce NBT/BCIP Plus Suppressor	100mL

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 2).

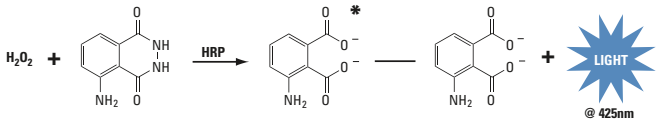


Figure 2. Luminol is oxidized in the presence of HRP and hydrogen peroxide to form an excited state product (3-aminophthalate). The 3-aminophthalate emits light at 425nm as it decays to the ground state.

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 # PI34095), 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.

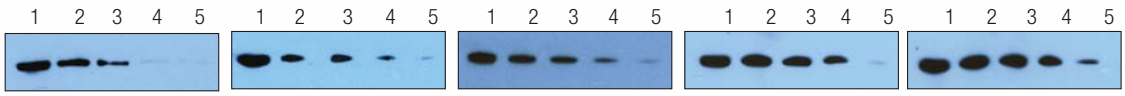
Chemiluminescent substrates differ from other substrates in that the light detected is a transient product of the reaction that is only present while the enzyme-substrate reaction is occurring. This is in contrast to substrates that produce a stable, colored product; these colored precipitates remain 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 Chemiluminescent Substrates.

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

Substrate Comparison

	Thermo Scientific Pierce ECL Substrate	Thermo Scientific SuperSignal West Pico Substrate	Thermo Scientific Pierce ECL Plus Substrate	Thermo Scientific SuperSignal West Dura Substrate	Thermo Scientific SuperSignal West Femto Substrate
					
Advantage	Same signal, lower price than other entry-level ECL substrates	Better signal, lower price than competing ECL substrates	Same signal and lower price than competing ECL Plus substrates	Best for use with imaging equipment	Most sensitive substrate for HRP detection
Detection Level	~20 picograms	~1 picograms	~0.5 picograms	~250 femtograms	~60 femtograms
Signal Duration	30 minutes-two hours	6-8 hours	5 hours	24 hours	8 hours
Detection Methods	X-ray film, CCD imager	X-ray film, CCD imager	X-ray film, CCD imager, fluorescence imager	X-ray film, CCD imager	X-ray film, CCD imager
Recommended Primary and Secondary Antibody Dilutions	1° 1:1K 2° 1:1K-1:15K	1° 1:1K 2° 1:20K-1:100K	1° 1:1K 2° 1:25K-1:200K	1° 1:5K 2° 1:50K-1:250K	1° 1:5K 2° 1:100K-1:500K
Select when ...	Target is abundant, sample is abundant and for everyday use	Target is less abundant, sample is limited and for more sensitivity than an entry level ECL substrate	Target is less abundant, sample is limited and for chemifluorescent detection	Target is less abundant, sample is limited and for CCD image capture	Target is least abundant, sample is precious and for maximum sensitivity
Room Temperature (RT) Working Solution Stability	1 hour	24 hours	1 hour at RT	24 hours	8 hours
Stock Solution Shelf Life	1 year at 4°C	1 year at RT	1 year at 4°C	1 year at RT	1 year at 4°C or 6 months at RT

For data above: Hsp86 detection in HeLa cell lysate (Lane 1: 10µg total protein; Lanes 2-5: serially diluted 1:1) was performed using Thermo Scientific Pierce HRP Chemiluminescent Substrates. The blots were developed using anti-Hsp86 Antibody (Product # PA3-013) and Goat Anti-rabbit HRP (Product # PI31460).

Standard Chemiluminescent Western Blotting Substrates and Kits

Thermo Scientific™ Pierce™ ECL Western Blotting Substrate

A reliable ECL formulation without inflated prices.

Paying more than what you should for an enhanced chemiluminescent (ECL) substrate? For researchers interested in a quality product at a fair price, there is an 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.

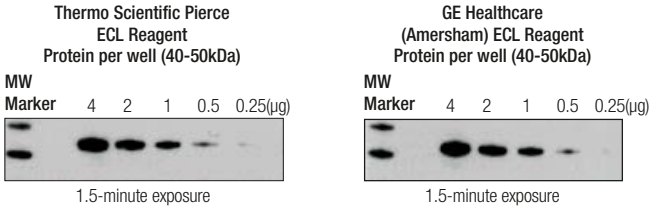


Figure 3. Thermo Scientific Pierce ECL Substrate Western blot detection of actin (beta) from HeLa cell lysate. Dilutions of HeLa cell lysate were prepared and separated by electrophoresis. The proteins were transferred to nitrocellulose membranes (Product # PI88025). Membranes were blocked with 5% skim milk and then incubated with Mouse Anti-Human Actin (US Biological, Swampscott, MA) at 1µg/mL. The membranes were washed and then incubated with 0.2µg/mL of HRP-conjugated Goat Anti-Mouse IgG (Product # PI31430) 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 # PI34090) for 90 seconds.

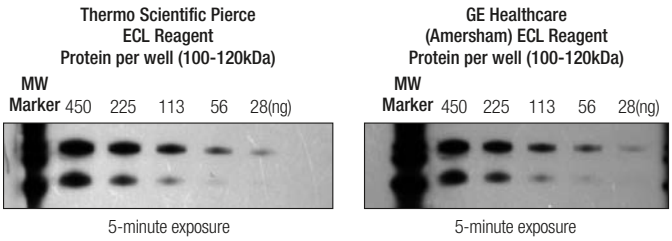


Figure 4. Thermo Scientific Pierce ECL Substrate Western blot detection of β-galactosidase (expressed) from *Escherichia coli* lysate. Dilutions of *E. coli* cell lysate were prepared and separated by electrophoresis. The proteins were transferred to PVDF membranes (Product # PI88585). Membranes were blocked with 5% skim milk and then incubated with Mouse Anti-β galactosidase AB-1 (Lab Vision, Fremont, CA) at 1µg/mL. The membranes were washed and then incubated with 0.2µg/mL of HRP-conjugated Goat Anti-Mouse IgG (Product # PI31430) 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 # PI34090) for five minutes.

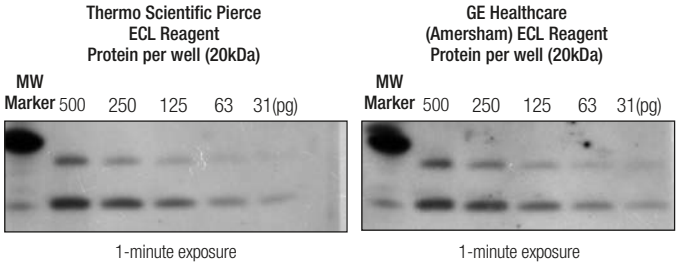


Figure 5. Thermo Scientific Pierce ECL Substrate Western blot detection of recombinant bovine TNF-α. Dilutions of recombinant bovine TNF-α (Product # PIRBOTNFAI) were prepared and electrophoresed. The proteins were transferred to nitrocellulose membranes (Product # PI88025). Membranes were blocked with 5% skim milk and then incubated with rabbit anti-bovine TNF-α at 4µg/mL. The membranes were washed and then incubated with 0.4µg/mL of HRP-conjugated Goat Anti-Rabbit IgG (Product # PI31460) 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:

- Same substrate, but for much less – about half the cost of other ECL substrates
- No optimization required – truly a plug-and-play reagent – our ECL formulation is the same so there should be no need for optimization or protocol changes

Ordering Information

Product #	Description	Pkg. Size
PI32106	Pierce ECL Western Blotting Substrate	500mL kit
PI32209	Pierce ECL Western Blotting Substrate	250mL kit
PI32109	Pierce ECL Western Blotting Substrate	50mL kit

Thermo Scientific™ Pierce™ ECL Plus Western Blotting Substrate

Pierce ECL Plus Substrate is acridan-based, generating acridinium esters when it reacts with HRP. As these ester intermediates react with peroxide, they produce strong and sustained chemiluminescence and a robust fluorescent signal at 440nm that can be captured by CCD and fluorescence imagers.

Highlights:

- **High sensitivity** – detect targets down to the low-picogram level
- **Long signal duration** – sustained light output for as long as five hours
- **More imaging options** – X-ray, CCD or laser-based imagers
- **More affordable** – same quality and performance, but now more affordable

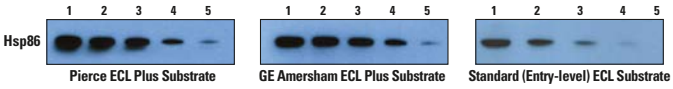


Figure 6. Thermo Scientific Pierce ECL Plus Substrate delivers the same performance you've come to expect. HeLa cell lysate was diluted in electrophoresis sample buffer and heated to 95˚C for five minutes. **Lane 1** contained 10µg of total protein. Four 1:1 dilutions were prepared and applied to **Lanes 2-5** at 10µL/well. After electrophoresis, the proteins were transferred to nitrocellulose membranes (Product # PI88013). 5% milk in TBST was used as a blocking buffer. Rabbit Anti-Hsp86 (Product # PA3-013) at 1:1000 dilution and Goat Anti-Rabbit HRP (Product # PI31460) at 6.6ng/mL (1:150,000 dilution of 1mg/mL stock solution) were used for target detection. Blots were prepared using CL-XPosure Film (Product # PI34090), Pierce ECL Plus Substrate (Product # PI32132) or other indicated substrates.

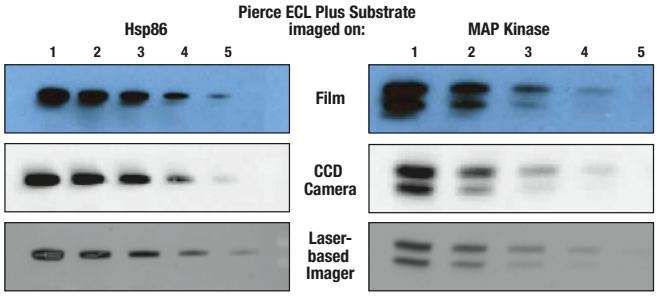


Figure 7. One substrate detected with three methods: X-ray film, CCD imager and laser-based imager. HeLa cell lysate was diluted in electrophoresis sample buffer and heated to 95˚C for five minutes. **Lane 1** contained 10µg of total protein. Four 1:1 dilutions were prepared and applied to **Lanes 2-5** at 10µL/well. After electrophoresis, proteins were transferred to nitrocellulose membranes (Product # PI88013). 5% milk in TBST was used as a blocking buffer. The membranes were incubated with primary antibody (Rabbit Anti-MAP Kinase, Millipore or Rabbit Anti-Hsp86, Product # PA3-013) at 1:1000 dilution and then with Goat Anti-Rabbit HRP (Product # PI31460) at 6.6ng/mL (1:150,000 dilution of 1mg/mL stock solution). Pierce ECL Plus Substrate (Product # PI32132) was used for detection. The membranes were exposed to CL-XPosure Film (Product # PI34090) for five seconds and scanned using the Typhoon™ 9410 Variable Mode Imager (Excitation at 457nm, Emission at 510nm) and Syngene™ G:Box iChemiXT Imager (1 minute exposure).

