WESTERN BLOTTING HANDBOOK

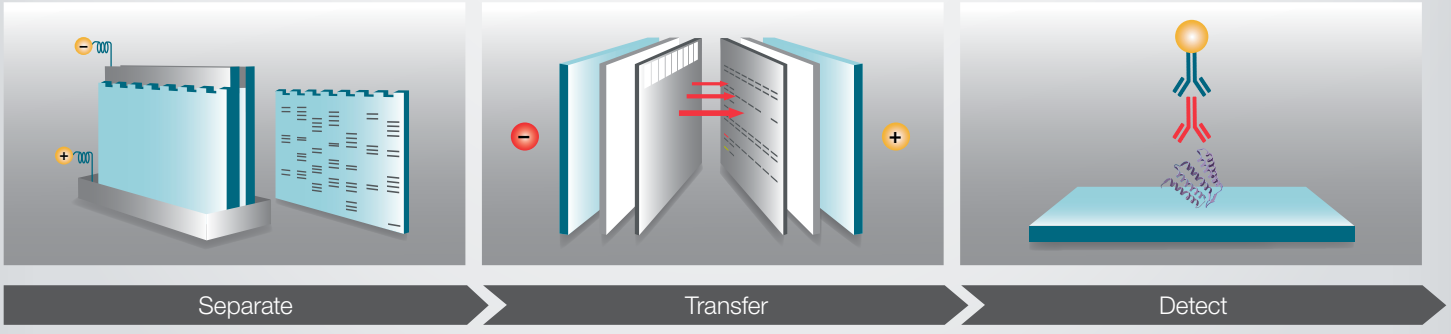
Techniques and tools for publication-quality results

ThermoFisher SCIENTIFIC

Contents

Overview of western blotting

Western blotting was introduced by Towbin et al. [1] in 1979 and is now a routine and fundamental technique for protein analysis. Western blotting, also called protein blotting or immunoblotting, uses antibodies to identify specific protein targets after they have been separated by electrophoresis and transferred to a membrane. The specificity of the antibody–antigen interaction enables a target protein to be detected in a complex protein mixture, such as cell or tissue lysate. Western blotting can be used to generate qualitative and semi-quantitative data for a protein of interest. In this handbook, you will find information to help you choose the right protein transfer and detection methods for your western blots. You will also find troubleshooting tips, how-to videos, selection guides, and buffer recipes, to help you achieve optimal results.



For a complete listing of all available products and more, visit **thermofisher.com/western**
Watch an overview video of western blotting **here**.

Transfer

Introduction to transfer methods for western blotting

Protein transfer from gel to membrane is necessary for two reasons: better handling capability offered by the membrane than the fragile gel during western blot processing, and better target accessibility on the membrane by macromolecules like antibodies. In general, all electrotransfer methods rely on the electrophoretic mobility of proteins to move them out of a gel. The techniques involve placing a protein-containing polyacrylamide gel in direct contact with a piece of nitrocellulose or polyvinylidene difluoride (PVDF) membrane or other suitable protein-binding support. Next, the gel and membrane are sandwiched between two electrodes, which are typically submerged in a conducting solution (transfer buffer) (Figure 1). When an electric field is applied, the proteins move out of the gel and become tightly attached on the surface of the membrane. The resulting membrane is a copy of the protein pattern that was in the gel. For a complete workflow, see Figure 2. Prior to electrotransfer, the most popular method for protein transfer was diffusion blotting, which took an average of 1.5 to 2 days. In comparison, a typical dry electrotransfer now averages less than 10 minutes. In this handbook, we will only be discussing electrotransfer and will refer to this as transfer or blotting.

There are three ways to transfer proteins from SDS-PAGE or native gels to nitrocellulose or PVDF membranes for the purpose of western blotting: wet transfer (also known as tank transfer), semi-dry transfer, and dry transfer. This handbook will focus on these three techniques and other considerations to help improve your protein transfer efficiency for better western blot results.

Did you know

W. Neal Burnette in Tobert Nowinski's lab at the Fred Hutchinson Cancer Research Center in Seattle was the first to coin the term "western blotting". The term honors Edwin Southern, who described blotting of DNA, and is a reference to the West Coast location of Nowinski's lab.

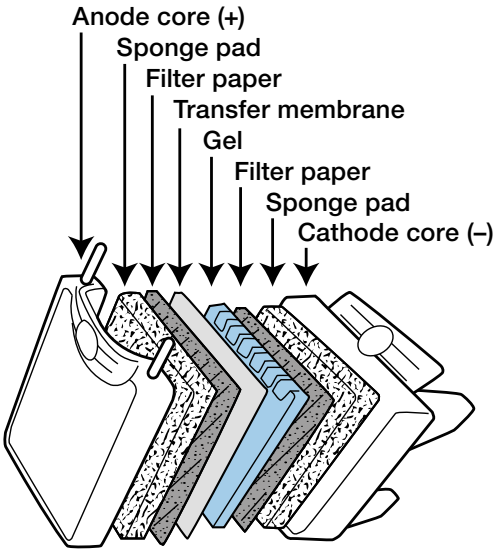


Figure 1. Western blot electrotransfer of proteins from gel to membrane. While this diagram depicts the setup of a typical wet transfer, many of the principles apply to semi-dry and dry methods of protein transfer to membranes.

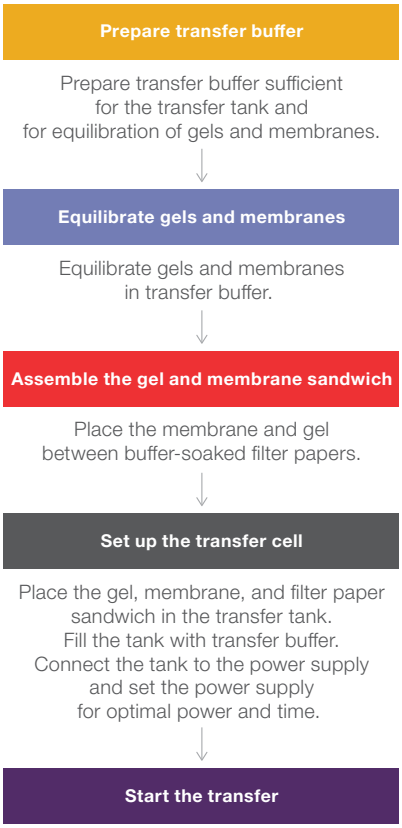


Figure 2. Workflow of the tank electrotransfer of proteins for western blotting.

Transfer systems for western blotting

All modern western blot transfer methods rely on the electrophoretic mobility of proteins. When an electric field is applied across the transfer sandwich or stack, the proteins move out of the polyacrylamide gel and onto the nitrocellulose or PVDF membrane, creating a copy of the protein separation pattern in the original gel. Wet, semi-dry, and dry transfer procedures are commonly used electroblotting methods. The key differences between these systems are the amount of buffer used and the speed of the transfer. In traditional wet transfer systems, the filter paper–membrane–gel sandwich is submerged in a tank that contains transfer buffer. A current passes through the buffer to move proteins from the gel to the membrane. For semi-dry transfer, the membrane and gel are sandwiched between filter paper soaked with transfer buffer. This wetted filter paper serves as the buffer reservoir to support transfer. Charge is driven through the filter paper to move the proteins from the gel to the membrane. In dry transfer systems, the membrane–gel sandwich is placed between specialized gel matrices that contain ions. These ions move when current is applied, resulting in transfer of the proteins from the protein gel to the membrane. The transfer systems we offer for each method are summarized in Table 1.

Table 1. Characteristics of Invitrogen™ transfer systems.

Wet transfer			Semi-dry transfer	Dry transfer
				
Mini Blot Module	**XCell II Blot Module**	**SureLock Tandem Midi Blot Module**	**Power Blotter Systems**	**iBlot 2 Gel Transfer Device**
Capacity: 1 mini gel per blot module; 1–2 blot modules per tank	**Capacity:** Up to 2 mini blots	**Capacity:** 1 midi gel per blot module; 1–2 blot modules per tank	**Capacity:** 1–4 mini or 1–2 midi gels	**Capacity:** 1–2 mini or 1 midi gel
Transfer time: 60 min	**Transfer time:** 60–120 min	**Transfer time:** 30 min	**Transfer time:** 7–10 min	**Transfer time:** 7 min
Blotting area: 9 x 9 cm	**Blotting area:** 9 x 9 cm	**Blotting area:** 9.2 x 14.4 cm	**Blotting area:** 10 x 18 cm or 21 x 22.5 cm	**Blotting area:** 8.5 x 13.5 cm
Transfer buffer volume: 220 mL per blot module	**Transfer buffer volume:** 200 mL	**Transfer buffer volume:** 300 mL per blot module	**Transfer buffer volume:** Pre-cut membranes and filters: 50–100 mL; pre-assembled Select transfer stacks: buffer not required	**Transfer buffer volume:** Buffer not required
Power supply: External	**Power supply:** External	**Power supply:** External	**Power supply:** Internal	**Power supply:** Internal
Required equipment: Mini Gel Tank	**Required equipment:** XCell SureLock Mini-Cell	**Required equipment:** SureLock Tandem Midi Gel Tank	–	–

Wet transfer (tank transfer)

Tank transfer systems are the most commonly used transfer systems, with semi-dry and dry transfer techniques being an evolution of traditional wet tank transfer. In this method, the gel is first equilibrated in transfer buffer. The gel is then placed in the transfer sandwich (filter paper–gel–membrane–filter paper), cushioned by pads and pressed together by a support grid. The supported gel sandwich is placed vertically in a tank between stainless-steel/platinum wire electrodes and immersed in transfer buffer (Figure 3).

Multiple gels may be electrotransferred in the standard field option, which is performed either at constant current (0.1 to 1 A) or voltage (5 to 30 V) for as little as 30 minutes to overnight. Transfers are typically performed with an ice pack or at 4°C to mitigate the heat produced.

In wet transfer, transfer efficiencies are better for lower molecular weight proteins than higher molecular weight proteins, with typical efficiencies of 80–100% for proteins between 14 and 116 kDa [2]. The transfer efficiency improves with increased transfer time. However, with increasing time and the use of membranes with larger pore sizes (0.45 µm), the risk of transferring the proteins completely through the membrane increases (also known as blow-through), especially for lower molecular weight (<30 kDa) proteins.

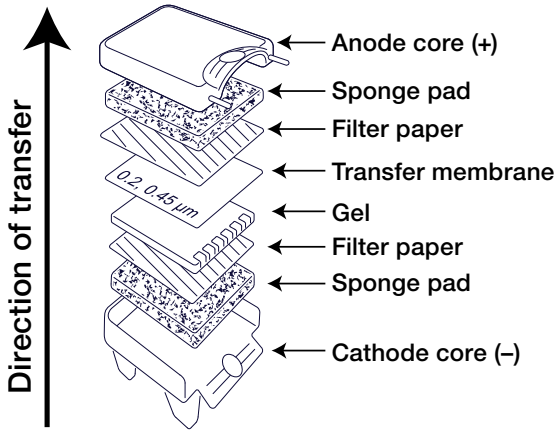


Figure 3. Tank (wet) transfer apparatus for western blotting, with gel–membrane sandwich detail. Schematic showing the assembly of a typical western blot apparatus with the position of the gel and transfer membrane and direction of protein transfer in relation to the electrode position.

Did you know

In many cases, ethanol can be substituted for methanol in transfer buffers without impacting transfer efficiency.

Mini Blot Module

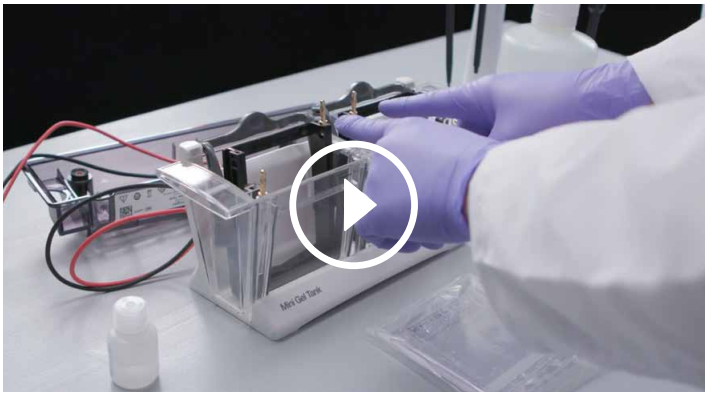
Leak-free, less-buffer wet transfer system

The Invitrogen™ Mini Blot Module is a wet transfer device used exclusively with the Mini Gel Tank and is designed to make your western transfers simple and easy to perform. The tank accommodates one Mini Blot Module per chamber, or two blot modules total with the side-by-side layout. The universal connection and molded gasket make the blot module easy to use, while the inner core of the blot module allows for use of less methanol-based transfer buffer per wet transfer than other commercially available transfer systems. At the recommended conditions and constant voltage, proteins can typically be transferred to nitrocellulose or PVDF membranes in 30 to 60 minutes (Figure 4).