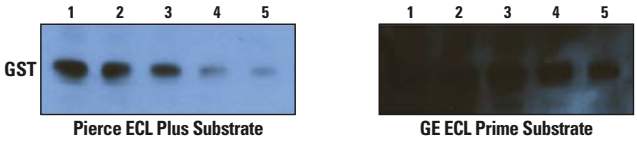


Figure 8. Better sensitivity does not always translate to better results. Purified GST (Product # PI20237) was diluted in Thermo Scientific™ Pierce™ LDS Sample Buffer (Product # PI84788) and heated to 95ºC for five minutes. **Lane 1** contained GST 50ng at 10µL/well. Four 1:1 serial dilutions were prepared and applied to **Lanes 2-5**. After electrophoresis proteins were transferred to nitrocellulose membranes (Product # PI88024). StartingBlock Blocking Buffer (Product # PI37542) was used for blocking. Biotinylated Anti GST (SantaCruz, sc-459) at 1:2000 dilution and Streptavidin HRP (Product # PI21130) at 6.66ng/mL were used for target detection. Blots were prepared using Pierce ECL Plus (Product # PI32132) or GE ECL Prime Substrate (Product # RPN2232) and CL-XPosure Film (Product # PI34090).

Ordering Information

Product #	Description	Pkg. Size
PI32132X3	**Pierce ECL Plus Western Blotting Substrate** *Sufficient reagents for 3,000cm² of membrane or thirty (10 x 10cm) blots.* Includes: Detection Reagent A Detection Reagent B	Kit 3 x 100mL 3 x 2.5mL
PI32132	**Pierce ECL Plus Western Blotting Substrate** *Sufficient reagents for 1,000cm² of membrane or ten (10 x 10cm) blots.* Includes: Detection Reagent A Detection Reagent B	Kit 100mL 2.5mL
PI32134	**Pierce ECL Plus Western Blotting Substrate** *Sufficient reagents for 250cm² of membrane.* Includes: Detection Reagent A Detection Reagent B	Kit 20mL 0.625mL

Pierce ECL Plus Substrate can be purchased through Fisher Scientific and VWR as Pierce ECL 2 Substrate (Product # PI80196). Pierce ECL 2 Substrate is specifically packaged for our channel partners and is the same formulation as Pierce ECL Plus Substrate.

Thermo Scientific™ SuperSignal™ West Pico Chemiluminescent Substrate

Twice as much signal for about 30% 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 discernible 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 9)
- **Picogram sensitivity** – highly sensitive for the rapid development of a wide range of protein levels (Figure 10)
- **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

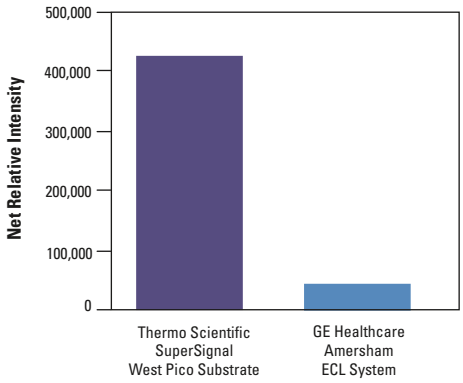


Figure 9. Enhanced light emission kinetics: Thermo Scientific SuperSignal Substrate vs. GE Healthcare Amersham ECL System. Net relative intensity six hours after incubation is much greater for SuperSignal West Pico Substrate than for the ECL System.

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

Step-by-step Conversion Protocol	Amersham ECL Substrate	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.

Reference

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.

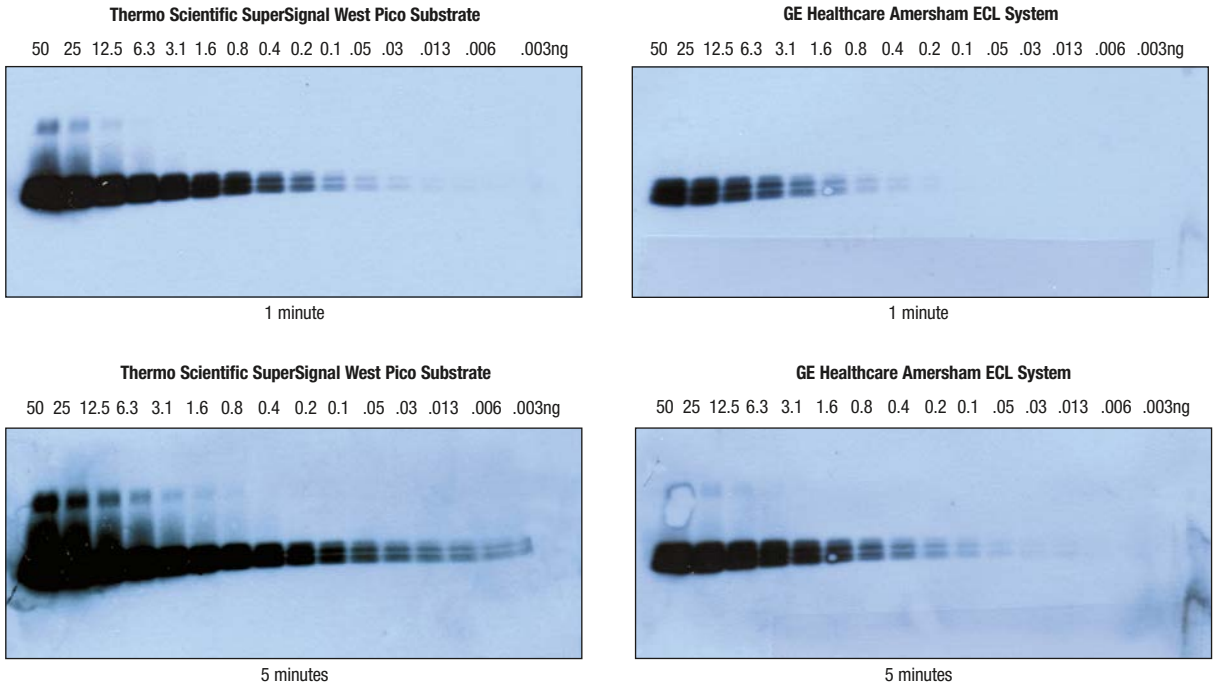


Figure 10. Thermo Scientific SuperSignal West Pico Substrate is more sensitive than GE Healthcare Amersham ECL Substrate. Recombinant mouse IL-2 was serially diluted (50-0.003ng) and electrophoresis was performed. The gels were transferred to nitrocellulose membranes, blocked and incubated with a 1µg/mL dilution of Rat Anti-Mouse IL-2. After washing, the membranes were incubated with 20ng/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.

Ordering Information

Product #	Description	Pkg. Size
PI34078	SuperSignal West Pico Chemiluminescent Substrate *Sufficient materials for 10,000cm² membrane.* Includes: Luminol/Enhancer Stable Peroxide Buffer	1L 500mL
PI34087	SuperSignal West Pico Chemiluminescent Substrate *Sufficient materials for 2,000cm² membrane.* Includes: Luminol/Enhancer Stable Peroxide Buffer	200mL 100mL
PI34080	SuperSignal West Pico Chemiluminescent Substrate *Sufficient materials for 5,000cm² membrane.* Includes: Luminol/Enhancer Stable Peroxide Buffer	500mL 250mL
PI34077	SuperSignal West Pico Chemiluminescent Substrate *Sufficient materials for 1,000cm² membrane.* Includes: Luminol/Enhancer Stable Peroxide Buffer	100mL 2 x 25mL
PI34079	SuperSignal West Pico Chemiluminescent Substrate Trial Kit *Sufficient materials for 1,000cm² membrane.* Includes: Luminol/Enhancer Stable Peroxide Buffer	50mL 25mL

Thermo Scientific™ SuperSignal™ West Pico 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		
PI34082	SuperSignal West Pico Mouse IgG Detection Kit	Kit
PI34083	SuperSignal West Pico Rabbit IgG Detection Kit	Kit
Complete Detection Kits		
PI34081	SuperSignal West Pico Complete Mouse IgG Detection Kit	Kit
PI34084	SuperSignal West Pico CompleteRabbit IgG Detection Kit	Kit

For a list of kit components, visit our website and search on the 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 benefits greatly with a substrate that produces an intense signal that is strong enough, and of long enough duration. 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. Femtogram sensitivity with up to a 24-hour light emission makes SuperSignal West Dura Substrate the right choice for our Western blot needs and the perfect companion for your imager.

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

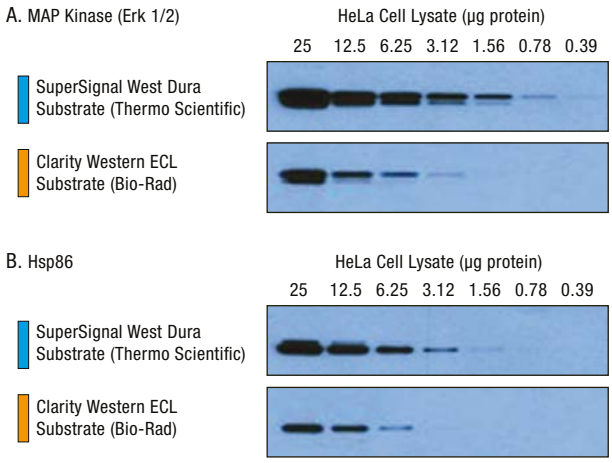


Figure 11. Thermo Scientific SuperSignal West Dura Substrate is more sensitive than Bio-Rad™ Clarity™ Western ECL Substrate. Detection of MAP Kinase (Erk 1/2) and Hsp86 proteins in HeLa cell lysate on PVDF membranes. Membranes were probed, processed and exposed to X-ray film using identical conditions, except that incubations in substrates were according to respective manufacturer's product instructions.

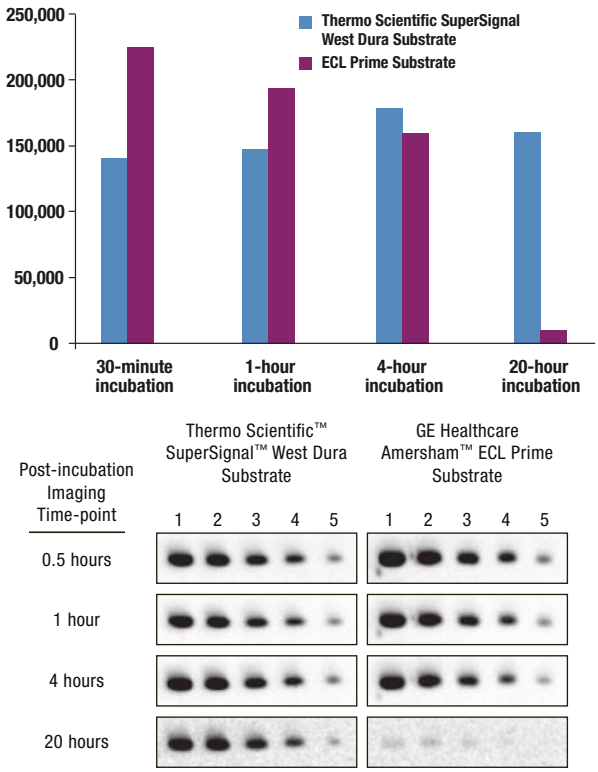


Figure 12. Thermo Scientific SuperSignal West Dura Substrate has better signal duration than GE ECL Prime Substrate. HeLa cell lysate was diluted in sample buffer and heated to 95°C for 5 minutes. **Lane 1** contained 10µg total protein (total 10µL/well). Four 1:1 serial dilutions were then prepared and loaded at 10µL/well (**Lanes 2-5**). After electrophoresis proteins were transferred to Thermo Scientific™ Nitrocellulose Membranes (Product # PI88024). Starting Block in TBS Blocking Buffer (Product # PI37542) was used for blocking. Rabbit Anti-Hsp 86 (Product # PA3-013) was used as primary antibody at 1:2000 dilution and Goat Anti-rabbit HRP (Product # PI31460) was used as secondary antibody at 6.6ng/mL. SuperSignal West Dura Substrate (Product # PI34076) and GE ECL Prime Substrate (Product # RPN2232) was used for detection. The blots were imaged using Syngene G:Box iChemiXT Imager at the indicated times. Quantitation of signal intensity was performed using GeneSnap image aquisition software.

Ordering Information

Product #	Description	Pkg. Size
PI34076	**SuperSignal West Dura Extended Duration Substrate** *Sufficient materials for 2,000cm² membrane.* Includes: Luminol/Enhancer Stable Peroxide Buffer	200mL 100mL 100mL
PI34075	**SuperSignal West Dura Extended Duration Substrate** *Sufficient materials for 1,000cm² membrane.* Includes: Luminol/Enhancer Stable Peroxide Buffer	100mL 50mL 50mL
PI37071	**SuperSignal West Dura Extended Duration Substrate** *Trial Kit Sufficient materials for 200cm² membrane.* Includes: Luminol/Enhancer Stable Peroxide Buffer	10mL 10mL 10mL

Reference

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

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 14); that's zeptomole-level detection
- **Economical** – conserve precious antibodies with up to 1:100,000 primary antibody dilutions and 1:500,000 secondary antibody 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**[1]

Lower detection limit

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

Signal duration

- 8 hours

Suggested antibody dilutions (from 1mg/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

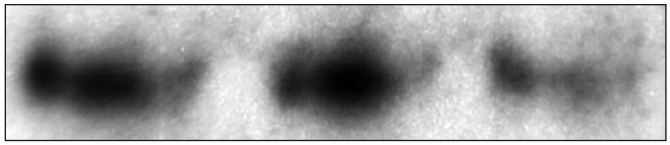


Figure 13. True femtogram detection of IκBα using Thermo Scientific SuperSignal West Femto Maximum Sensitivity Substrate. Serially diluted samples from 100 to 1fg were run on 4-20% Precise Precast Gels. The protein was then transferred to PVDF membrane and blocked with StartingBlock Blocking Buffer for 1 hour at room temperature (RT). The blot was incubated in Rabbit Anti-IκBα (1mg/mL) at 1:1,000 dilution overnight at 4°C, followed by incubation in Goat Anti-Rabbit HRP (1mg/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
PI34096	**SuperSignal West Femto Maximum Sensitivity Substrate** *Sufficient materials for 2,000cm² membrane.* Includes: Luminol/Enhancer Solution Stable Peroxide Solution	200mL 100mL 100mL
PI34095	**SuperSignal West Femto Maximum Sensitivity Substrate** *Sufficient materials for 1,000cm² membrane.* Includes: Luminol/Enhancer Solution Stable Peroxide Solution	100mL 50mL 50mL
PI34094	**SuperSignal West Femto Maximum Sensitivity Substrate Trial Kit** *Sufficient materials for 200cm² membrane.* Includes: Luminol/Enhancer Solution Stable Peroxide Solution	20mL 10mL 10mL

References

1. Feissner, R., *et al.* (2003). *Anal. Biochem.* **315**, 90-94.
2. Adilakshmi, T. and Laine, R.O. (2002). *J. Biol. Chem.* **277**, 4147-4151.
3. Conti, L.R., *et al.* (2001). *J. Biol. Chem.* **276**, 41270-41278.
4. Guo, Y., *et al.* (2001). *J. Biol. Chem.* **276**, 45791-45799.

Fast Western Blotting Kits

Thermo Scientific Pierce Fast Western Blot Kits accelerate the Western blotting process with streamlined protocols and optimized reagents that provide accuracy, sensitivity and reliability. The kits reduce hands-on and overall blotting time to approximately one hour using any of the Pierce ECL and SuperSignal Chemiluminescent Substrates.

Thermo Scientific™ Pierce™ Fast Western Blot Kit, ECL Substrate

Perform a Western blot in 55 minutes.

The Pierce Fast Western Blot Kit, ECL Substrate contains optimized reagents that shorten the time to perform a typical Western blot from 4 hours down to approximately 55 minutes. The kit provides all the reagents necessary to complete a Western blot being probed with a mouse or rabbit primary antibody. The protocol requires minimal hands-on time and yields results comparable to classic Western blotting with ECL. The included Pierce ECL Substrate produces a chemiluminescent signal, which is detected using photographic or other imaging methods. Blots can be repeatedly exposed to film to obtain optimal results or stripped of the primary antibody and immunodetection reagents and reprobed.

The Pierce Fast Western Blot Kit is a reagent-based system that provides optimized reagents for blocking, antibody dilution and detection of Western blots with Pierce ECL Substrate. The protocol is a quick, efficient and economic way to obtain Western blot results without the hassle of buying an instrument and the consumables required by other popular rapid Western blotting systems. No vacuum pump is needed so there are never any clogged lines or membranes that can occur when using instrument-based systems. Because there is no apparatus, there is no need to buy disposable blotting trays that limit the number of blots processed at one time.

Highlights:

- **Fast** – all the sensitivity of Pierce ECL Substrate and saves 4-5 hours per blot
- **Convenient** – no expensive hardware or vacuum required; no clogging issues
- **Simple** – optimized protocol makes Western blot analysis easier than ever
- **Economical** – cost as little as $8 per blot; no expensive consumables or extra equipment
- **Excellent stability** – kit is stable for 1 year stored at 4°C
- **Easy** – complete kit gives you all the components you need to block and probe and develop a blot with your mouse or rabbit primary antibody

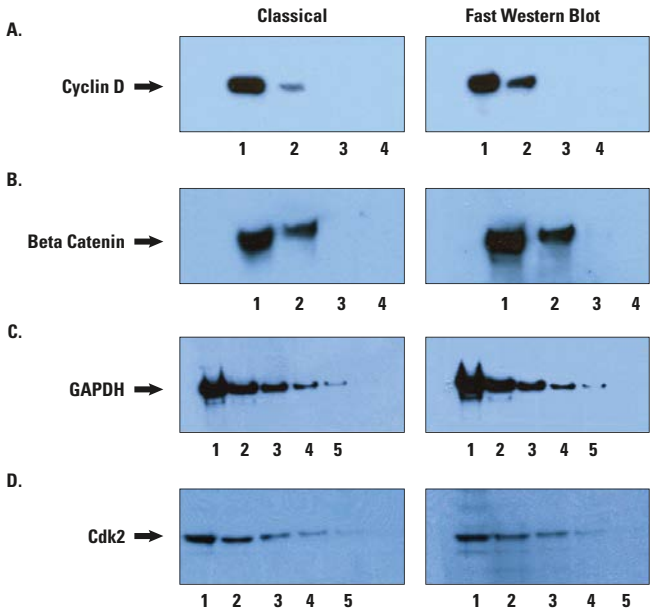


Figure 14. Obtain comparable results to the classic Western blotting protocol using Thermo Scientific Pierce Fast Western Blot Kit, ECL Substrate. The detection sensitivity for various target proteins was compared using the Fast Western Blot Kit and the classical Western blot protocol, **Panels A and B** contained 10, 2, 0.4 and 0.08µg of protein from A549 cell lysates in **Lanes 1-4**, respectively. **Panels C and D** contained 2, 1, 0.5, 0.25 and 0.125µg of 293T cell lysate in **Lanes 1-5** of **Panel C** and 25, 12.5, 6.25, 3.1 and 0.39µg in **Lanes 1-5** of **Panel D:** Protein detection was achieved as follows: **Panel A:** Mouse anti-cyclin D1 antibody (BD Pharmingen) on PVDF. **Panel B:** Rabbit anti-beta catenin antibody (LabVision) on PVDF. **Panel C:** Mouse anti-GAPDH antibody (Millipore) on nitrocellulose. **Panel D:** Rabbit anti-cdk2 antibody on PVDF.

Ordering Information

Product #	Description	Pkg. Size
PI35050	**Pierce Fast Western Blot Kit, ECL Substrate** *Sufficient reagents for 25 Western blots (8 x 10cm) probed with mouse or rabbit antibody.*	Kit
	Includes: Antibody Diluent	500mL
	10X Wash Buffer	250mL
	Optimized HRP Reagent	25mL
	Pierce ECL Detection Reagent 1	125mL
	Pierce ECL Detection Reagent 2	125mL
PI35055	**Pierce Fast Western Blot Kit, ECL Substrate** *Sufficient for 5 mini blots probed with mouse or rabbit primary antibodies.*	200mL Kit
	Includes: Fast Western Antibody Diluent	100mL
	Fast Western 10X Wash Buffer	50mL
	Fast Western Optimized HRP Reagent	5mL
	Pierce ECL Detection Reagent 1	25mL
	Pierce ECL Detection Reagent 2	25mL

Thermo Scientific™ Pierce™ Fast Western SuperSignal™ Kits

Sensitivity AND speed in Western blotting … all in one kit.