Features:

- **Universal module design**—allows modules to fit in either chamber of the tank, simplifying the transfer setup
- **Unique gasket seal**—helps prevent buffer leakage to minimize mess during setup of your western transfer
- **1/2-inch buffer chamber**—requires only 220 mL per blot of methanol-based transfer buffer, helping save you money on buffer and disposal costs
- **Standard 60-minute transfer protocol**—accelerates your western workflow so you can get results faster
- **Robust electrodes, sturdy steel plates**—for highly efficient and reliable western transfers

View how-to video:



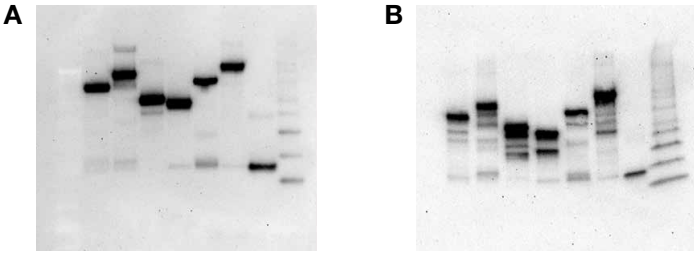


Figure 4. Using Bolt Bis-Tris Plus gel chemistry for electrophoresis followed by wet transfer using the Mini Blot Module results in a western blot with sharp protein signals corresponding to only full-length proteins, whereas a western blot of a Bio-Rad™ TGX™ gel shows multiple low molecular weight degradation products. Protein kinases implicated in cancer (IKKB, HCK, EPHB3, MAPK14, FLT1, and DDR2) were analyzed on **(A)** a Bolt Bis-Tris Plus gel and **(B)** a Bio-Rad TGX Tris-glycine gel. Protein samples were prepared for electrophoresis according to each manufacturer's protocol. The purified kinases (50 ng each) as GST fusion proteins, along with MagicMark XP Protein Standard and purified recombinant GST, were loaded in a Bolt 4–12% gel and a Bio-Rad TGX 4–20% gel. The samples were separated and transferred to PVDF membranes using the Mini Blot Module for the Bolt gel or on the Bio-Rad transfer system. Blot detection was performed using an anti-GST antibody and an Invitrogen™ WesternBreeze™ Chemiluminescent Kit.

Specifications	
Mode of transfer	Wet
Gel compatibility	NuPAGE Bis-Tris, Tris-acetate, Tris-glycine, Tricine, and Bolt Bis-Tris Plus gels; pour-your-own gels cast with the Invitrogen™ SureCast™ system
Running dimension	Vertical
Capacity	≤2 blot modules/mini-gel tank; 1 mini gel/blot module
Gel size	Mini (8 x 8 cm)

Recommended products

The Invitrogen™ PowerEase™ Touch 350W Power Supply and pre-cut nitrocellulose and PVDF membranes are recommended for use with the Mini Blot Module.

Learn more at **thermofisher.com/minigeltank**

XCell II Blot Module

Expands your XCell SureLock and XCell II Mini-Cell systems into western transfer devices

The Invitrogen™ XCell II™ Blot Module allows you to easily transfer proteins or nucleic acids from mini gels to membranes. It fits neatly into the Invitrogen™ XCell SureLock™ and XCell II™ Mini-Cell systems in place of the gel/buffer core assembly. It requires less than 200 mL of transfer buffer for western, Southern, and northern transfers. Tough platinized-titanium and stainless-steel electrodes create a uniform electrical field without clamps or hinged gel holders.

Features:

- **Economical**—requires only 200 mL of methanol-based transfer buffer and helps save you money
- **Flexible**—fits gel sizes up to 8 x 8 cm
- **Robust electrodes, sturdy steel plates**—for highly efficient and reliable western transfers

Specifications	
Mode of transfer	Wet
Gel compatibility	NuPAGE Bis-Tris and Tris-Acetate, Bolt Bis-Tris Plus, Novex Tris-Glycine, Novex TBE, and Novex TBE-Urea mini gels; pour-your-own gels cast with the SureCast system
Running dimension	Vertical
Capacity	Up to 2 mini gels
Gel size	Mini (8 x 8 cm)

Recommended products

The PowerEase Touch 350W Power Supply and pre-cut nitrocellulose and PVDF membranes are recommended for use with the XCell II Blot Module.

SureLock Tandem Midi Blot Module

The Invitrogen™ SureLock™ Tandem Midi Gel Tank is uniquely designed to enable convenient, reliable gel electrophoresis and protein transfer of high-performance Invitrogen™ midi gels. With the SureLock Tandem Midi Blot Module, the tank performs efficient room-temperature wet transfers for downstream western blot analysis in 30 minutes. The tank accommodates two blot modules, allowing transfer of one or two gels at a time. The unique design of the SureLock Tandem Midi Blot Module uses considerably less transfer buffer (only ~300 mL per transfer) than other midi-sized wet transfer systems. This lower buffer requirement reduces the amount of hazardous methanol waste.

Features:

- **2-in-1 midi gel electrophoresis and transfer tank**—run and transfer high-performance Invitrogen midi gels using the same tank
- **Two separate gel chambers**—run 1 or 2 gels or transfers using only the necessary amount of buffer for each gel, minimizing buffer cost and waste
- **Optimal performance with fast transfer protocols**—efficient, room-temperature transfers in 30 minutes; eliminate the need to prechill buffers and the hassle and messiness of ice baths

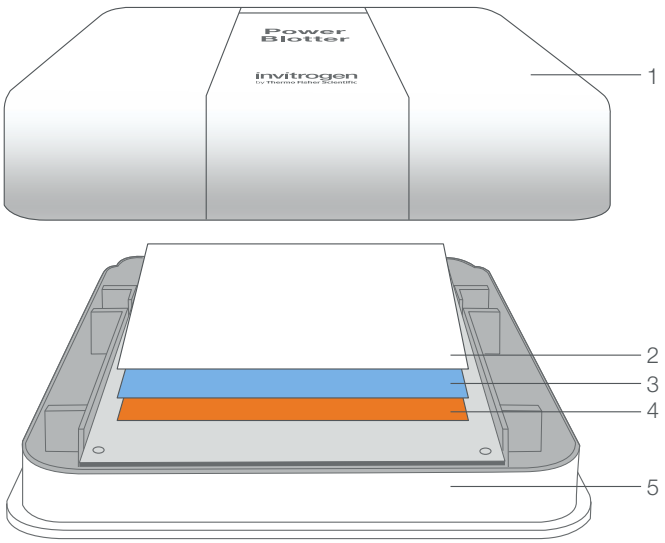
Specifications	
Mode of transfer	Wet
Gel compatibility	NuPAGE Bis-Tris, NuPAGE Tris-Acetate, and Novex Tris-Glycine Plus midi gels
Running dimension	Vertical
Capacity	1 midi gel per blot module
Gel size	Midi (8 x 13 cm)

View a how-to video on the SureLock Tandem Midi Gel Tank and Blot Module **here**.

Learn more at **thermofisher.com/wettransfer**

Semi-dry transfer

Semi-dry transfer became available as the need for faster results became an issue for researchers. For semi-dry protein transfer, the transfer sandwich is placed horizontally between two plate electrodes in a semi-dry transfer apparatus (Figure 5). The key to improving the speed of transfer with this method is to maximize the current passing through the gel versus around it. To do this, the amount of buffer used in the transfer is limited to that contained in the transfer sandwich. Hence, it is critical that the membrane and filter paper sheets are cut to the gel size without overhang and that the gel and filter paper are thoroughly equilibrated in transfer buffer. Also, the use of extra-thick filter paper (approximately 3 mm thickness) is helpful in certain semi-dry transfer devices because these sheets can hold more transfer buffer.



1 Cathode
2 Transfer gel layer (cathode)
3 Gel
4 Transfer gel layer (anode), membrane on top
5 Anode

Figure 5. Semi-dry electroblotting transfer. The filter paper–gel–membrane–filter paper sandwich is placed horizontally between two plate electrodes for semi-dry transfer. The presoaked filter paper acts as an ion reservoir that enables fast transfer.

Methanol may be included in the transfer buffer, but other organic solvents, including aromatic hydrocarbons, chlorinated hydrocarbons, and acetone, should not be added to avoid damage to the electrode plates. Fast-blotting, semi-dry techniques use higher ionic strength transfer buffers and a high current power supply to decrease transfer times to under 10 minutes. In rapid methods, amperage is held constant and voltage is limited to a maximum of 25 V. Transfer with traditional Towbin buffers can be preformed in a semi-dry apparatus either at constant current (0.1 up to approximately 0.4 A) or voltage (10 to 25 V) for 30 to 60 minutes.

Did you know

Addition of SDS to the transfer buffer increases the relative current, power, and heating during transfer, and may also affect the recognition of some proteins by antibodies.

Power Blotter System

High performance semi-dry transfers in less than 10 minutes

The Invitrogen™ Power Blotter and Power Blotter XL Systems are designed specifically for rapid semi-dry transfer of proteins from polyacrylamide gels to nitrocellulose or PVDF membranes in 7 to 10 minutes (Figure 6). The Power Blotter features an integrated power supply optimized to enable consistent and high-efficiency protein transfer.

Features:

- **Efficient**—high transfer efficiency with a broad range of protein sizes compared to conventional semi-dry or wet transfer methods (Figure 7)
- **Fast**—7- to 10-minute transfer when used with Invitrogen™ Power Blotter Select Transfer Stacks or Invitrogen™ Power Blotter 1-Step Transfer Buffer
- **Integrated power supply**—seamless operation between control unit and cassette; no external power supply necessary
- **Easy-touch programming**—use convenient preprogrammed transfer methods or program customized transfer methods
- **Flexible**—transfer 1–2 mini-size or 1 midi-size gel using the Power Blotter Cassette; transfer 1–4 mini-size or 1–2 midi-size gels with the Power Blotter Cassette XL
- **Versatile**—compatible with Power Blotter Select Transfer Stacks and Invitrogen™ Pierce™ 1-Step Transfer Buffer for rapid blotting programs or Towbin transfer buffer for conventional semi-dry transfer methods

Both the Power Blotter System and Power Blotter XL System have an easy-to-use color LCD touchscreen interface with preprogrammed transfer methods for different numbers and sizes of gels and protein molecular weight ranges. The easy-touch programming feature allows custom transfer settings to be quickly created, saved, and run. Both systems achieve highly efficient transfer in 7 minutes when used with Power Blotter Select Transfer Stacks or 10 minutes with Power Blotter 1-Step Transfer Buffer. The instruments are also effective for standard 30- to 60-minute semi-dry transfer protocols based on traditional buffers.

Power Blotter System
Transfer 1–2 mini-size gels
or 1 midi-size gel

Power Blotter XL System
Transfer 1–4 mini-size gels
or 2 midi-size gels

Figure 6. Power Blotter System formats. Each system includes the Power Blotter Station and either the Power Blotter Cassette or Power Blotter Cassette XL. The station and cassettes are also available separately.

Specifications	
Mode of transfer	Semi-dry
Gel compatibility	Denaturing or native polyacrylamide gels, either homemade or precast
Running dimension	Horizontal
Capacity	Up to 2 mini or 1 midi with standard system; up to 4 mini and 2 midi with XL system
Gel size	Mini (8 x 8 cm), midi (8 x 13 cm)

Watch how to use the Power Blotter at **thermofisher.com/powerblotter**

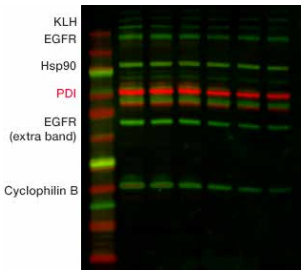
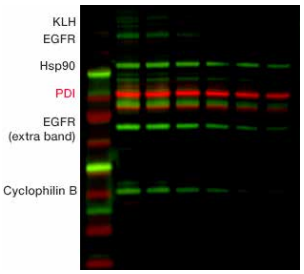
The Power Blotter System offers high transfer efficiency for a broad range of protein sizes		
Apparatus and consumables	Power Blotter System with Power Blotter Select Transfer Stacks	Power Blotter System with Power Blotter Pre-cut Membranes and Filters, and Power Blotter 1-Step Transfer Buffer
Transfer time	7–10 min	7–10 min
Transfer efficiency		

Figure 7. Power Blotter Select Transfer Stacks or Power Blotter Pre-cut Membranes and Filters efficiently transfer high, medium, and low molecular weight proteins. Western blot analysis of several targets (KLH, EGFR, Hsp90, PDI, and cyclophilin B protein) was performed by loading serially diluted HeLa cell lysate with KLH spike (starting at 7.5 µg HeLa cell lysate, 7.5 µg KLH spike per well, serially diluted 2:3) onto Bolt 4–12% Bis-Tris Plus gels. Proteins were transferred in 7 minutes using a Power Blotter Select Transfer Stack (left, Cat. No. PB5310; PB3310) or Power Blotter Pre-cut Membranes and Filters stack (right, Cat. No. PB9320; PB7320), and probed with target-specific primary antibodies and fluorescently conjugated secondary antibodies. Images were captured using automatic exposure on an Invitrogen™ iBright™ Imaging System.

Learn more at **thermofisher.com/powerblotter**

Dry transfer

Dry transfer methods use a transfer sandwich containing innovative components that eliminate use of traditional transfer buffers. A unique gel matrix (transfer stack) that incorporates buffer is used instead of buffer tanks or soaked filter papers (Figure 8). The high ionic density in the gel matrix enables rapid protein transfer. During dry blotting the copper anode does not generate oxygen as a result of water electrolysis, unlike in wet and semi-dry techniques. This absence of oxygen generation reduces blot distortion. Typically, transfer time is reduced by the shortened distance between electrodes, high field strength, and high current. As dry blotting does not require the setup time of wet or semi-dry transfer, not only is the speed of transfer a major benefit, but the overall time investment is improved.

Did you know

The use of impure methanol instead of analytical grade methanol can increase transfer buffer conductivity and result in poor protein transfer.

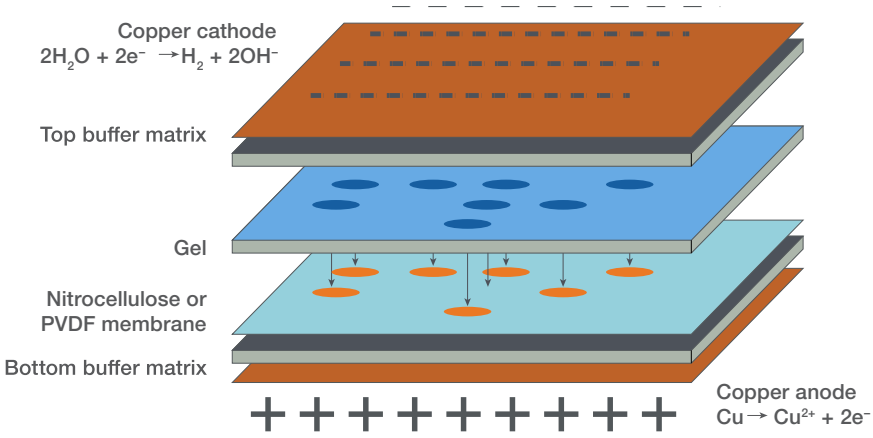


Figure 8. Schematic of Invitrogen™ iBlot™ 2 Transfer Stack for dry western blot transfer.

iBlot 2 Gel Transfer Device

Dry western transfer in 7 minutes, with no transfer buffer needed

The Invitrogen™ iBlot™ 2 Gel Transfer Device is for dry electroblotting of proteins from mini-, midi-, and Invitrogen™ E-PAGE™ gels onto nitrocellulose or PVDF membranes for western detection. The iBlot 2 system offers exceptional transfer efficiency, convenience, and speed, producing crisp and clear bands that remain sharp and straight (Figure 9). Buffer ion reservoirs incorporated into the gel matrix (transfer stacks as opposed to buffer tanks or soaked filter papers) enable rapid protein transfer to either nitrocellulose or PVDF membranes. The shortened distance between electrodes, along with high field strength and current, reduce run times to 7 minutes (Table 2). With the iBlot 2 system, there is no need to prepare buffers, pretreat your gel, presoak filter papers, or clean up after blotting.

Features:

- **Engineered for rapid transfer**—the short distance between electrodes, along with high field strength and current, reduces transfer time to just 7 minutes
- **Minimal preparation and cleanup**—transfer stacks streamline transfer setup and teardown
- **Convenient**—touchscreen interface, preprogrammed and optimized transfer protocols, and prepackaged ready-to-use stacks for transferring midi and mini blot formats (1 midi blot or up to 2 mini blots at a time)

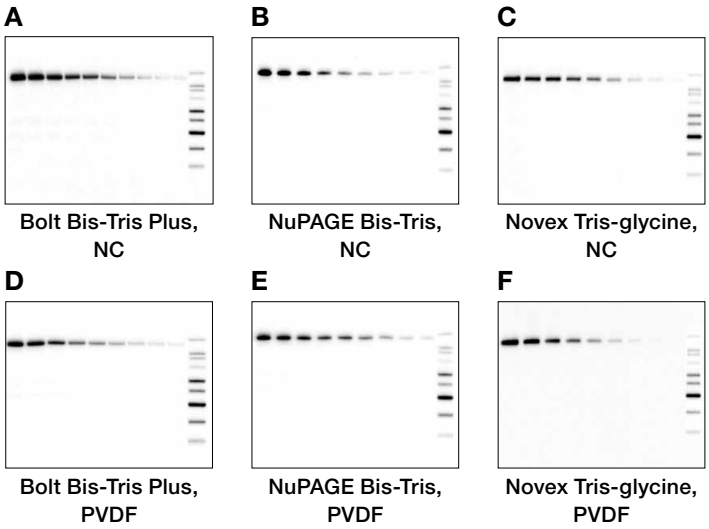


Figure 9. Membranes processed on the iBlot 2 Gel Transfer Device show consistent transfer across various protein gel chemistries to both nitrocellulose (NC) and PVDF membranes. Total cell extracts from A431 cells were transferred to NC (**A–C**) or PVDF (**D–F**) membranes from Invitrogen™ Bolt™ 4–12% Bis-Tris gels, Invitrogen™ NuPAGE™ 4–20% Bis-Tris gels, and Invitrogen™ Novex™ 4–20% Tris-glycine gels.

View how-to video:

Learn more and see the video at **thermofisher.com/iblot2**

Table 2. Comparison of elapsed time for protein transfer with the iBlot 2 Gel Transfer Device to other blotting methods.

	iBlot 2 Gel Transfer Device	Conventional semi-dry transfer	Wet transfer
Buffer preparation	0 min	30 min	30 min
Soaking gel in transfer buffer	0 min	20 min	0 min
Assembling layers	2 min	10 min	10 min
Transfer	7 min	45–90 min	1–3 hr
Cleanup	0 min	10 min	10 min
Total elapsed time	9 min	1 hr, 55 min–2 hr, 40 min	1 hr, 50 min–3 hr, 50 min
Time saved with the iBlot 2 Gel Transfer Device	NA	1 hr, 45 min– 2 hr, 30 min	1 hr, 40 min– 3 hr, 40 min

Specifications	
Mode of transfer	Dry
Gel compatibility	NuPAGE Bis-Tris and Tris-Acetate, Bolt Bis-Tris Plus, Novex Tris-Glycine, and E-PAGE gels
Running dimension	Horizontal
Capacity	Up to 2 mini gels or 1 midi gel
Gel size	Mini (8 x 8 cm), Midi (8 x 13 cm)

Recommended products

We recommend the use of **iBlot 2 Transfer Stacks** with the iBlot 2 Gel Transfer Device. These are available in nitrocellulose or PVDF, and in regular or mini-gel size.

Did you know

Although nitrocellulose membranes were originally used to filter out particles, such as bacteria, they are now used primarily to bind macromolecules in western and Southern blotting. Macromolecules such as proteins are thought to bind to nitrocellulose membranes by hydrophobic interactions.

Pre-transfer considerations

Choice of transfer system

When choosing a transfer methodology, convenience, speed, flexibility, and throughput are important considerations. While the devices the laboratory currently has may be the most convenient, consider how alternative methods may improve steps such as transfer preparation and cleanup. A dry transfer system, with ready-to-use consumables, provides greater convenience, requiring very little setup and cleanup time. Regarding transfer speed, wet transfer systems are typically the slowest, followed by semi-dry transfer (using Towbin buffer), with dry transfer systems being the fastest. However, with the specially designed transfer buffers for semi-dry systems, semi-dry systems can match the speeds offered by dry transfer systems. Wet tank systems come in a variety of capacities and designs and typically offer the greatest flexibility in throughput, but this attribute should be weighed against the transfer speed. One may be able to complete the same number of transfers (or more) using a fast, lower-throughput, semi-dry or dry transfer system compared to using a slower wet tank transfer system.

With respect to transfer efficiency, most modern transfer systems will transfer typical protein molecular weight ranges with high efficiency. Most modern semi-dry systems and dry systems offer preprogrammed optimized methods for particular molecular weight ranges, including high and low molecular weights. Transferring very high or very low molecular weight proteins often requires optimization regardless of the system used. However, because of the flexibility of wet transfer (for example, transfer membranes of different pore sizes can be swapped easily and transfer buffer formulations can be modified), wet tank transfer is a good place to start when transferring very high or low molecular weight proteins. See Table 1 on page 6 for a summary of Invitrogen transfer system characteristics.

Building the western transfer sandwich

After proteins have been separated by electrophoresis, the next step of western blotting is the assembly of the "transfer sandwich". For wet and semi-dry electrotransfer devices, the transfer sandwich typically consists of a filter paper–gel–membrane–filter paper arrangement, where the filter paper aids in wicking of the transfer buffer. For some commercially available wet and semi-dry transfer devices, pre-assembled transfer stacks are available with pre-cut membrane and filter paper, in which the polyacrylamide gel is inserted.

Dry transfer devices do not require filter paper for wicking transfer buffer. Instead a unique gel matrix transfer stack incorporates buffer, eliminating the need for buffer tanks and wetted filter paper.

Choosing the western blot membrane

The most common immobilization membranes for western blotting are nitrocellulose, PVDF, and nylon. The following characteristics make these membranes suitable for protein transfer:

- A large surface area:volume ratio
- A high binding capacity
- Extended storage of immobilized proteins
- Ease of use
- Possible optimization for low background, signal, and reproducibility

Western blot membranes are typically supplied in either sheets or rolls, and commonly have a thickness of 100 µm, with typical pore sizes of 0.1, 0.2, or 0.45 µm. Most proteins can be successfully blotted using a membrane with a pore size of 0.45 µm, while low molecular weight proteins or peptides (<20 kDa) usually require a pore size of 0.1 or 0.2 µm. For some transfer instruments, pre-assembled membrane and blotting paper stacks are available.

Learn more at **thermofisher.com/transfer**

Nitrocellulose membranes

High protein-binding affinity and compatibility with a variety of detection methods make nitrocellulose a popular matrix. Protein immobilization on the membrane is thought to occur by hydrophobic interactions. Use of high salt and low methanol concentrations in transfer conditions improves protein immobilization on the membrane, especially with proteins of higher molecular weights.

We offer a variety of nitrocellulose membranes to support most of your transfer needs. A comparison of the various offerings can be found in Table 3.

Features:

- **High quality**—pure, 100% nitrocellulose membranes with high surface area and excellent uniformity
- **Selection**—available in 0.2 µm and 0.45 µm pore sizes for peptide and protein applications, respectively
- **Convenient**—available in pre-cut sheets, rolls, ready-to-use formats with membrane included, or in specialized device-specific formats
- **High sensitivity**—provides high-affinity protein binding, blocks easily, and exhibits very low background in chemiluminescent western blotting

Table 3. Thermo Scientific™ and Invitrogen™ nitrocellulose membranes.

Membrane size	Pore size			
	0.2 µm		0.45 µm	
	Membrane with filter papers	Membrane only	Membrane with filter papers	Membrane only
Mini	Nitrocellulose/Filter Paper Sandwiches, 0.2 µm, 8.3 x 7.3 cm (Cat. No. LC2000)	Nitrocellulose Membranes, 0.2 µm, 8 x 8 cm (Cat. No. 88024)	Nitrocellulose/Filter Paper Sandwiches, 0.45 µm, 8.3 x 7.3 cm (Cat. No. LC2001)	Nitrocellulose Membranes, 0.45 µm, 8 x 8 cm (Cat. No. 88025)
Midi	Nitrocellulose/Filter Paper Sandwiches, 0.2 µm, 8.5 x 13.5 cm (Cat. No. LC2009) SureLock Tandem Midi Pre-cut Membranes and Filters, 0.2 µm, nitrocellulose, 8.6 x 14 cm (Cat. No. STM2007)	Nitrocellulose Membranes, 0.2 µm, 8 x 12 cm (Cat. No. 77012)	Nitrocellulose/Filter Paper Sandwiches, 0.45 µm, 8.5 x 13.5 cm (Cat. No. LC2006) SureLock Tandem Midi Pre-cut Membranes and Filters, 0.45 µm, nitrocellulose, 8.6 x 14 cm (Cat. No. STM2008)	Nitrocellulose Membranes, 0.45 µm, 8 x 12 cm (Cat. No. 77010)
Roll	NA	NA	NA	Nitrocellulose Membrane, 0.45 µm, 30 cm x 3.5 m (Cat. No. 88018)

Learn more at **thermofisher.com/membranes**

PVDF membranes

PVDF membranes are ideal for western blotting applications as well as for amino acid analysis and sequencing of small amounts of proteins (as little as 10 pmol). PVDF membranes are highly hydrophobic and must be pre-wetted with methanol or ethanol prior to submersion in transfer buffer. PVDF membranes have a high binding affinity for proteins, with binding likely occurring via dipole and hydrophobic interactions, and offer better retention of adsorbed proteins than other supports. PVDF is also less brittle than nitrocellulose and can be stripped and reprobed without a loss of sensitivity or increased background.

We offer a variety of PVDF membranes to support most of your transfer needs. A comparison of the various offerings can be found in Table 4.

Features:

- **High quality**—PVDF transfer membranes manufactured especially for protein transfer and western blot applications; more resistant to discoloration than other commercially available PVDF membranes
- **Durable**—PVDF is compatible with most organic solvents, acids, and mild bases; doesn't tear or become brittle like nitrocellulose
- **Selection**—available in 0.2 µm and 0.45 µm pore sizes; available in pre-cut sheets, rolls, ready-to-use formats with membrane included, or in specialized device-specific formats
- **Versatile**—compatible with chemiluminescent, chromogenic, and fluorescent western blot detection

While our 0.2 µm PVDF membrane performs well for western blotting, amino acid analysis, and protein sequencing applications, our high-quality 0.45 µm PVDF membrane is suited for high-sensitivity and low-background immunoblotting.

Table 4. Thermo Scientific™ and Invitrogen™ PVDF membranes.