Now there is a Western blotting product that yields higher sensitivity in 55-60 minutes. The Pierce Fast Western Blot Kits containing our popular SuperSignal Substrates perform better when more sensitive detection is required and you need the results fast. These Pierce Fast Western Blot Kits rely on the proprietary SuperSignal technology that yields longer light emission and higher intensity over other luminol-based systems, allowing you to make several exposures. Three kits containing the various SuperSignal Substrates are now available:

Highlights:

- Pierce Fast Western Kit, SuperSignal West Pico Substrate – for picogram detection levels
- Pierce Fast Western Kit, SuperSignal West Dura Substrate – for femtogram detection levels
- Pierce Fast Western Kit, SuperSignal West Femto Substrate – for low- to mid-femtogram detection levels, the maximum sensitivity

All Pierce Fast Western Kits, SuperSignal Substrates are compatible with nitrocellulose and PVDF membranes. Separate kits for use with mouse or rabbit antibodies are available.

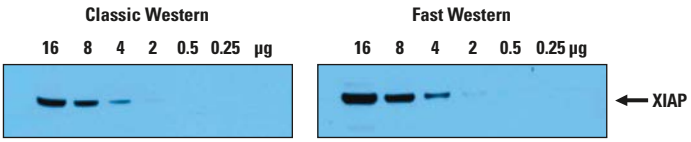


Figure 15. Classic vs. fast Western protocol using Thermo Scientific SuperSignal West Pico Substrate. 293T cell lysate was diluted in reducing sample buffer and loaded to a gel at 16, 8, 4, 2, 0.5, and 0.25µg/well. After electrophoresis, proteins were transferred to nitrocellulose membranes using the Thermo Scientific™ Pierce™ Fast Semi-Dry Blotter for 10 minutes at 25V. One membrane was developed using the classic Western protocol, and the other was developed using the fast Western protocol. The blots were probed with mouse anti-XIAP antibody (1µg/mL, BD Pharmingen). Each blot was incubated in SuperSignal West Pico Substrate Working Solution for 5 minutes and exposed to film for 1 minute.

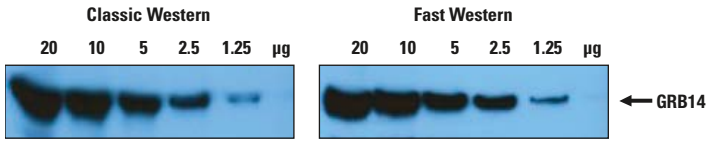


Figure 16. Classic vs. fast Western protocol using Thermo Scientific SuperSignal West Dura Substrate. HeLa cell lysate was diluted in reducing sample buffer and loaded onto a gel at 20, 10, 5, 2.5 and 1.25µg/well. After electrophoresis, the proteins were transferred to PVDF membranes using the Pierce Fast Semi-Dry Blotter for 10 minutes at 25V. The membranes were probed with rabbit anti-GRB14 antibody (0.5µg/mL, Millipore) Membranes were developed using the classic Western protocol or the fast Western protocol. Each blot was incubated in SuperSignal West Dura Substrate Working Solution for 5 minutes and exposed to film for 1 minute.

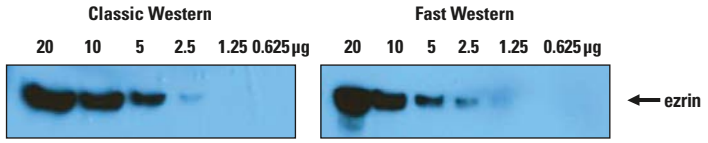


Figure 17. Classic vs. fast Western protocol using Thermo Scientific SuperSignal West Femto Substrate. HeLa cell lysate was diluted by 50% and loaded onto a gel at 20, 10, 5, 2.5, 1.25, and 0.625µg. After electrophoresis, the proteins were transferred to PVDF membranes using the Pierce Fast Semi-Dry Blotter for 10 minutes at 25V. Membranes were developed using either the classic Western blotting protocol or the fast Western protocol. The membranes were probed with mouse anti-ezrin antibody (1µg/mL, Lab Vision). Each blot was incubated in SuperSignal West Femto Substrate Working Solution for 5 minutes and exposed to film for 1 minute.

Ordering Information

Product #	Description	Pkg. Size
PI35065	**Pierce Fast Western Blot Kit, SuperSignal West Pico Substrate, Mouse Trial Size** *Sufficient reagents to perform five Western blots (8cm x 10cm) probed with a mouse primary antibody.*	Kit
PI35060	**Pierce Fast Western Blot Kit, SuperSignal West Pico Substrate, Mouse** *Sufficient reagents to perform 20 Western blots (8cm x 10cm) probed with a mouse primary antibody.*	Kit
PI35066	**Pierce Fast Western Blot Kit, SuperSignal West Pico Substrate, Rabbit Trial Size** *Sufficient reagents to perform five Western blots (8cm x 10cm) probed with a rabbit primary antibody.*	Kit
PI35061	**Pierce Fast Western Blot Kit, SuperSignal West Pico Substrate, Rabbit** *Sufficient reagents to perform 20 Western blots (8cm x 10cm) probed with a rabbit primary antibody.*	Kit
PI35075	**Fast Western Blot Kits, SuperSignal West Dura, Mouse** *Sufficient reagents to perform 2 Western blots (8cm x 10cm) probed with a mouse primary antibody.*	Kit
PI35070	**Fast Western Blot Kits, SuperSignal West Dura, Mouse** *Sufficient reagents to perform 10 Western blots (8cm x 10cm) probed with a mouse primary antibody.*	Kit
PI35076	**Fast Western Blot Kits, SuperSignal West Dura, Rabbit** *Sufficient reagents to perform 2 Western blots (8cm x 10cm) probed with a rabbit primary antibody.*	Kit
PI35071	**Fast Western Blot Kits, SuperSignal West Dura, Rabbit** *Sufficient reagents to perform 10 Western blots (8cm x 10cm) probed with a rabbit primary antibody.*	Kit
PI35080	**Fast Western Blot Kits, SuperSignal West Femto, Mouse** *Sufficient reagents to perform 10 Western blots (8cm x 10cm) probed with a mouse primary antibody.*	Kit
PI35081	**Fast Western Blot Kits, SuperSignal West Femto, Rabbit** *Sufficient reagents to perform 10 Western blots (8cm x 10cm) probed with a rabbit primary antibody.*	Kit

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.

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 poly-histidine 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 19)
- **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 10cm blots**

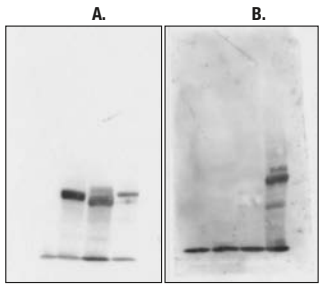


Figure 18. Specificity comparison of polyhistidine-tagged (PHT) fusion protein detection methods. Panel A: using HisProbe-HRP shows high specific binding and low background. **Panel B:** using anti-polyHis failed to recognize two of the three fusion proteins.

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
PI15165	**HisProbe-HRP**	2mg
PI15168	**SuperSignal West Pico HisProbe Kit**	Kit
	Includes: HisProbe-HRP	2mg
	SuperSignal West Pico Chemiluminescent Substrate	500mL
	Blocker BSA in TBS (10X)	1 x 125mL
	BupH Tris Buffered Saline Packs	10 x 500mL
	Surfact-Amps 20 (10%) Ampules	6 x 10mL

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. For more information on Far-Western blotting, please refer to the Thermo Scientific™ Protein Interaction Handbook.

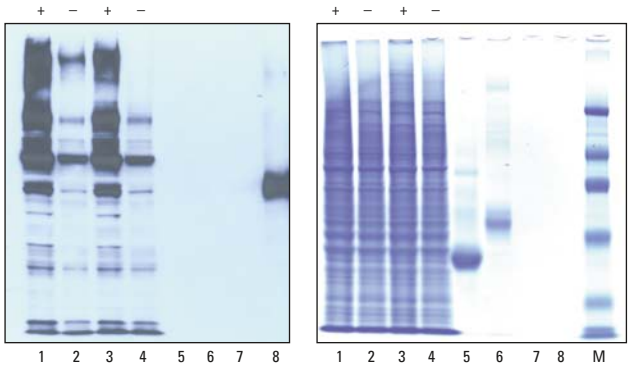


Figure 19. Western blot detection of O-GlcNAc-modified proteins after SDS-PAGE. Lanes 1-4 are proteins from the Jurkat cell extract. Lanes 5, 6 and 7 are the negative controls: ovalbumin (5µg), fetuin (5µg) and *O*-β-GalNAc-modified BSA (10ng). **Lane 8** is *O*-β-GlcNAc-modified BSA (5ng, positive control). The (+) and (-) refer to plus and minus treatment with PUGNAc and glucosamine, and M represents the molecular weight marker.

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

High-specificity monoclonal against O-GlcNAc.

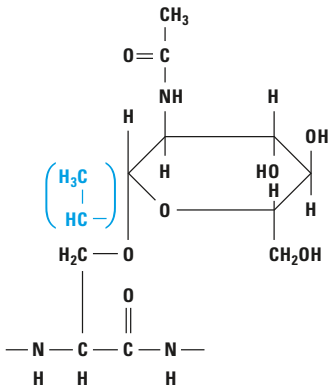
The Pierce *O*-GlcNAc Western Blot Detection Kit contains the most highly specific mouse monoclonal antibody available for the detection of the *O*-GlcNAc post-translational modification. Reaction of the monoclonal antibody in this Western blotting kit is confined to the β-*O*-linked serine or threonine GlcNAc modification. There is no cross-reactivity with the α-*O*-GlcNAc linkage, the α/β-O-GalNAc modification or theother N-linked oligosaccharides (Figure 20).

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.



β-O-GlcNAc Modified Serine/Threonine in Peptide Linkage

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

Target Detection

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 Thermo Scientific™ Pierce™ Background Eliminator Solution (Product # PI21065), 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 75.