Membrane size	Pore size			
	0.2 µm		0.45 µm	
	Membrane with filter papers	Membrane only	Membrane with filter papers	Membrane only
Mini	PVDF/Filter Paper Sandwiches, 0.2 µm, 8.3 x 7.3 cm (Cat. No. LC2002)	Low-Fluorescence PVDF Transfer Membranes, 0.2 µm, 7 x 8.4 cm (Cat. No. 22860)	Invitrolon™ PVDF/Filter Paper Sandwiches, 0.45 µm, 8.3 x 7.3 cm (Cat. No. LC2005)	PVDF Transfer Membranes, 0.45 µm, 10 x 10 cm (Cat. No. 88585)
Midi	NA	NA	Invitrolon™ PVDF/Filter Paper Sandwiches, 0.45 µm, 8.5 x 13.5 cm (Cat. No. LC2007) SureLock Tandem Midi Pre-cut Membranes and Filters, 0.45 µm, PVDF, 8.6 x 14 cm (Cat. No. STM2006)	NA
Roll	NA	PVDF Transfer Membrane, 0.2 µm, 26.5 cm x 3.75 m (Cat. No. 88520)	NA	PVDF Transfer Membrane, 0.45 µm, 26.5 cm x 3.75 m (Cat. No. 88518)

Table 5. Transfer stacks for the iBlot Gel Transfer Device.

Membrane size	Mini size (8 x 8 cm)	Regular size (13 x 8.3 cm)
Nitrocellulose	iBlot 2 Transfer Stacks, nitrocellulose, mini (Cat. No. IB23002)	iBlot 2 Transfer Stacks, nitrocellulose, regular size (Cat. No. IB23001)
PVDF	iBlot 2 Transfer Stacks, PVDF, mini (Cat. No. IB24002)	iBlot 2 Transfer Stacks, PVDF, regular size (Cat. No. IB24001)

Table 6. Transfer stacks and pre-cut membranes with filters for the Power Blotter System.

Membrane size	Mini		Regular (2 mini or 1 midi)	
	Pre-assembled stacks	Membrane with filter papers	Pre-assembled stacks	Membrane with filter papers
Nitrocellulose	Power Blotter Select Transfer Stacks, nitrocellulose, mini (Cat. No. PB3210)	Power Blotter Pre-cut Membranes and Filters, nitrocellulose, mini (Cat. No. PB7220)	Power Blotter Select Transfer Stacks, nitrocellulose, regular size (Cat. No. PB3310)	Power Blotter Pre-cut Membranes and Filters, nitrocellulose, regular size (Cat. No. PB7320)
PVDF	Power Blotter Select Transfer Stacks, PVDF, mini (Cat. No. PB5210)	Power Blotter Pre-cut Membranes and Filters, PVDF, mini (Cat. No. PB9220)	Power Blotter Select Transfer Stacks, PVDF, regular size (Cat. No. PB5310)	Power Blotter Pre-cut Membranes and Filters, PVDF, regular size (Cat. No. PB9320)

Membranes for western transfer devices

Nitrocellulose and PVDF membranes are available as pre-assembled stacks for the Invitrogen™ iBlot™ Gel Transfer Device and Invitrogen™ Power Blotter System (Tables 5 and 6). The Power Blotter System is also compatible with pre-cut membranes with filter papers (Table 6).

Low-fluorescence membranes for fluorescent western blotting

To eliminate a major source of background fluorescence, we recommend using membranes with low autofluorescence, including nitrocellulose and specialty low-fluorescence PVDF membranes (Table 7 and Figure 10).

All Invitrogen™ iBlot™ 2 and Power Blotter PVDF pre-assembled stacks are equipped with low-fluorescence PVDF membranes for use in fluorescent western blot applications.

Table 7. Thermo Scientific™ and Invitrogen™ nitrocellulose and PVDF membranes for fluorescent western blotting applications.

	Mini	Midi	Roll
Nitrocellulose	Nitrocellulose/Filter Paper Sandwiches, 0.2 µm, 8.3 x 7.3 cm (Cat. No. LC2000)	Nitrocellulose/Filter Paper Sandwiches, 0.2 µm, 8.5 x 13.5 cm (Cat. No. LC2009)	Nitrocellulose Membrane, 0.45 µm, 30 cm x 3.5 m (Cat. No. 88018)
	Nitrocellulose/Filter Paper Sandwiches, 0.45 µm, 8.3 x 7.3 cm (Cat. No. LC2001)	Nitrocellulose/Filter Paper Sandwiches, 0.45 µm, 8.5 x 13.5 cm (Cat. No. LC2006)	NA
Low-fluorescence PVDF	Low-Fluorescence PVDF Transfer Membrane, 0.2 µm, 7 x 8.4 cm (Cat. No. 22860)	NA	NA

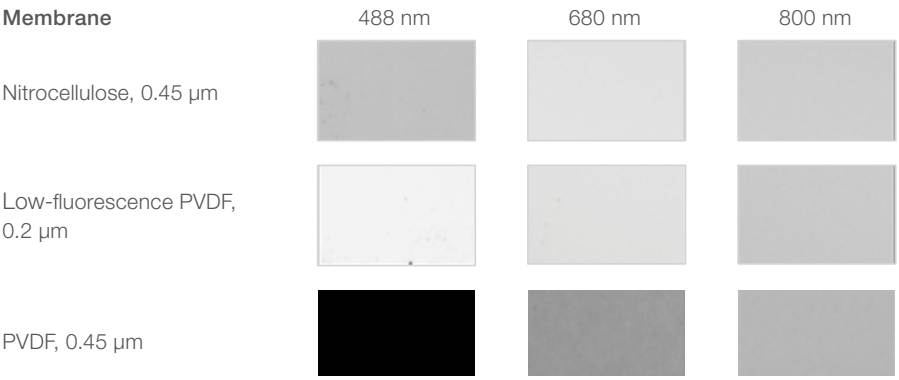


Figure 10. Background fluorescence of common membranes.

Learn more at **thermofisher.com/membranes**

Blotting paper

Blotting (or filter) paper is an essential component for the transfer sandwich in wet and semi-dry transfer methods. The filter paper is first wetted in transfer buffer before building the transfer sandwich. The paper serves to aid the wicking of transfer buffer through the gel, helping the proteins move out of the gel onto the membrane. Dry electrotransfer conditions do not use filter paper.

Filter paper should be made of high-quality materials so that it doesn't contribute to possible background issues during the western blotting detection step. The paper thickness may also be of concern with some transfer systems. For example, in a semi-dry transfer setup, the thickness of the filter paper can affect how much buffer reservoir is available for transfer. A thicker filter paper can typically hold more transfer buffer. When using a transfer device, it is important to follow the vendor's recommended filter paper thickness.

Pierce Western Blotting Filter Papers

Thermo Scientific™ Pierce™ Western Blotting Filter Papers are pre-cut cotton sheets to support wet or semi-dry electrophoretic transfer of proteins from polyacrylamide gels (SDS-PAGE) to PVDF, nitrocellulose, or other membranes (Table 8). Pierce Western Blotting Filter Papers are suitable for use with alcohol or other organic solvents commonly used in protein and nucleic acid blotting applications.

Features:

- **High quality**—clean cotton cellulose fiber paper manufactured with additive-free ultrapure water to minimize sources of background signal and artifacts
- **Easy to use**—pre-cut filter paper sheets in several convenient sizes for use with most mini gel sizes, tank transfer cassettes, and semi-dry blotters
- **Verified**—tested for use with various protein methods, including wet and semi-dry transfer
- **Two thicknesses**—standard-thickness filter paper for traditional procedures; extra-thick filter paper for high-capacity blotting or as a replacement for multiple sheets

Table 8. Pre-cut filter papers.

Thickness	Mini size	Midi size	Special sizes
0.83 mm	Western Blotting Filter Paper, 7 cm x 8.4 cm (Cat. No. 84783)	Western Blotting Filter Paper, 8 cm x 13.5 cm (Cat. No. 84784)	Western Blotting Filter Paper, 8 cm x 10.5 cm (Cat. No. 88600)
2.5 mm	Western Blotting Filter Paper, Extra Thick, 7 cm x 8.4 cm (Cat. No. 88605) Blotting Filter Papers, 2.5 mm thick, 7.5 cm x 8.4 cm (Cat. No. LC2010)	Western Blotting Filter Paper, Extra Thick, 8 cm x 13.5 cm (Cat. No. 88615) Blotting Filter Papers, 2.5 mm thick, 8.6 cm x 13.5 cm (Cat. No. LC2008)	Western Blotting Filter Paper, Extra Thick, 8.5 cm x 9 cm (Cat. No. 88610) Western Blotting Filter Paper, Extra Thick, 20 cm x 20 cm (Cat. No. 88620)

Did you know

The addition of 0.05% SDS to the transfer buffer helps with transfer of large molecular weight proteins.

Learn more at **thermofisher.com/filterpaper**

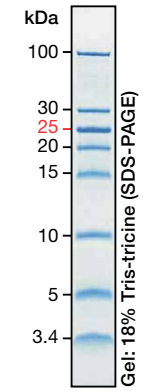
Protein ladder considerations

Typically, protein ladders or standards are included during gel electrophoresis for protein molecular weight estimation (sizing) after separation (if not performing western blotting) or after the detection step (when performing western blotting). Protein ladders can also be used to monitor transfer efficiencies, although only qualitatively. In this use, prestained ladders are the recommended choice (Table 9) because they are visible on the membrane post-transfer. Special-purpose protein ladders designed specifically for western blots (Table 9) enable easy and convenient protein molecular weight estimation directly on western blots or indirectly by using various blotting detection systems (e.g., chemiluminescent, fluorescent, or chromogenic). The protein markers consist of recombinant proteins with an IgG binding site. These IgG binding sites bind the primary or secondary antibody used for detection of the target protein, allowing visualization of the standard on the western blot.

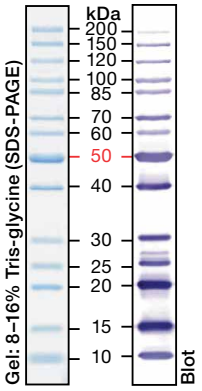
Use our **interactive protein ladder selection tool** to find the most appropriate ladder for your application and gel system.

Table 9. Protein ladder selection guide.

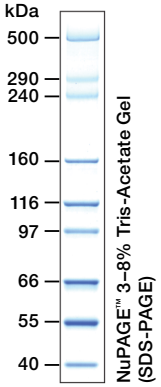
	MW range	Product	No. of proteins	Range
Unstained	Low	PageRuler Unstained Low Range Protein Ladder	8	3.4–100 kDa
	Broad	PageRuler Unstained Protein Ladder	14	10–200 kDa
	High	HiMark Unstained Protein Standard	9	40–500 kDa
Prestained	Low	PageRuler Prestained Protein Ladder	10	10–170 kDa
	Broad	PageRuler Plus Prestained Protein Ladder	9	10–250 kDa
	High	HiMark Pre-stained Protein Standard	9	30–460 kDa
Multicolor prestained	Low	Spectra Multicolor Low Range Protein Ladder	6	1.7–40 kDa
	Broad	Spectra Multicolor Broad Range Protein Ladder	10	10–260 kDa
	High	Spectra Multicolor High Range Protein Ladder	8	40–300 kDa
Western	Broad	iBright Prestained Protein Ladder	10	11-250 kDa
		MagicMark XP Protein Standard	9	20–220 kDa
Specialty	Broad	PageRuler Prestained NIR Protein Ladder	10	11–250 kDa
		BenchMark Fluorescent Protein Standard	7	11–155 kDa
		IEF Marker 3–10	13	pI 3–10



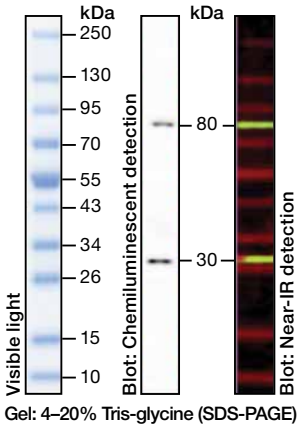
Thermo Scientific™ PageRuler™ Unstained Low Range Protein Ladder
Cat. No. 26632



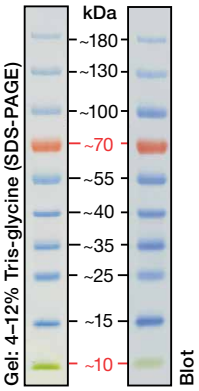
Thermo Scientific™ PageRuler™ Unstained Protein Ladder
Cat. No. 26614



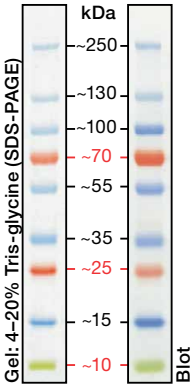
Invitrogen™ HiMark™ Unstained Protein Standard
Cat. No. LC5688



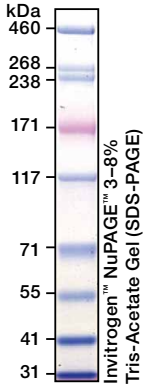
Invitrogen™ iBright™ Prestained Protein Ladder
Cat. No. LC5615



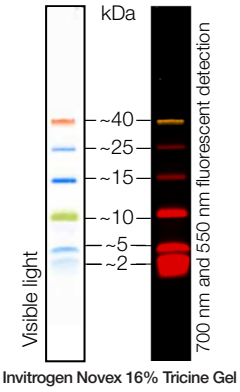
Thermo Scientific™ PageRuler™ Prestained Protein Ladder
Cat. No. 26616



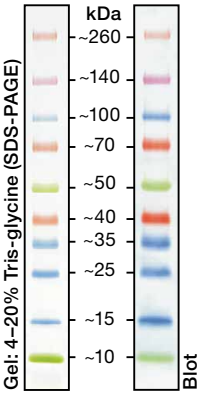
Thermo Scientific™ PageRuler™ Plus Prestained Protein Ladder
Cat. No. 26619



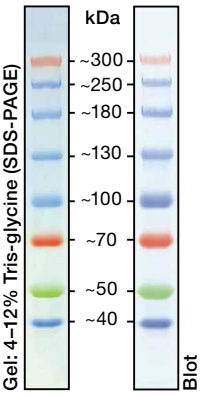
Invitrogen™ HiMark™ Pre-stained Protein Standard
Cat. No. LC5699



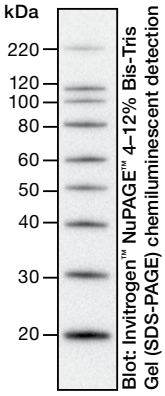
Thermo Scientific™ Spectra™ Multicolor Low Range Protein Ladder
Cat. No. 26628



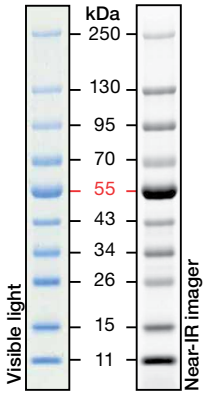
Thermo Scientific™ Spectra™ Multicolor Broad Range Protein Ladder
Cat. No. 26634



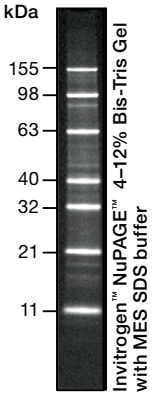
Thermo Scientific™ Spectra™ Multicolor High Range Protein Ladder
Cat. No. 26625



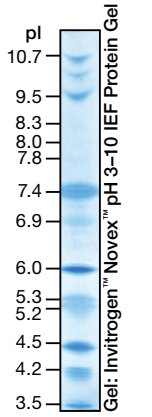
Invitrogen™ MagicMark™ XP Western Protein Standard
Cat. No. LC5602



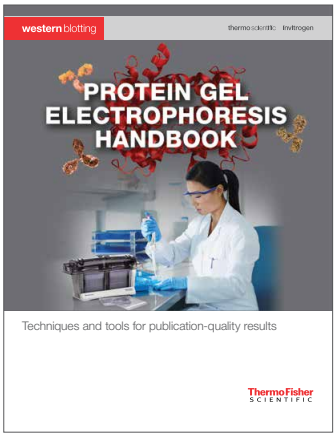
Thermo Scientific™ PageRuler™ Prestained NIR Protein Ladder
Cat. No. 26635
4–20% Tris-glycine (SDS-PAGE)



Invitrogen™ BenchMark™ Fluorescent Protein Standard
Cat. No. LC5928



Invitrogen™ IEF Marker 3–10
Cat. No. 39212-01



For additional information on protein ladders, refer to the protein gel electrophoresis technical handbook at **thermofisher.com/pagehandbook** or **thermofisher.com/proteinladders**

Transfer buffers

Common buffers used for western blotting are the Towbin buffer system (25 mM Tris-HCl, pH 8.3, 192 mM glycine, 20% (v/v) methanol) [1] and the CAPS buffer system (10 mM CAPS, pH 10.5, 10% (v/v) methanol). However, the final choice of transfer buffer may depend on the transfer device and will be noted in the device instruction manual.

In most experiments, SDS should be omitted from the western transfer buffer because the negative charge imparted to proteins can cause them to pass through the membrane as opposed to binding the membrane (also known as blow-through). Typically, residual SDS associated with the proteins in SDS-PAGE gels is sufficient to effectively carry them out of the gel and onto the membrane support. For proteins that tend to precipitate, the addition of low concentrations of SDS (<0.01%) may be necessary. It should be noted that adding SDS to the transfer buffer may require optimization of other transfer parameters (e.g., time, current) to prevent proteins from blowing through the membrane.

Methanol in the transfer buffer aids in stripping off SDS from proteins in SDS-PAGE gels, thus increasing their ability to bind to support membranes. However, methanol can inactivate enzymes required for downstream analyses and can shrink the gel and membrane, which may increase the transfer time of large molecular weight proteins (>150 kDa) with poor solubility in methanol. In the absence of methanol, though, protein gels may swell in low ionic strength buffers, and therefore it is recommended to pre-swell gels for 30 minutes to one hour to prevent band distortion.

To increase speed of transfer with semi-dry methods, high ionic strength buffers are the choice. These buffers, when combined with a suitable constant high-current power source (1.5 to 5.0 A), will decrease protein transfer times to under 10 minutes.

We offer several ready-to-use buffers for standard wet, semi-dry, and fast semi-dry blotting systems (Table 10).

Table 10. Transfer buffer selection guide.

Wet transfer		
Protein sample	**Gel chemistry**	**Transfer buffer**
Broad-range MW (6–400 kDa)	Bolt Bis-Tris Plus	**Bolt Transfer Buffer** Reduced samples: **Bolt Antioxidant**
Broad-range MW (6–400 kDa)	NuPAGE Bis-Tris	**NuPAGE Transfer Buffer** Reduced samples: **NuPAGE Antioxidant**
High MW (40–500 kDa)	NuPAGE Tris-Acetate	
Broad-range MW (6–400 kDa)	Novex Tris-Glycine	**Novex Tris-Glycine Transfer Buffer**
Low-range MW (2.5–40 kDa)	Novex Tricine	
Semi-dry transfer		
Power Blotter 1-Step Transfer Buffer (5X)		

Did you know

The addition of up to 20% methanol or ethanol to a transfer buffer can improve small molecular weight protein transfer.

Learn more at **thermofisher.com/transferbuffer**

Bolt Transfer Buffer (20X)

Invitrogen™ Bolt™ Transfer Buffer (20X) is optimized for the transfer of proteins from Invitrogen™ Bolt™ Bis-Tris Plus gels to membranes for western blotting. When combined with 10% methanol, Bolt Transfer Buffer can be used with the Invitrogen™ Mini Blot Module or XCell™ II Blot Module for wet transfer. It can also be used with Invitrogen™ Bolt™ Antioxidant to enhance transfer of reduced proteins to membranes.

NuPAGE Transfer Buffer (20X)

Invitrogen™ NuPAGE™ Transfer Buffer (20X) is the buffer choice for transfer of proteins from Invitrogen™ NuPAGE™ Bis-Tris and Tris-Acetate gels to membranes for western blotting. NuPAGE Transfer Buffer can be used with the Mini Blot Module, XCell II Blot Module, or SureLock Tandem Midi Blot Module for wet transfer. It can also be used with Invitrogen™ NuPAGE™ Antioxidant to enhance transfer of reduced proteins to membranes.

BupH Tris-Glycine Buffer Packs

Each Thermo Scientific™ BupH™ Tris-Glycine Buffer Pack yields 500 mL of 25 mM Tris, 192 mM glycine at a pH of approximately 8 when dissolved in 400 mL deionized water and 100 mL of methanol.

Pierce 10X Tris-Glycine Buffer

Thermo Scientific™ Pierce™ 10X Tris-Glycine Buffer is a space-saving stock solution that is ideal for quickly preparing standard Tris-glycine (pH 8.5) transfer buffer used for western blotting. Simply dilute with deionized water or 20% methanol.

Novex Tris-Glycine Transfer Buffer (25X)

Invitrogen™ Novex™ Tris-Glycine Transfer Buffer (25X) is optimized for western blot transfer applications using Tris-glycine gels. The buffers are made with high-purity reagents and are strictly quality controlled. The concentrated buffer requires a simple dilution with deionized water before use.

Pierce 10X Western Blot Transfer Buffer, Methanol-free

Thermo Scientific™ Pierce™ 10X Western Blot Transfer Buffer, Methanol-free does not require cooling or the addition of methanol. Simply dilute the 10X solution with water and use directly in tank or conventional semi-dry transfer.

Power Blotter 1-Step Transfer Buffer

Invitrogen™ Power Blotter 1-Step™ Transfer Buffer is designed for rapid semi-dry transfer of proteins from polyacrylamide gels (SDS-PAGE) to nitrocellulose or PVDF membranes using the Power Blotter System. The high–ionic strength buffer coupled with a high-current power supply allows for protein transfers in 7 to 10 minutes when used with a compatible system, such as the Power Blotter System. Transfer efficiencies are similar to conventional western transfer techniques.

Did you know

Harry Towbin's group in Basel, Switzerland, was the first to describe electroblotting of proteins to membranes and the use of secondary antibodies. The method he described in 1979 is the closest technique to what we know as modern-day western blotting.

Post-transfer

Once the protein transfer process is completed, the membrane is removed from the transfer sandwich and is ready for any post-transfer membrane treatment. Before committing valuable time and detection reagents to western blot processing and detection, it is prudent to assess the efficiency of the transfer step. Signal enhancers may be used at this stage, before the membrane is incubated with blocking buffer, if there is a need for increased sensitivity.

Monitoring transfer efficiency

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, contact of the gel with the membrane, the position of the electrodes, transfer time, field strength, and the presence of detergents, as well as the size and composition of the protein of interest.

Some researchers may use prestained protein ladders to assess transfer efficiency. Others may stain the gel to confirm that proteins have migrated out of the gel. However, these methods are unreliable because they do not necessarily reveal how effectively proteins have transferred to the membrane. A better method to monitor transfer efficiency relies on staining the membrane for total protein with a dye such as Ponceau S or amido black 10B. Because dyes may interfere with antibody binding and detection, a protein stain that is easily removable is ideal. Ponceau S stain is the most widely used reagent for reversibly staining proteins on a membrane, although it has limited sensitivity, does not photograph well, and fades quickly, making documentation difficult. Superior alternatives for staining protein on nitrocellulose or PVDF membranes are available that are easily photographed and do not fade until removed. These dyes also allow the detection of low-nanogram levels of protein on membranes. No-Stain Protein Labeling Reagent (page 84) can also be used for total protein labeling after gel transfer. It labels proteins covalently and can be used to normalize for protein sample loading variations when performing quantitative western blotting experiments.

Pierce Reversible Protein Stain Kits for Nitrocellulose or PVDF Membranes

A great alternative to Ponceau S

Thermo Scientific™ Pierce™ Reversible Protein Stain Kits for either nitrocellulose or PVDF membranes provide a rapid and sensitive alternative to Ponceau S stain for protein detection on nitrocellulose or PVDF membranes after transfer from polyacrylamide gels (Figures 11 and 12). The lower limit of detection with this method is 25–50 ng per band (at least 5x more sensitive than traditional Ponceau S staining). The simple and quick staining protocol results in turquoise-blue bands that do not fade and are easily photographed for future reference. The stain can be easily reversed in less than 15 minutes. Subsequent western blot detection is unaffected because the stain does not alter the protein and is completely removed. The treated membrane does not interfere with conventional chemiluminescent or chromogenic detection using horseradish peroxidase (HRP) and alkaline phosphatase (AP) substrates. In addition, the stain is compatible with N-terminal sequence analysis of proteins excised and eluted from the membrane.

Features:

- **Better than Ponceau S**—more sensitive, easier to document, permanent until reversed (Table 11)
- **Sensitive**—high-avidity, total-protein stain; lower limit of detection equals 25 to 50 ng per band
- **Specific**—detects only protein; does not bind or interact with other electrophoresis or sample components
- **Rapid**—stains in less than 5 minutes (nitrocellulose membranes) or in less than 30 minutes (PVDF membranes); erase and reverse staining in less than 15 minutes
- **Stable**—components are stored at room temperature to help save refrigerator space and eliminate equilibration steps

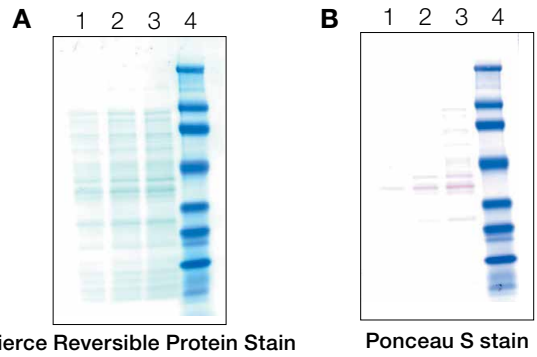


Figure 11. Pierce Reversible Protein Stain and Ponceau S stain: a comparison of staining of bacterial lysate with overexpressed GST protein, on nitrocellulose. Increasing amounts of lysate were separated on two 4–20% Tris-glycine polyacrylamide gels and transferred to nitrocellulose membranes. **(A)** One blot was treated with Pierce Reversible Protein Stain for 30 seconds and destained according to the protocol. **(B)** Another blot was stained with 0.1% Ponceau S stain for 5 minutes and destained. The blot stained with Pierce Reversible Protein Stain demonstrates superior visual detection of bands. GST lysate loading volumes: lane 1: 5 µL, lane 2: 10 µL, lane 3: 15 µL. Lane 4: 10 µL MW marker.