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

Troubleshooting tips for chemiluminescence imaging using cooled CCD cameras:

1. Although any chemiluminescent substrate can be used for detection using digital imaging technology, SuperSignal West Dura, SuperSignal West Femto and SuperSignal West Pico Substrates are recommended because of strong and stable light output.
2. Use a higher binning setting in the CCD camera to increase the sensitivity of image. However, increasing binning reduces image resolution.
3. A cooled CCD camera in an imaging system increases sensitivity by decreasing "dark" or thermal noise.
4. Because of the increased signal dynamic range of a digital imaging system, it may be necessary to adjust image contrast and/or invert the image on the imaging instrument in order to view weak signal.
5. To obtain the image with the best signal dynamic range, a longer exposure time may be required in an imaging system than with X-ray film.
6. No darkroom is necessary when using imaging instruments. These instruments essentially replace the dark room saving space and X-ray developing reagents. Refer to the instrument manufacturer's instructions for more information on an individual instrument.

For more information on how CCD imaging compares to x-ray film, visit **thermoscientific.com/pierce** and look under the product blog articles.

Digital Imaging

Detect your protein.

The Western blot is now ready for detection, based on the type of substrate used. If a chromogenic substrate is used, the bands on the blot are detected visually. For chemiluminescence, the bands can be visualized in a cooled-CCD imaging system or using X-ray film. While X-ray film can be used to obtain semi quantitative data, digital imaging is more sensitive because of the broad dynamic range of detection, allowing researchers to obtain quantitative data from Western blots.

Thermo Scientific™ MYECL™ Imager

One-touch Western blot and gel imaging at your finger tips.

The powerful and easy-to-use MYECL Imager delivers a complete set of high sensitivity Western blot and gel documentation tools through a streamlined and intuitive touchscreen interface, convenient acquisition options, and analysis software supplied with the instrument. The MYECL Imager incorporates advanced CCD technology that results in greater than two times the sensitivity of X-ray film and 10 times the dynamic range. The imager can be used in chemiluminescent, ultraviolet and visible modes to image Western blots, stained nucleic acid gels or stained protein gels.

Highlights:

- **One-touch image acquisition** – press any one of several optimized presets in each mode and the imager does all the rest; no focusing or camera settings need to be adjusted
- **Multi-exposure feature** – automatically capture a series of images using up to five different preset or user-defined exposure times
- **Automatic visible image capture** – system automatically takes a corresponding visible image with every chemiluminescent image exposure; allows overlay alignment with prestained MW markers
- **Remote Tech Support access** – share your MYECL Imager screen in a live session with Technical Support to receive immediate help while using the instrument
- **Live camera setting** – in any mode see a live view of the illuminated platform on the display screen while the door is open so you can place and center the sample
- **Shoot-and-review convenience** – imager keeps the last five captured images immediately available in on-screen tabs so you can quickly review, compare, choose and make adjustments to results
- **Interactive Chemi** – automatically calculates the exposure time of a Western blot with maximum dynamic range and minimal pixel saturation from a short, 15-second exposure image
- **File manager** – easily copies, deletes, exports and edits image information of one or more image files in multiple gallery folders
- **Create dark and bias images** – creates new dark and bias master files to compensate for noise coming from the CCD camera during image acquisition
- **Adjust image** – adjust the black, white and gamma levels of acquired images to increase sample visibility
- **Intensity display** – select a point of interest on the acquired image to view the pixel intensity and pixel coordinates of the corresponding region

The instrument is controlled with an easy-to-use touchscreen interface that simplifies image acquisition. Researchers can set custom exposure ranges with up to five different exposure times or use preset versions. Images are stored to an internal drive in a nonproprietary data format for easy sharing. The file management system has sort and search functionalities to find locally saved images. Files are easily transferred to any computer using one of three USB ports and the included 2GB Flash Drive or through the Ethernet connection at the back of the instrument.

Analyze images by adjusting display settings (saturation, invert, contrast) on-screen with the imager to ensure that the captured data are of suitable quality for the intended use. Then, once the original image is transferred to another computer that is running the Thermo Scientific MyImageAnalysis Software, it can be fully analyzed to identify lanes and bands, overlay molecular weight markers, calculate molecular weights, measure densitometry, etc.

The MyECL Imager is easier to set up and use than most instruments of its type, allowing researchers to adapt and use the new technology quickly without the need for an engineering visit. The imager eliminates the need for a darkroom, replacing it with a machine that has a small footprint. Unlike film, the MyECL Imager does not require the use of silver-containing chemicals that require hazardous materials handling and disposal.

MyECL Imager Accessories (Included)

- MyImageAnalysis Software, 5-user network license
- Orange Filter (565-620nm) for ethidium bromide or SYPRO™ Orange Stain
- Chemiluminescence Exposure Screen
- White Light Conversion Screen
- UV Exposure Screen
- Imaging Reference Target
- Transilluminator Glass Plate
- Quick Start Guide
- USB Flash Drive, 2GB
- Touchscreen Stylus and Holder

MyECL Imager Accessories (Optional)

- UV Transilluminator Bulb, 306nm
- Green Filter (510-550nm)
- Red Filter (600-640nm)

Hardware specifications.

CCD camera	16-bit, 4.2 megapixel; thermoelectrically regulated at -25°C (±0.1)
Lens	50mm, *f*/0.95
Array size (pixels)	2048 x 2048
Pixel size	7.4 x 7.4µm
Field of view	15.0cm x 15.0cm
Dynamic range	>4.0 orders of magnitude
Binning modes	1 x 1, 2 x 2, 3 x 3 (default), 4 x 4, 8 x 8
Image capture modes	Chemiluminescence, UV transilluminator, epi-white light
Image exposure modes	Automatic or manual
Image file format	TIFF (16-bit grayscale)
Excitation source	306nm UV transilluminator; Epi-white light
Filter wheel	Motorized, 4 position
Computer	Internal with 250 GB hard drive†
Touchscreen display	10.4-inch LCD
Ports	3 USB, 1 network
Instrument size, L x W x H	56cm x 31cm x 53cm (22in x 12.2in x 21in)
Weight	29.5kg (65lbs)

† Approximately 200 GB are available for storage of acquired images, providing storage for more than 200,000 image files captured using the default image acquisition setting (3 x 3 binning).

Ordering Information

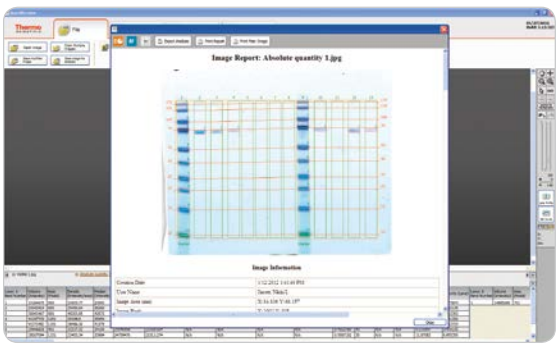
Product #	Description	Pkg. Size
PI62236	**MYECL Imager** **Instrument:** CCD Camera: 16-bit, 4.2 MP; thermoelectrically regulated at -25°C Motorized Fixed Lens: 50mm, f/0.95 Light Source: 306nm Trans-UV, Epi-white Filter Wheel: 4 position, motorized Orange Filter: (565-620nm bandpass) Integrated Computer: 250GB hard drive (approx. 200GB for data storage) Touchscreen: 10.4 inch LCD Ports: 3 USB (2.0), 1 ethernet **Accessory Kit:** MYECL Imager Manual,1 CD MYECL Imager Quick Start Guide Chemiluminescence Exposure Screen, 1 screen White Light Conversion Screen, 1 screen UV Exposure Screen, 1 screen Touchscreen Stylus and Holder, 1 each Imaging Reference Target, 1 target Power Cord with C/13 Connector, 1 device USB Flash Drive, 2GB, 1 device MYIMAGEANALYSIS Software, 1 CD (5 licenses)	1 unit
PIEW84857	**Extended Warranty, 1-year** *Covers all parts (non-consumable) and labor; includes shipping and handling.*	1 warranty
PI62238	**Orange Filter** *Sufficient for replacement for the pre-installed orange filter in the MYECL Imager for detection of colorimetric protein stains (silver, coomassie) and wavelengths of common fluorescent nucleic acid gel stains (e.g., ethidium bromide) and protein stains, such as SYPRO Orange Protein Gel Stain.*	1 filter
PI62239	**Green Filter** *Sufficient for addition of a green filter to one of two open positions in the MYECL Imager for detection (emission) wavelengths of common fluorescent nucleic acid stains, such as SYBR™ Green Nucleic Acid Gel Stain and SYBR™ Safe DNA Gel Stain.*	1 filter

Product #	Description	Pkg. Size
PI62240	**Red Filter** *Sufficient for addition of a red filter to one of two open positions in the MYECL Imager for detection (emission) wavelengths of common fluorescent protein stains, such as SYPRO™ Red Protein Gel Stain, SYPRO™ Ruby Protein Gel Stain and Deep Purple™ Total Protein Stain.*	1 filter
PI62241	**Blue Filter** *Sufficient for addition of a blue filter to one of two open positions in the MYECL Imager for detection of blue-fluorescent dyes and stains.*	1 filter
PI62242	**Chemiluminescence Exposure Screen** *Sufficient for replacement of MYECL Imager accessory; used on the transilluminator for acquiring chemiluminescent images.*	1 screen
PI62243	**White Light Conversion Screen** *Sufficient for replacement of MYECL Imager accessory; used on the transilluminator for acquiring images in the epi-white (visible mode).*	1 screen
PI62244	**UV Exposure Screen** *Sufficient for replacement of MYECL Imager accessory; used on the transilluminator for acquiring images in the UV mode.*	1 screen
PI62267	**Imaging Reference Target** *Sufficient for replacement of MYECL Imager accessory; used for focusing in all imaging modes.*	1 target
PI62255	**UV Transilluminator Bulb, 365nm** *Sufficient for substitution (replacement) of the pre-installed 306nm UV bulbs in the MYECL Imager with long-wave 365nm bulbs; All six lamps must be the same bulb type (365nm or 306nm).*	1 bulb
PI62258	**UV Transilluminator Bulb, 306nm** *Sufficient for replacement of one of six pre-installed 306nm UV bulbs in the MYECL Imager; All six lamps must be the same bulb type (306nm or 365nm).*	1 bulb
PI62261	**Touchscreen Stylus and Holder** *Sufficient for replacement of MYECL Imager accessory; for interaction with the imager touchscreen.*	1 set

Thermo Scientific™ MYIMAGEANALYSIS™ Software

Thermo Scientific MYIMAGEANALYSIS Software is a full-featured, multifunction computer program to analyze and edit digital images of electrophoresis gels and blots that were acquired using gel documentation imagers or scanners.

The MYIMAGEANALYSIS Software comprises a complete set of easy-to-use research tools for the quantitative digital analysis of image data from electrophoresis gels and Western blots. The software uses a powerful algorithm to automatically select and identify lanes and band-boundaries for calculation of migration distances or densitometry. Intuitive, ready-to-use functions (buttons) provide molecular weight determination, relative and absolute quantitation, and purity calculation. Images are easily loaded, viewed, compared, adjusted (e.g., contrast, rotation, crop) and annotated. The program reads and writes to standard file formats, providing compatibility with nearly any source image or final presentation method.



Highlights:

- **Intuitive design** – sleek task-bars (ribbons) organize functions into four main tabs: File, Image, Lanes/Bands, Annotation
- **Compatible file types** – load images in popular file formats (TIFF, JPEG, PNG, etc.); analyze images from nearly any source, and easily share the results
- **Image comparison** – open two to six image files at once to review them simultaneously or toggle between them to edit them in parallel
- **Auto-identification** – accurate, customizable, automatic lane and band identification creates numbered, selectable objects for lane profile densitometry and analysis
- **MW determination** – versatile, trouble-free calculation of migration distances and molecular weights based on pre-installed or custom-added markers
- **Advanced quantitation** – automatically calculate sample purity based on band and lane intensities, or determine the relative or absolute quantities of bands or regions
- **Image refinement and annotation** – crop, rotate, invert, display saturation and adjust contrast; then add simple labels, notes and arrows
- **Exporting and sharing** – easily transfer data and images to Microsoft™ Excel™, Word™ and PowerPoint™ Programs for further analysis and presentation

Ordering Information

Product #	Description	Pkg. Size
PI62237	MYIMAGEANALYSIS Software	2 licenses

Film

Thermo Scientific™ CL-XPosure™ Radiography Film

Economical X-ray film.

Highlights:

- Up to one-third the price of other suppliers' 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 24cm 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)	$1.29
Kodak X-Omat Blue Film (Blue X-ray Film) (Sigma Aldrich)	$2.08
Kodak BioMax MR-1 (Gray X-ray Film) (Sigma Aldrich)	$5.34

Source: 2014 Online Catalogs

Ordering Information

Product #	Description	Pkg. Size
PI34090	CL-XPosure Film, 5 x 7 in (13 x 18cm)	100/pkg.
PI34089	CL-XPosure Film, 7 x 9.5 in (18 x 24cm)	100/pkg.
PI34091	CL-XPosure Film, 8 x 10 in (20 x 25cm)	100/pkg.
PI34093	CL-XPosure Film, 8 x 10 in (20 x 25cm)	50/pkg.

Amersham Hyperfilm product conversion.

GE Amersham Hyperfilm Blue product			Thermo Scientific alternative		
Product #	Description	Pkg. Size	Product #	Description	Pkg. Size
28-9888-22	Amersham Hyperfilm Blue, 5 x 7in. (12.7 x 17.8cm)	100 sheets	PI34088	CL-XPosure Film, 5 x 7in. (12.5 x 17.5cm)	50 sheets
			PI34090	CL-XPosure Film, 5 x 7in. (13 x 18cm)	100 sheets
28-9888-21	Amersham Hyperfilm Blue, 7.1 x 9.5in. (18 x 24cm)	100 sheets	PI34089	CL-XPosure Film, 7 x 9.5in. (18 x 24cm)	100 sheets
28-9888-20	Amersham Hyperfilm Blue, 8 x 10in. (20.3 x 25.4cm)	100 sheets	PI34091	CL-XPosure Film, 8 x 10in. (20 x 25cm)	100 sheets

GE Amersham Hyperfilm ECL product			Thermo Scientific alternative		
Product #	Description	Pkg. Size	Product #	Description	Pkg. Size
28-9068-40	Amersham Hyperfilm ECL, 10 x 12in. (24 x 30cm)	50 sheets	PI34097	CL-XPosure Film, 9.5 x 11.8in. (24 x 30cm)	100 sheets
28-9068-41	Amersham Hyperfilm ECL, 11.8 x 15.8in. (35 x 43cm)	50 sheets	PI34099	CL-XPosure Film, 14 x 17in. (35 x 40cm)	100 sheets

GE Amersham Hyperfilm MP product			Thermo Scientific alternative		
Product #	Description	Pkg. Size	Product #	Description	Pkg. Size
28-9068-47	Amersham Hyperfilm MP, 10 x 12in. (24 x 30cm, single film bag)	50 sheets	PI34097	CL-XPosure Film, 9.5 x 11.8in. (24 x 30cm)	100 sheets
28-9068-48	Amersham Hyperfilm MP, 11.8 x 15.8in. (35 x 43cm, single film bag)	50 sheets	PI34099	CL-XPosure Film, 14 x 17in. (35 x 40cm)	100 sheets

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 3).

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.

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.

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

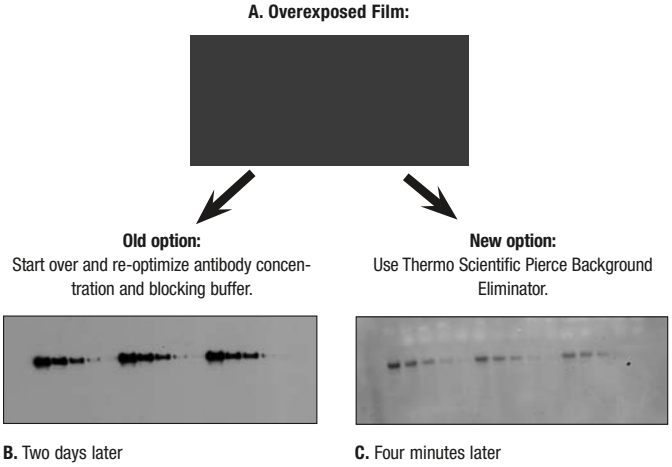


Figure 2. Thermo Scientific Pierce Background Eliminator lightens the entire film evenly in four minutes vs. the two days traditional methods require to start over and reoptimize experiment conditions. A431 cell lysate was electrophoresed on a 4-12% NuPage™ Gel (Novex) and transferred overnight to nitrocellulose. The membrane was blocked with SuperBlock Blocking Buffer in PBS (Product # PI37515) for 1 hour and incubated with 1.25ng/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 2.5ng/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)**.

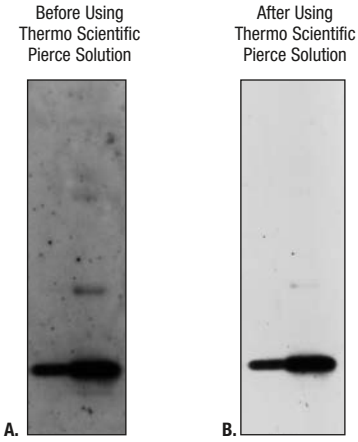


Figure 3. Thermo Scientific Pierce Background Eliminator erases speckling. Recombinant Human TNFα was electrophoresed on a 4-20% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane. The membrane was blocked and detected with Mouse anti-Human TNFα followed by Goat anti-Mouse-HRP (Product # PI31434) and SuperSignal West Dura Substrate (Product # PI34075). 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)**.

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

- In-gel detection systems
- Gel-shift assays
- Ribonuclease protection assays (RPA)

Ordering Information

Product #	Description	Pkg. Size
PI46428	**Pierce Background Eliminator**	Kit
	Sufficient reagent to prepare 3L of working solution.	
	Includes: Pierce Reagent A	100mL
	Pierce Reagent B	100mL

Stripping

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. Thermo Scientific™ Restore Western Blot Stripping Buffer (Product # PI21059) and Restore PLUS Western Blot Stripping Buffer (Product # PI46430) 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 few as 15 minutes (Figure 5, page 72).

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.

Stripping Buffers

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, reperforming 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!

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

Optimize assay conditions
Using Pierce SuperSignal West Substrates, the secondary antibody concentrations are optimized after a single stripping and re-probing cycle (Figure 4, page 72).

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 5, page 72).

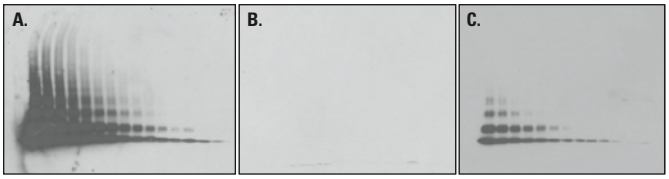


Figure 4. Antibody optimization study. Western blots of Interleukin-2 (diluted 20-0.156ng) were detected using SuperSignal West Pico Chemiluminescent Substrate. The first blot **(A)** used the primary antibody diluted to 1:1,000 (0.5µg/mL) of Rat Anti-Mouse IL-2 (BD PharMingen; San Jose, CA) and the HRP-labeled Goat Anti-Rat secondary antibody (Product # PI31470) 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.

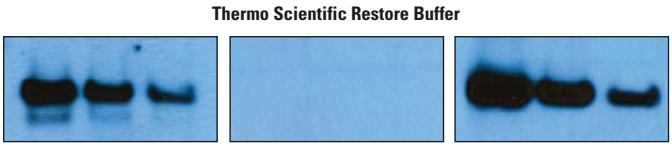


Figure 5. Strip and reprobe effectively with Thermo Scientific Restore Stripping Buffer. HeLa cell lysate was serially diluted and transferred to membrane. **Panel A:** The blot was probed for Src. Signal was detected with Pierce ECL Chemiluminescent Substrate and a 5-minute film exposure. **Panel B:** The blot was stripped with Restore Western Blot Stripping Buffer and evaluated by incubating in anti-mouse HRP with subsequent Pierce ECL detection. No signal was detected after a 15-minute film exposure. **Panel C:** The blot was reprobed for GAPDH and detected with a 1-minute film exposure.

References

Baolin Zhang, B., *et al.* (2003). *Mol. Cell. Biol.* **23**, 5716-5725.
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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
PI21059	**Restore Western Blot Stripping Buffer** *Sufficient for stripping 25 (8cm x 10cm) blots.*	500mL
PI21062	**Restore Western Blot Stripping Buffer** *Sufficient for stripping one (8cm x 10cm) blot.*	30mL
PI21063	**Restore Western Blot Stripping Buffer** *Sufficient reagent to strip 500 (8cm x 10cm) blots.*	5L

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.