Learn more at **thermofisher.com/proteinstains**

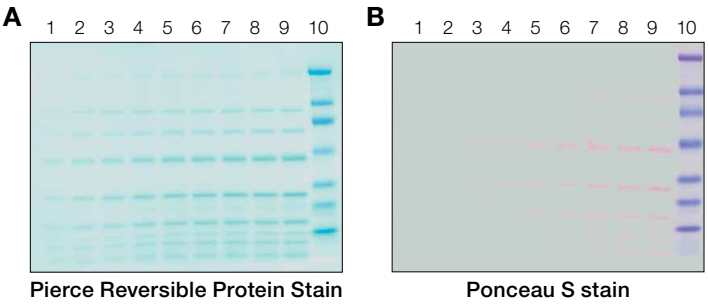


Figure 12. Comparison of Pierce Reversible Protein Stain with Ponceau S stain on PVDF membrane. Unstained protein MW markers were serially diluted (lanes 1–9), applied to two 4–20% Tris-glycine SDS-polyacrylamide gels, and electroblotted to PVDF membranes. **(A)** Blot was stained with Pierce Reversible Protein Stain for 1 minute and destained according to the protocol. **(B)** Blot was stained with 0.1% Ponceau S stain in 5% acetic acid for 5 minutes and destained according to the published protocol. Lane 10: MW marker.

Table 11. Comparison of Pierce Reversible Protein Stain with Ponceau S stain.

Pierce Reversible Protein Stain	Ponceau S stain
• Tightly binding, higher-sensitivity general protein stain	• Weakly binding, low-sensitivity general protein stain
• Detection limit: 25–50 ng	• Detection limit: 250 ng
• Turquoise blue bands are photographed easily	• Red bands are difficult to photograph
• Turquoise bands do not fade over time, but they can be reversed	• Stained protein bands fade within hours
• Typical staining time: 60 seconds	• Typical staining time: 5 minutes
• Background eliminated quickly with low-pH wash	• No background elimination step

Did you know

Towbin buffer as described by Harry Towbin in 1979 is still the most widely used wet transfer buffer for western blotting.

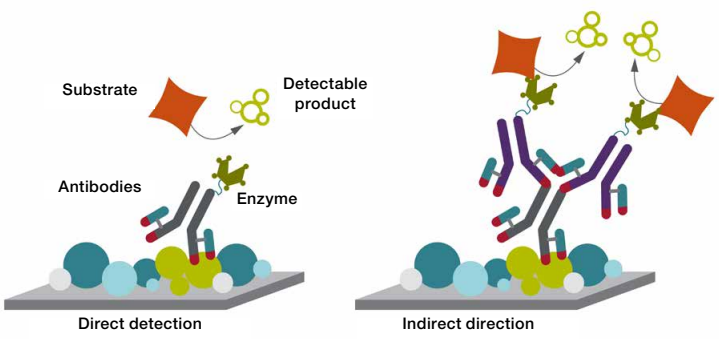
Detect

Introduction to western blot detection considerations

Western blots detect specific protein from cells or tissues in a convenient, flexible format for rapid evaluation. The western blot format can also be quantitative and offer a high degree of sensitivity. With a variety of detection techniques, including chemiluminescent, fluorescent, or chromogenic to choose from, you can select a technology to match your experimental requirements and the instruments you have available. We discuss below a few key factors to consider before performing western blotting.

Signal-to-noise ratio

Signal-to-noise ratio compares the level of desired or relevant signal to the level of background noise or irrelevant signal; the higher the ratio, the better the result. In western blotting, the signal is the density of the specific probed protein band of interest; the noise is the density of the background. In western blotting applications, optimization of the signal-to-noise ratio is often more important than increasing the sensitivity of the system. The sensitivity of the system is irrelevant if the signal cannot be adequately distinguished from the noise. For information on western blot optimization methods, see page 80.



Advantages:

- Quicker since only one antibody is used
- No concern over cross-reactivity of a secondary antibody

Disadvantages:

- Labeling may reduce immunoreactivity of primary antibody
- Potentially high background if the primary antibody is not highly specific for the target protein or if the antibody cross-reacts with the blocking protein
- Labeled primary antibodies are expensive
- Low flexibility in choice of primary antibody label
- Little signal amplification

Advantages:

- Secondary antibodies can amplify signal
- A variety of labeled secondary antibodies are available
- One secondary antibody may be used with many primary antibodies
- Use of a labeled secondary antibody does not affect primary antibody immunoreactivity
- Changing the secondary antibody allows a change of detection method

Disadvantages:

- Secondary antibodies may produce nonspecific signals due to cross-reactivity
- Additional steps required compared to the direct method

Figure 13. Comparison of direct and indirect western blot detection methods.

Direct vs. indirect detection

The antibody that recognizes a target protein is called the primary antibody. If this antibody is labeled with a tag for visualization purposes (typically an enzyme or fluorophore), direct detection of the target is possible. Typically, the primary antibody is not labeled for direct detection. Instead a secondary antibody that has been labeled with a detectable tag is used to probe for the primary antibody, which is bound to the target. Thus, the target is detected indirectly. Indirect detection with secondary antibodies requires more steps than direct detection, but it can also offer significant advantages over using primary antibodies that are directly labeled (Figure 13).

Indirect methods can offer increased sensitivity through the signal amplification that occurs as multiple secondary antibody molecules bind to a single primary antibody. In addition, a given secondary antibody will recognize most primary antibodies of the same isotype and target species, making it a more versatile reagent than individually labeled primary antibodies.

Several variants of these probing and detection strategies exist. However, each variant depends on a specific probe (e.g., a primary antibody) whose presence is linked directly or indirectly to some sort of measurable tag. In this handbook, most methods discussed use indirect detection, as this has emerged as the most popular detection strategy.

Manual vs. automated western blot processing

Traditionally, probing a western blot prior to data visualization involved a series of manual steps, many of which were individually short but collectively required significant hands-on time. Today, instruments are available to automate some of these tasks, tremendously decreasing hands-on time.

Manual and automated procedures share three essential steps: blocking the membrane, probing with primary and secondary antibodies, and washing the membrane (Figure 14).

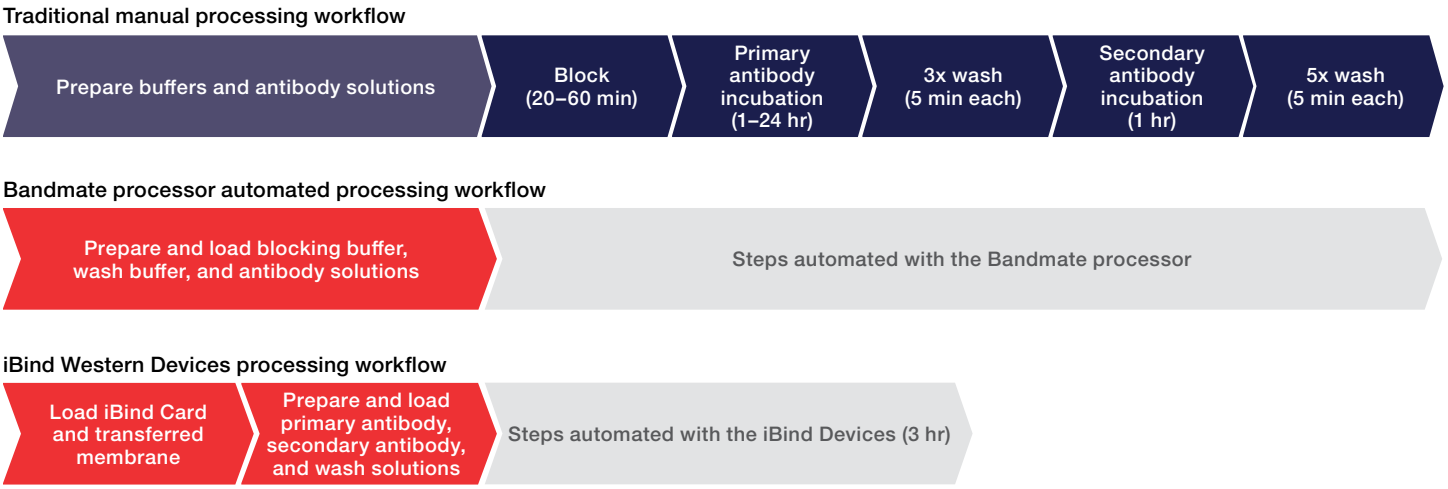


Figure 14. Comparison of traditional manual vs. automated western blot processing workflows. The traditional manual western blot processing workflow requires more than 10 hands-on steps and at least 4 hours before final detection can occur. Automated western blot processing using the Invitrogen™ Bandmate™ Automated Western Blot Processor or Invitrogen™ iBind™ or iBind™ Flex Western Device requires only a 15–30 minute setup time and no additional hands-on steps before you can move to final detection.

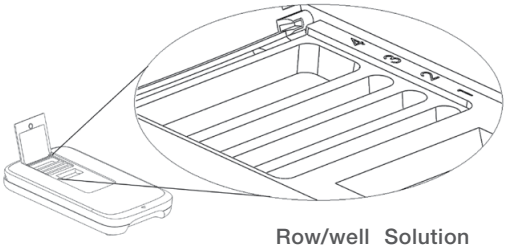
iBind western systems

The iBind western systems are nonpowered devices that automate immunodetection steps. The traditional manual process involves preparing and replacing multiple antibody and wash solutions over several hours in a tray containing the blot of interest. By contrast, iBind western systems allow all solutions to be prepared and loaded in the device at the start of the procedure (Figure 15). Subsequent steps proceed automatically and uninterrupted by sequential lateral flow (SLF) technology, i.e., simple capillary action—no electricity or batteries are required.

Two iBind western systems are available: the original Invitrogen™ iBind™ Western Device, which accommodates one mini blot at a time, and the Invitrogen™ iBind™ Flex Western Device, which accommodates up to one midi blot, two mini blots, or up to six vertically cut strips at a time. Table 12 compares the two systems.

Features:

- **Flexibility**—pick the system that matches your throughput; process 1 mini or midi blot, 2 mini blots, or 6 vertically cut strips using the same or different conditions
- **Antibody savings**—use up to 80% less primary antibody
- **Load and go**—the system processes solutions using SLF technology, with no batteries, shakers, trays, or timers required
- **Reproducibility**—automated blot processing enables improved blot-to-blot consistency
- **Sensitivity**—iBind systems offer greater sensitivity than manual methods for many monoclonal and polyclonal antibodies (Figure 16)



Row/well	Solution
1	Primary antibody
2	1X iBind/iBind FD Solution
3	Secondary antibody
4	1X iBind/iBind FD Solution

Figure 15. Sample loading wells for the iBind western system.

Table 12. Characteristics of iBind western systems.

	iBind Western Device	iBind Flex Western Device
		
Mini blot (8 x 8 cm), single	Yes	Yes
Mini blot (8 x 8 cm), dual	No	Yes
Midi blot (13 x 8 cm)	No	Yes
Vertically cut strips (up to 6)	No	Yes

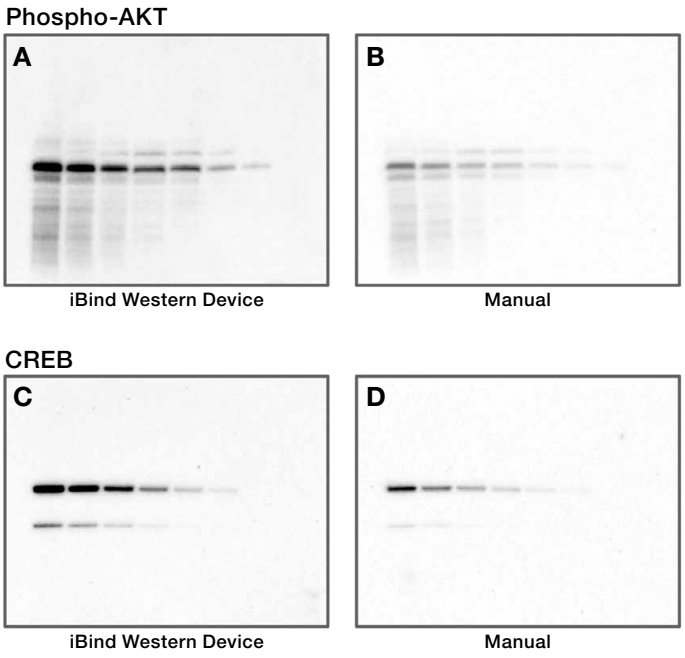


Figure 16. Western blots processed on the iBind device show superior sensitivity compared to western blots processed manually. (A and B) Western blots with phosphorylated AKT (left to right: 30 µg–500 ng cell lysate load) were processed either on the iBind device or using standard manual western processing protocols as specified by the antibody manufacturer (monoclonal phospho-AKT [pT308] (C31E5E) primary antibody; HRP-conjugated anti-rabbit secondary antibody). The blot processed with the iBind device detected phospho-AKT in 500 ng of cell lysate, while the target was detected in 4 µg on the manually processed blot. **(C and D)** Western blots with cell lysate expressing CREB (left to right: 30 µg–1 µg cell lysate load) were processed either on the iBind device or using standard manual western processing protocols as specified by the antibody manufacturer (polyclonal CREB primary antibody; HRP-conjugated anti-rabbit secondary antibody). The blot processed with the iBind device detected CREB in 6 µg of cell lysate, while 10 µg of lysate was needed to detect CREB on the manually processed blot. For all blots, proteins were separated using Bolt Bis-Tris Plus mini gels and transferred to PVDF membranes using the iBlot dry blotting system.

Watch how to use the iBind devices at **thermofisher.com/ibind**

Bandmate Automated Western Blot Processor

The Invitrogen™ Bandmate™ Automated Western Blot Processor is a programmable blot rocking system that automates the tedious hands-on blocking, washing, and antibody incubation steps of western blot processing. Minimal effort is required to set up the Bandmate device to process up to 2 midi blots or 4 mini blots using your current optimized reagents and protocols for blot processing, freeing up time for other important tasks.