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 6, page 73)

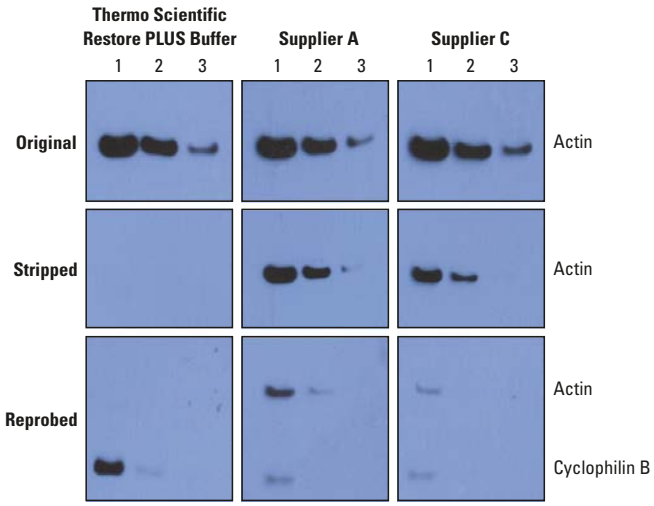


Figure 6. Reprobing with different antibodies. HeLa cell lysate was probed for actin and detected with Pierce ECL Substrate (**Original Panel**). Blots were then stripped with either Restore PLUS Stripping Buffer or other suppliers' stripping buffers (**Stripped Panel**). The blots were then re-blocked and reprobed for cyclophilin B and detected with Pierce ECL Substrate (**Reprobed Panel**).

Ordering Information

Product #	Description	Pkg. Size
PI46428	**Restore PLUS Western Blot Stripping Buffer** *Sufficient reagent to strip one to two (8cm x 10cm) blots.*	30mL
PI46430	**Restore PLUS Western Blot Stripping Buffer** *Sufficient reagent to strip 25 (8cm x 10cm) blots.*	500mL
Complementary Products		
PI32106	**Pierce ECL Substrate**	500mL
PI34080	**SuperSignal West Pico Chemiluminescent** Includes: Luminol/Enhancer Stable Peroxide Buffer	500mL 250mL 250mL
PI34075	**SuperSignal West Dura Chemiluminescent Substrate** Includes: Luminol/Enhancer Solution Stable Peroxide Buffer HRP-Conjugated Goat Anti-Rabbit HRP HRP-Conjugated Goat Anti-Mouse HRP	100mL 50mL 50mL 1mL 1mL
PI34095	**SuperSignal West Femto Chemiluminescent Substrate** Includes: Luminol/Enhancer Solution Stable Peroxide Solution HRP-Conjugated Goat Anti-Rabbit HRP-Conjugated Goat Anti-Mouse	100mL 50mL 50mL 1mL 1mL

Thermo Scientific™ Restore™ Fluorescent Western Blot Stripping Buffer

The Restore Fluorescent Western Blot Stripping Buffer is a gentle and highly effective reagent for quickly removing primary and near-infrared (IR) dye-labeled secondary antibodies from Western blots. Restore Fluorescent Western Blot Stripping Buffer enables the reuse of PVDF membranes, simplifying the Western blot optimization process and allowing the same blot to be reprobed with different primary antibodies to detect alternative targets. Restore Fluorescent Western Blot Stripping Buffer is for use with low-fluorescence PVDF membrane only (Product # PI22860).

Highlights:

- **Fast** – strip blots in only 15 minutes at room temperature
- **Saves time** – no need to run new gels and prepare a new blot
- **Conserve samples** – reprobe the same PVDF membrane for multiple targets
- **Economical** – less expensive than other commercially available stripping buffers
- **Efficient** – effectively strips blots the first time

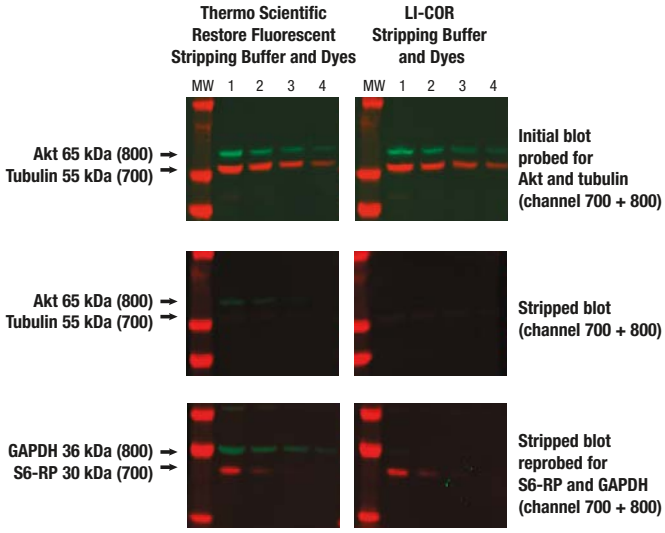


Figure 7. Effective stripping and reprobing of near-IR fluorescent Western blots on PVDF. A549 whole cell lysates (20, 10, 5, and 2.5µg of protein in **Lanes 1, 2, 3, and 4**, respectively) were transferred to PVDF membranes. **Top Panels:** The blots were probed with anti-Akt and anti-tubulin antibodies and detected using DyLight 680 Goat Anti-Mouse IgG (Product # PI35519) and DyLight 800 Goat Anti- Rabbit IgG (Product # PI35571), or IRDye™ 680 Goat Anti-Mouse and IRDye 800CW Goat Anti-Rabbit (LI-COR Biosciences). The LI-COR Odyssey™ Infrared Imaging System (channel 700 and 800) was used for imaging. **Middle Panels:** Blots were stripped with Restore Fluorescent Stripping Buffer or NewBlot™ PVDF Stripping Buffer for 15 minutes at room temperature. Blots were rinsed with TBS for 5 minutes and re-imaged using channel 700 and 800. **Bottom Panels:** Stripped membranes were reprobed with rabbit anti-S6 ribosomal protein and mouse anti-GAPDH antibodies. Targets were detected using DyLight 800 Goat Anti-Mouse IgG (Product # PI35521) and DyLight 680 Goat Anti-Rabbit IgG (Product # PI35568), or IRDye 800CW Goat Anti-Mouse and IRDye 680 Goat Anti-Rabbit and imaged as described above.

Ordering Information

Product #	Description	Pkg. Size
PI62299	**Restore Fluorescent Western Blot Stripping Buffer** *Sufficient for 10 mini blots or 800cm² membrane.*	20mL
PI62300	**Restore Fluorescent Western Blot Stripping Buffer** *Sufficient for 50 mini blots or 4000cm² membrane.*	100mL

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 nonrelevant 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 ratio, 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.

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 20mL for an 8 x 10cm blot). Alternatively, the blot can be incubated with a solution of 2% w/v SDS, 62.5mM Tris•HCl, 100mM 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

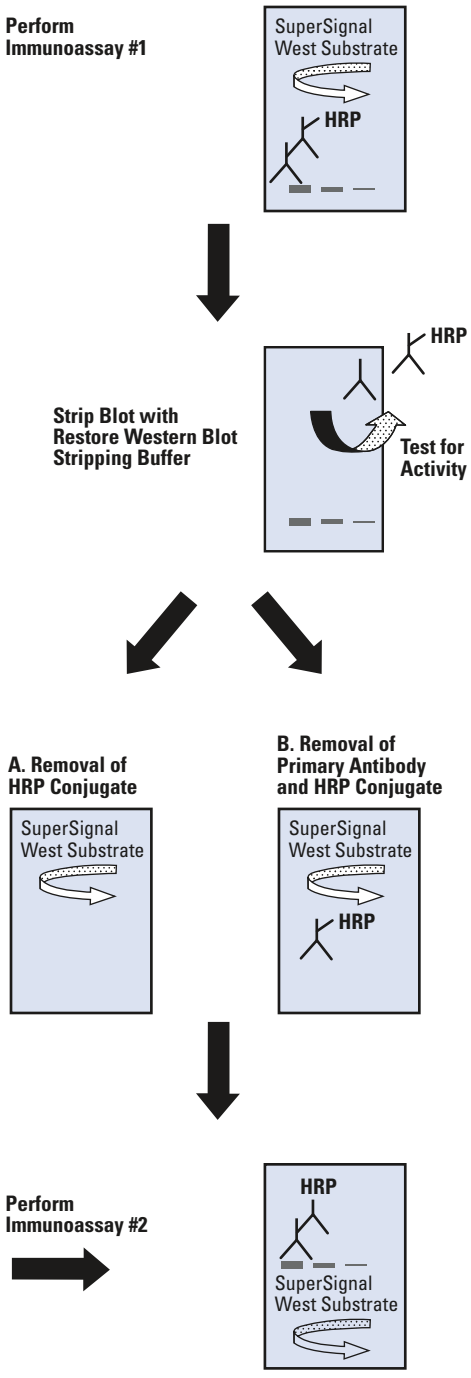
   B. To test for complete removal of the primary antibody, incubate the membrane with the HRP-labeled secondary antibody, followed by a wash in wash buffer. Apply SuperSignal West Working Solution. If no signal is detected with a 5-minute exposure, the primary antibody has been successfully removed from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



Thermo Scientific Restore Western Blot Stripping Buffer protocol.

Troubleshooting

Optimizing Chemiluminescence Blotting

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 the next page.

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.

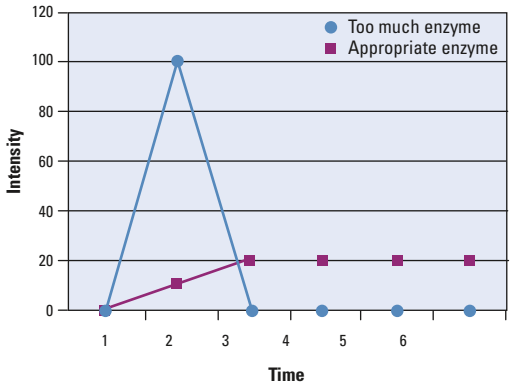
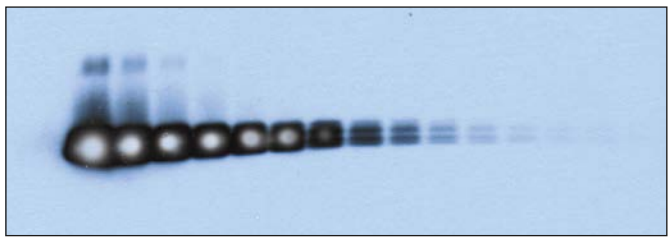


Figure 1. A signal that peaks and terminates quickly is usually caused by the use of too much enzyme.

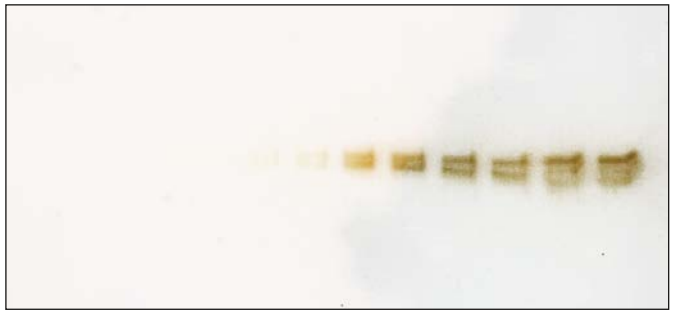
The following is a list of several indicators of too much enzyme:

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



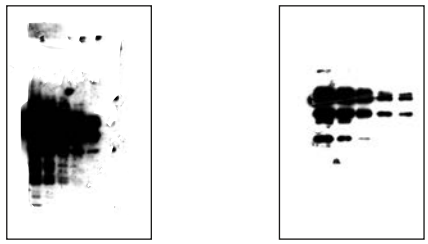
A "ghosting" of bands where the protein of interest is expected could be caused by using too much enzyme.

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



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

4. High background and/or unwanted bands



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

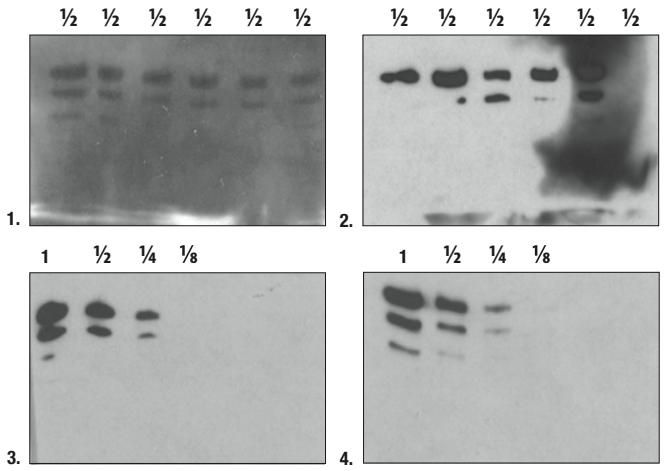
Primary Antibody
1:5,000
Secondary Antibody
1:50,000

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 overexpose sensitive X-ray film

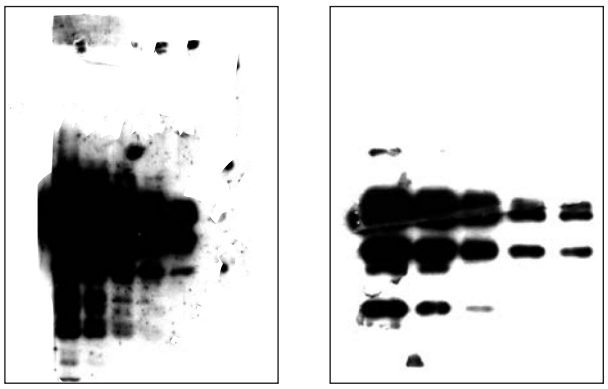
Optimize Antibody Concentration

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 2-3). 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 17).



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

Figure 2. 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.



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

2. **Primary Antibody 1:5,000 Secondary Antibody 1:50,000**

Figure 3. 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.

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 ~1mg/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 (2-5µL 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 Substrate	Recommended Primary Antibody Dilutions (from 1mg/mL stock)
Pierce ECL Substrate	1:1,000-1:10,000
Pierce ECL Plus Substrate	1:1,000-1:20,000
SuperSignal West Pico Substrate	1:1,000-1:5,000
SuperSignal West Dura Substrate	1:1,000-1:50,000
SuperSignal West Femto Substrate	1:5,000-1:100,000

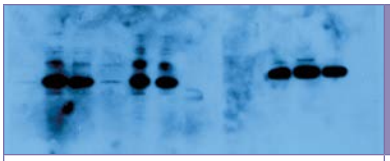
6. Wash the membrane four to six times in TBS or PBS, using as large a volume of wash buffer as possible. Add 0.05% Tween-20 Detergent to the wash buffer to help reduce nonspecific background. For each wash, suspend the membrane in wash buffer and agitate for approximately 5 minutes. Pour off the wash 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 Substrate	Recommended Secondary Antibody Dilutions (from 1mg/mL stock)
Pierce ECL Substrate	1:1,000-1:15,000
Pierce ECL Plus Substrate	1:25,000-1:200,000
SuperSignal West Pico Substrate	1:20,000-1:100,000
SuperSignal West Dura Substrate	1:50,000-1:250,000
SuperSignal West Femto Substrate	1:100,000-1:500,000

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: 0.1mL/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.

High Background that is Uniformly Distributed

Possible Causes	Precautions/Solutions
Antibody concentrations are too high	• High concentrations of primary and/or secondary antibody can cause high background. • Decrease antibody concentrations.
Incompatible blocking buffer was used	• 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 0.05% Tween-20 Detergent to the blocking buffer at a final concentration of 0.05%. This is not applicable to StartingBlock T20 Blocking Buffer in PBS or TBS or SuperBlock T20 Blocking Buffer in PBS or TBS. • Prepare antibody dilutions in blocking buffer that contains 0.05% Tween-20 Detergent.
Cross-reactivity of antibody with other proteins in blocking buffer	• Use blocking buffers that contain no proteins of plant or animal origin, for example Pierce Protein-Free Blocking buffers. • 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 at a final concentration of 0.05%. (Note: If the concentration of Tween-20 Detergent is too high, it can strip proteins off the membrane.) Skip this step if you use StartingBlock T20 Blocking Buffer in PBS or TBS or SuperBlock T20 Blocking Buffer in PBS or TBS.
Exposure time is too long	• Reduce the time the blot is exposed to film.
Membrane problems	• Wet membranes thoroughly according to the manufacturer's instructions. • Use new membranes. • Cover the membrane 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.

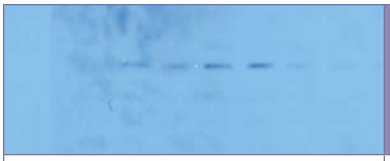


High Background that is Blotchy or Speckled

Possible Causes	Precautions/Solutions
Antibody concentrations are too high	• High concentrations of primary and/or secondary antibody can cause high background. • Decrease antibody concentrations.
Aggregate formation in the HRP conjugate can cause speckling	• Filter the conjugate through a 0.2µm filter. • Use a new, high-quality conjugate.
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 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 the blocking buffer to a final concentration of 0.05%. Skip this step if you use StartingBlock T20 Blocking Buffer in PBS or TBS or SuperBlock T20 Blocking Buffer in PBS or TBS. • 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. • Cover the membrane with 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	• Use only clean and contaminant-free electrophoresis equipment, blotting equipment and incubation trays. • No pieces of gel should be left on the membrane after transfer because proteins can stick to them, causing background.

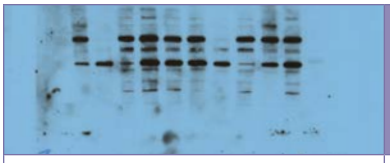
Black Blots with White Bands or Signal That Decreases Quickly

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.



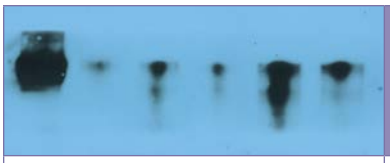
Weak Signal or No Signal

Possible Causes	Precautions/Solutions
Proteins did not transfer properly to the membrane	• After transfer, 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 Pierce Reversible Membrane Stain to check membrane for transfer efficiency. • Ensure sufficient contact between the gel and membrane during transfer. • Make sure the transfer sandwich is assembled correctly. • Wet membrane according to manufacturer's instructions. • 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 have not destroyed antigenicity of the sample. *(Note: Some proteins cannot be run under reducing conditions.)*
Insufficient binding to membrane	• Add 20% methanol to the transfer buffer to help 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 target protein. • 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.
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	• Do not use sodium azide, an inhibitor of HRP, as a preservative in buffers.
Exposure time is too short	• Lengthen the film exposure time.
Substrate incubation is too short	• SuperSignal Substrates require a 5-minute substrate incubation.
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 there is no cross-contamination between the two bottles of substrate, which can cause a decline in activity.
Membrane has been stripped and reprobed	• Optimize stripping procedure to prevent any loss of antigen or denaturation. • 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.



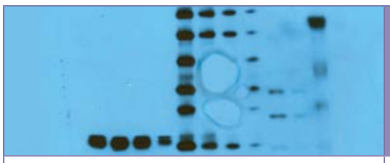
Nonspecific Bands

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.



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 100mM 2-mercaptoethanol, dithiothreitol or TCEP that will reduce the disulfide bonds present in the protein sample

   Adjust solution to pH 6.8.
2. Apply 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
Pierce ECL Plus 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

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 18.**
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 77.**

Thermo Scientific Substrate	Recommended Primary Antibody Dilutions (from 1mg/mL stock)
Pierce ECL Substrate	1:1,000-1:10,000
Pierce ECL Plus Substrate	1:1,000-1:20,000
SuperSignal West Pico Substrate	1:1,000-1:5,000
SuperSignal West Femto Substrate	1:1,000-1:50,000
SuperSignal West Dura Substrate	1:5,000-1:100,000

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 Detergent 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 for 1 hour with shaking at RT. For recommended antibody-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 SecondaryAntibody Dilutions (from 1mg/mL stock)
Pierce ECL Substrate	1:1,000-1:15,000
Pierce ECL Plus Substrate	1:25,000-1:200,000
SuperSignal West Pico Substrate	1:20,000-1:100,000
SuperSignal West Dura Substrate	1:50,000-1:250,000
SuperSignal West Femto Substrate	1:100,000-1:500,000

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.
11. Incubate the blot with SuperSignal Substrate Working Solution for 5 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 blot against the film and expose. Standard autoradiographic film in an imaging system such as the MYECL Imager can be used. A recommended first exposure time is 60 seconds. Vary exposure time to obtain optimum results.
14. Quantitate the bands using software provided with the imaging system such as MYImageAnalysis software, or, if using X-ray film, develop the film using appropriate developing solution and fixative for the type of film used.

Recommended Reading

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