Features:

- **Load-and-go setup**—prepare block, wash, and antibody solutions, load into the machine, select a program, and walk away
- **Works with traditional western blotting protocols**—no need to adapt from current protocols and no specialized reagents required (Figure 17); program the timing of steps based on preference or utilize preprogrammed options
- **Antibody recovery**—collection tubes can recover antibody for reuse in future experiments if desired

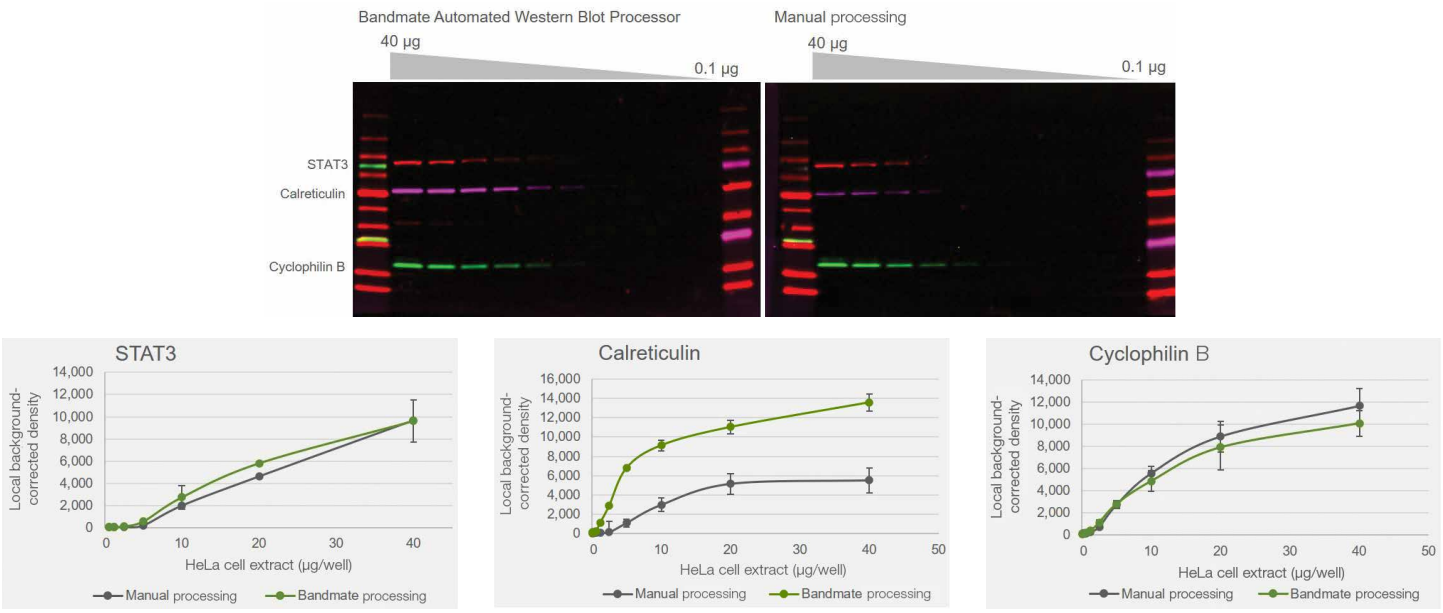


Figure 17. Comparison of mini blots processed with the Bandmate Automated Western Blot Processor vs. manual processing (probing and washing steps performed in a tray and on a shaker platform). The blots processed with the Bandmate device show comparable or higher intensity levels across multiple probed targets compared to the blots processed with a manual processing procedure.

Learn more and see the video at **thermofisher.com/bandmate**

Blocking the membrane

The membrane supports used in western blotting have a high affinity for proteins. In order to prevent nonspecific binding of detection antibodies during the steps after transfer, it is imperative to block the remaining binding sites on the membrane surface.

A variety of blocking buffers containing milk, normal serum, or highly purified proteins have been used to block free sites on a membrane. By blocking these free sites, the signal-to-noise ratio of the assay should improve through a reduction in background interference. Inadequate amounts of blocker will result in excessive background noise and a reduced signal-to-noise ratio, whereas excessive concentrations of blocker may mask antibody–target protein interactions or inhibit the marker enzyme, again causing a reduction of the signal-to-noise ratio. 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 (Figures 18 and 19).

For example, with applications using an alkaline phosphatase conjugate, a blocking buffer in Tris-buffered saline (TBS) should be selected because phosphate-buffered saline (PBS) interferes with alkaline phosphatase activity. Likewise, the use of milk as a blocking reagent should be avoided when using an avidin–biotin detection system. Empirically testing various blocking buffers with your system can help achieve the best possible results.

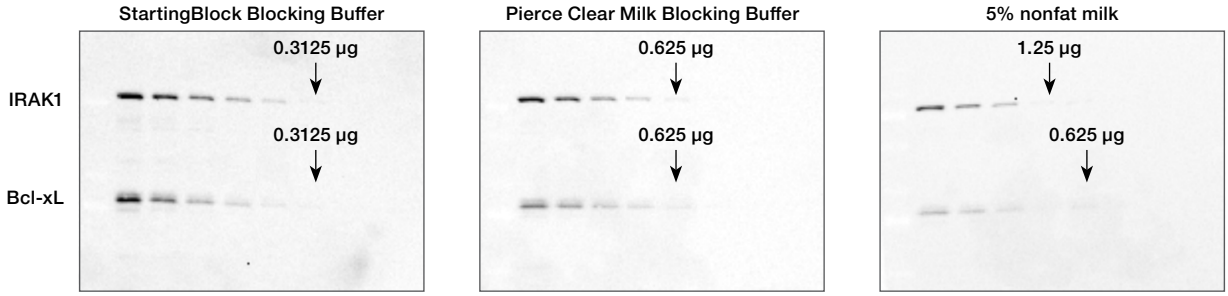


Figure 18. Comparison of Thermo Scientific™ StartingBlock™ Blocking Buffer, Pierce™ Clear Milk Blocking Buffer, and 5% nonfat milk blocking buffer. Serial dilutions of HeLa lysate were prepared, separated by SDS-PAGE, and transferred to membranes for detection of IRAK1 and Bcl-xL. The membranes were blocked with respective blocking buffers per product instructions. The proteins were probed with Invitrogen™ primary antibodies against IRAK1 (Cat. No. 4359S) and Bcl-xL (Cat. No. 2764S), followed by an Invitrogen™ HRP-conjugated goat anti–rabbit IgG secondary antibody (Cat. No. 32460) diluted per manufacturer's instructions. Blots were then incubated with SuperSignal West Atto Ultimate Sensitivity Substrate (Cat. No. A38558). Images were captured at 12 seconds using an Invitrogen™ iBright™ imager.

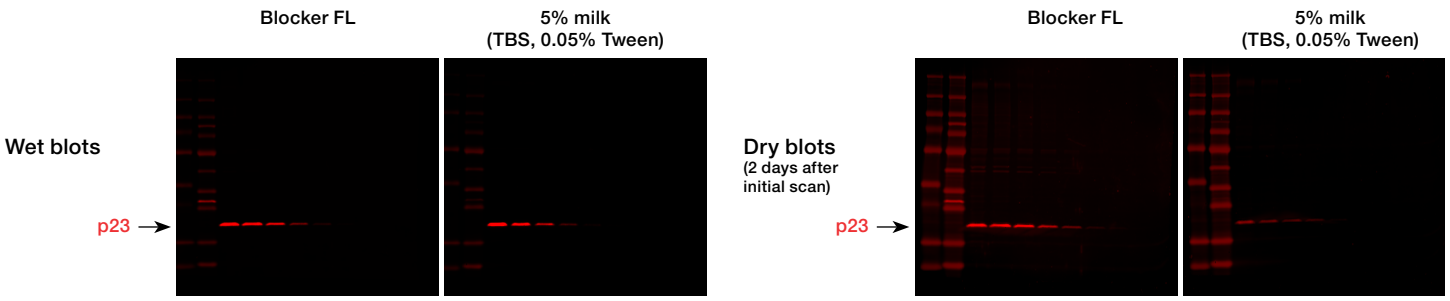


Figure 19. Comparison of Thermo Scientific™ Blocker™ FL Fluorescent Blocking Buffer and 5% nonfat milk blocking buffer. Western blotting was performed by loading serial dilutions of A431 cell lysate (15 mg, 3-fold serially diluted) and carrying out electrophoresis on an Invitrogen™ Novex™ 4–20% Tris-Glycine Plus Midi Gel (Cat. No. WXP42012BOX). The first 2 lanes were loaded with 5 µL PageRuler Prestained Protein Ladder (Cat. No. 26616) and 3 µL iBright Prestained Protein Ladder (Cat. No. LC5615). The proteins were transferred to a nitrocellulose membrane using the Power Blotter System (Cat. No. 22834). The membranes were blocked for 30 minutes with either 1X Blocker FL Fluorescent Blocking Buffer (Cat. No. 37565), or TBS with 5% nonfat milk and 0.05% Tween 20 detergent.

No single blocking agent is ideal for every occasion because each antibody–target pair has unique characteristics. For this reason, we offer a variety of buffers to suit your western blot conditions. See Table 13 for guidance in selecting the right blocking buffer for your experiment.

Table 13. Blocking buffer selection guide.

Select when …	Thermo Scientific™ product	Blocking agent	Highlights	When to use	Available formats
… optimizing a new western blot system	StartingBlock™ Blocking Buffer	Serum and biotin-free single purified protein	• Performs well with a wide range of antibodies and antibody combinations • Compatible with streptavidin systems • Blocks in less than 15 min	• With medium- to high-abundance proteins or strong antibody affinity • Current blocking buffer has high background • Stripping and reprobing western blots	PBS TBS PBST TBST
… performing fluorescent western detection	Blocker™ FL Fluorescent Blocking Buffer (10X)	Single purified protein	• Blocks excess nonspecific binding sites to help reduce background fluorescence • Works with both nitrocellulose and low-fluorescence PVDF membranes • Detergent-free • Blocks in 15–30 min	• Imaging and storage of dry fluorescence blots	10X concentrate
… you have high background with homemade milk buffers	Blocker™ Casein	Purified casein	• High-performance replacement for homemade milk blocking buffers in western blotting applications • Single-protein blocking buffer provides fewer chances of cross-reaction with assay components than serum or milk solutions	• Use when high background is seen with nonfat milk	PBS TBS
… targeting phosphoproteins	Blocker™ BSA	Purified bovine serum albumin (BSA)	• 10% solutions of high-quality BSA • Single purified protein provides fewer chances of cross-reaction with assay components than serum or milk solutions	• Use when targeting phosphoproteins • Best to use when storing reused antibodies in blocker	10X concentrate
… you need a protein-free blocker	Pierce™ Protein-Free Blocking Buffer	Non-protein blocking compound	• Helps minimize or eliminate cross-reactivity associated with protein-based blocking buffers • Designed for when sample + antibody combinations require the elimination of all possible exogenous animal proteins in the assay system to avoid cross-reaction or quenching of the desired probe function	• Use when protein-based blockers cause high background	PBS TBS PBST TBST

Did you know

Milk should not be utilized as a blocking buffer when probing for phosphoprotein targets because milk contains casein, a phosphoprotein, which can cause high background.

Learn more at **thermofisher.com/blockingbuffers**

Probing the membrane

Most western blot analysis methods depend on the use of target-specific probes that are detectable via chemical tags or labels to measure the presence of specific proteins in biological samples. Antibodies are the most common type of probe because their binding affinity for specific proteins enables "finding" and detecting them in a complex sample such as a lysate preparation of cells or tissues. However, antibodies are themselves proteins, and they are not specifically detectable in an assay system unless they are tagged for visualization (a primary conjugate) or are used in combination with a tagged secondary antibody.

Different types of chemical labels or tags can be conjugated to antibodies to facilitate their detection and measurement by various methods. The most common tags are enzymes and fluorophores. A number of advancements in reagents and instrumentation make these technologies more versatile and powerful. Chemiluminescence using enzymatic tags, such as AP and HRP, is the most common method of detection in a western blotting. However, the use of fluorescence technologies in western blot detection is rapidly advancing.

Primary antibody incubation

The choice of a primary antibody for a western blot will depend on the target protein to be detected and what antibodies are available against that protein. A large number of primary antibodies are available commercially. Alternatively, a primary antibody may be custom-made to recognize the protein 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 antigens. 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. Recombinant antibodies are those for which the DNA sequence of the antibody's variable region is available and the antibody is produced in an expression system. Recombinant antibodies offer lot-to-lot consistency and reproducible performance. To obtain antibodies with the greatest specificity, you can affinity-purify the antibody using the immobilized target molecule.

Specificity of primary antibodies

Once an antibody is obtained, it is important to determine if it is specific to its target. Antibodies that recognize proteins other than the intended one can give rise to multiple bands in a western blot, leading to challenges in data interpretation. Different types of specificity tests (**thermofisher.com/antibodyvalidation**) based on the target biology can be employed to determine antibody specificity.

Primary antibodies on thermofisher.com

With hundreds of thousands of antibodies, our portfolio is validated* and cited for flow cytometry, immunohistochemistry (IHC), immunofluorescence (IF), immunocytochemistry (ICC), western blotting, enzyme-linked immunosorbent assay (ELISA), and other applications. With search filters, references, data images, published figures, and additional antibody supplier options, utilize the comparison feature at **thermofisher.com/antibodies** to easily find the antibody you need. To help ensure superior antibody results, Invitrogen™ antibodies are tested for specificity using a 2-part approach for advanced verification, testing for target specificity and functional application.

Did you know

Thermo Fisher Scientific offers custom antibody services to help you develop novel polyclonal, monoclonal hybridoma, or recombinant antibodies.

* The use or any variation of the word "validation" refers only to research use antibodies that were subject to functional testing to confirm that the antibody can be used with the research techniques indicated. It does not ensure that the product(s) was validated for clinical or diagnostic uses.

Learn more at **thermofisher.com/antibodies**

Secondary antibody incubation

A secondary antibody aids in the detection of a target protein by binding to a primary antibody that has been directly bound to the target protein. The vast majority of primary antibodies are produced in just a few host animal species and most are of the IgG class, making it relatively easy to choose ready-to-use, labeled secondary antibodies for most applications and detection systems. Secondary antibodies can be polyclonal, monoclonal, or recombinant. They are available in different buffer formulations and forms, for example lyophilized or in solution. The secondary antibody incubation step in western blotting is usually performed for 1 hour at room temperature in blocking buffer. Longer incubations can be used, if needed.

Secondary antibodies for western blotting are typically diluted to working concentrations ranging from 1:500 for fluorescent western blotting to 1:500,000 for chemiluminescent detection of abundant targets when using a substrate designed for high sensitivity. The optimal dilutions vary significantly between fluorescent and chemiluminescent detection. Optimal dilution for chemiluminescent detection depends on several experimental factors, such as protein target abundance, choice of primary antibody, and, most importantly, choice of the chemiluminescent substrate. Dilutions are typically higher for enzyme-conjugated secondary antibodies due to enzymatic amplification of the chemiluminescent signal. We offer a wide variety of labeled secondary antibodies for use in western blotting. The labels include horseradish peroxidase (HRP) and alkaline phosphatase (AP) for chemiluminescent and chromogenic detection, poly-HRP and biotin conjugates for signal amplification, and Invitrogen™ Alexa Fluor™ and Alexa Fluor™ Plus secondary antibodies for fluorescent western blotting.

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 can be 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 shares the same kappa light chains as IgG). In contrast, immunizing a goat with only mouse IgG1 antibodies will only generate antibodies specific for mouse IgG1 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-adsorbed to achieve minimal cross-reaction with other immunoglobulins. Using the example described above, immobilized mouse IgG1 antibodies would be used to affinity-purify all goat antibodies that bind to mouse IgG1. These anti–mouse IgG1 antibodies would then be further purified by passage through one or more chromatography columns containing mouse IgG2a, IgG2b, IgG3, IgM, and other isotypes to remove any antibodies that cross-react with non-IgG1 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-adsorption (typically referred to as “cross-adsorbed” in product names) 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 (Table 14).

Table 14. Commonly used abbreviations for target species.

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

Enzyme labels for detection: alkaline phosphatase (AP) and horseradish peroxidase (HRP)

Enzymatic labels are most commonly used as secondary antibody tags for detection in western blotting applications. Enzymes provide detectable signal via their activity; reaction with a specific substrate chemical yields a colored or chemiluminescent product. AP and HRP

are the two enzymes used most extensively as labels for chemiluminescent protein detection. An array of chromogenic and chemiluminescent substrates is available for use with either enzyme (see pages 42–59).

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

HRP catalyzes the oxidation of substrates by hydrogen peroxide, resulting in a colored or chemiluminescent product or the release of light as a by-product of the reaction. HRP functions optimally at near-neutral pH and can be inhibited by cyanides, sulfides, and azides. Antibody-HRP conjugates are superior to antibody-AP conjugates with respect to the specific activities of both the enzyme and antibody. In addition, its high turnover rate, good stability, low cost, and wide availability of substrates makes HRP the enzyme of choice for most applications. Because of the relatively small size of the HRP enzyme, further increases in sensitivity may be achieved by using poly-HRP–conjugated secondary antibodies and may eliminate the need for using ABC-type amplification systems, which require the use of biotinylated secondary antibodies, and streptavidin–HRP conjugates. Streptavidin–HRP could bind nonspecifically to biotinylated proteins in the sample and thereby interfere with specific detection of the target protein.

Fluorescent labels for detection

Historically, fluorophore-labeled secondary antibodies and other probes were used in a small number of cell biology applications such as flow cytometry (FC), cell sorting, and IHC using fluorescence microscopy, but are now expanding into western blotting applications. The use of fluorophore-conjugated secondary antibodies in western blotting requires fewer steps compared to the use of enzymatic labels because there is no substrate development step to perform. While the protocol is shorter, fluorescent detection requires imaging instrumentation capable of capturing the fluorescent signal, and the sensitivity can be lower than what can be obtained with a well-optimized enzymatic chemiluminescent system. Although not as sensitive as enzymatic detection, fluorescent detection methods reduce chemical waste and have the added advantage of multiplex compatibility (leveraging multiple fluorescently tagged antibodies to detect multiple proteins on the same blot without the need to strip and reprobe the blot as one would do in a chemiluminescence-based western blot system).

The growing demand for multiplex assays has driven the development of many new fluorescent dyes and conjugation chemistries, making possible the development of improved fluorescent dye–labeled secondary antibodies, such as Alexa Fluor Plus secondary antibodies. These new fluorescent secondary antibodies are brighter and more photostable than the traditional fluorophore-conjugated secondary antibodies and comprise a broader range of nonoverlapping spectra. Together with advances in digital imaging instrumentation, these new fluorophores provide a compelling reason to consider this detection strategy versus a chemiluminescence detection strategy.

Biotin-binding proteins as probes

The highly specific affinity interaction between biotin and avidin or streptavidin protein is the basis for many kinds of detection and affinity-purification methods. Biotin is very small (244 daltons), so its covalent attachment to antibodies or other probes rarely interferes with their functions. Yet its presence as a tag on a probe allows efficient and specific secondary detection with either avidin, streptavidin, or Thermo Scientific™ NeutrAvidin™ Protein. Biotin-binding proteins are available in purified forms labeled with enzymatic or fluorescent tags that enable detection in many kinds of assays systems.

Both avidin and streptavidin bind very strongly and specifically to biotin. However, each protein has its limitations in certain assays. Avidin is glycosylated, which may lead to nonspecific lectin binding. Streptavidin contains a RYD motif, a bacterial recognition sequence, that can cause background binding with certain samples. An alternative is to use NeutrAvidin Protein, which is an exclusive, deglycosylated form of avidin that avoids the drawbacks of both native avidin and streptavidin.

Choosing a secondary antibody

Selecting an appropriate secondary antibody is a critical step in the western blot detection procedure. A careful assessment of the needs of the experiment is required. The stepwise considerations below are useful in the process of choosing a secondary antibody.

1 **Determine origin of the primary antibody.**

- Example 1—mouse monoclonal IgG1
- Example 2—rabbit recombinant IgG

2 **Select an appropriate host species and target reactivity for the secondary antibody:**

- Potential secondary antibody for example 1—goat anti–mouse IgG1
- Potential secondary antibody for example 2—goat anti–rabbit IgG

3 **Consider cross-reactivity or specificity issues of the secondary antibody.**

- Cross-adsorbed—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 method.**

- Label—appropriately conjugated to the correct enzyme, tag, or fluorophore for the chosen detection method

5 **Consider requirements of the supplied secondary.**

- Supplied state—such as sterile liquid or lyophilized, suspended in PBS or Tris buffer

6 Visit **thermofisher.com/secondaries** for a complete list.

Storage conditions for antibodies and antibody–enzyme conjugates

Antibody solutions for western blotting are typically diluted from 1:100 to 1:500,000 beginning from a 1 mg/mL stock solution. These 1 mg/mL stocks often can be stored at 4°C for days to weeks without significant loss in activity. However, for increased stability, glycerol or ethylene glycol may be added to a final concentration of 50% and the antibody can be stored at –20°C. Alternatively, the antibody solution may be stored in small working aliquots at –20°C to avoid repeated freeze/thaw cycles. Antimicrobial agents such as sodium azide or thimerosal may be added to avoid microbial growth.

Generally, antibody conjugates are best stored at –20°C with glycerol or ethylene glycol added at a final concentration of 50%. Although some enzyme conjugates may be stored at –20°C without cryoprotectant, frozen stocks must be as single-use aliquots to prevent repeated freeze/thaw cycles; AP conjugates are particularly sensitive to freezing. Conjugates typically maintain good activity for 1–2 years if stored at –20°C with glycerol or ethylene glycol. However, contaminants in cryoprotectants may affect enzyme activity, and few researchers take steps to ensure the purity of the cryoprotectant used. For both unlabeled and labeled antibodies, it is best to refer to the manufacturer's recommendations for specific storage conditions.

Ethylene glycol

Thermo Scientific™ Ethylene Glycol provides exceptional antifreeze protection and storage stability for antibody–enzyme conjugates because it is purified to remove impurities commonly found in traditional glycerol stocks. The 50% (w/v) aqueous solution, when mixed in equal volumes with purified protein samples such as primary antibodies, stabilizes and maintains the mixture as a liquid during freezer storage (–20°C).

Features:

- Specially purified to remove impurities such as aldehydes, peroxides, iron, and UV-absorbing hydrocarbons
- Suitable for enzyme storage without the worry of losing enzymatic activity
- Stable for months

Guardian Peroxidase Conjugate Stabilizer/Diluent

Thermo Scientific™ Guardian™ Peroxidase Conjugate Stabilizer/Diluent preserves the functional integrity and activity of HRP-conjugated antibodies and other proteins at very dilute concentrations for long-term storage. With Guardian Peroxidase Conjugate Stabilizer/Diluent, typical 1 mg/mL antibody or streptavidin peroxidase conjugates can be diluted as much as 100,000-fold for storage at 4°C. The Guardian solution enables these working concentrations of HRP conjugate to be prepared in advance and stored at 4°C for 12 months or at room temperature for 6 months. For blotting applications, it is usually best to prepare a concentration for storage that can be diluted 10-fold in assay buffer (which may contain blocking components) for final use.

Features:

- **Preserves HRP activity**—no significant loss of HRP activity over a 6 month period at room temperature (1:1,000 dilution) or 12 months at 4°C
- **Convenient**—store ready-to-use dilutions (1:1,000 to 1:100,000) that maintain enzyme activity in the refrigerator; no aliquoting or freezing needed
- **Assay compatible**—simply add your favorite blocking buffer to create the ideal diluent for your HRP-based ELISA system or store the HRP conjugate as a 1:1,000 stock solution for western blots and dilute in the final assay buffer
- **Helps save money**—less expensive than alternative formulations or ordering new HRP conjugates
- **Superior stability**—better maintenance of HRP activity over time (Figure 20)

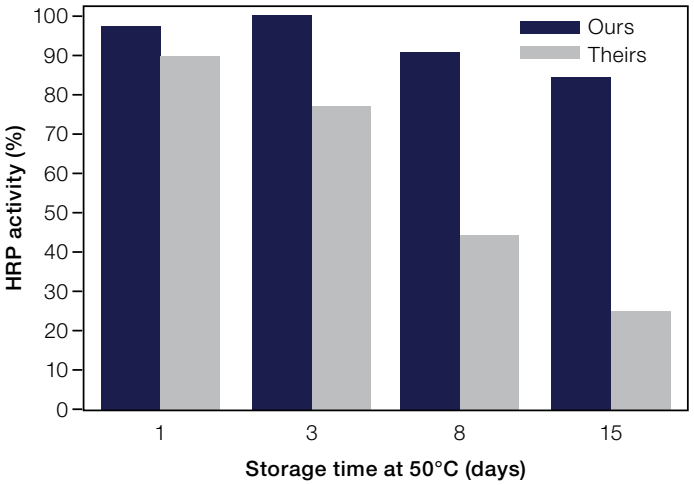


Figure 20. Better stability of HRP conjugate with Guardian Peroxidase Conjugate Stabilizer/Diluent. Streptavidin-HRP (1 mg/mL) was diluted 1:1,000 and stored at 50°C in Guardian stabilizer/diluent ("Ours") or another supplier's HRP stabilizer ("Theirs"). At each time point, the HRP conjugate was diluted 1:5,000,000 with Thermo Scientific™ SuperBlock™ (PBS) Blocking Buffer and incubated for 1 hour in a white plate that had been coated with biotinylated BSA. The plate was then washed 3 times with 200 µL PBST. Thermo Scientific™ SuperSignal™ ELISA Femto Substrate (100 µL) was added to the plate and incubated for 1 minute and then measured in a luminometer. Samples at each time point were compared to control (i.e., HRP conjugate that had not been stored in diluted form). Storing enzymes at 50°C for 2 weeks is equivalent to 12 months at 4°C.

Did you know

Detergents such as Tween 20 surfactant can help minimize background when added to a blocking buffer, but too much detergent can prevent sufficient blocking. A final concentration of 0.05% often works well but may need to be optimized for the specific application.

Washing the membrane

Like other immunoassay procedures, western blotting consists of a series of incubations with different antibodies separated by wash steps. Washing steps are necessary to remove unbound or weakly bound reagents and to reduce background, 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 or target protein from the blot. As with other steps in performing a western blot, a variety of buffers may be used (Table 15). 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 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.

Although detergents are available from several commercial sources and used routinely in many research laboratories, the importance of detergent purity and stability is not widely appreciated. Detergents often contain trace impurities from their manufacture. Some of these impurities, especially peroxides that are found in most nonionic detergents, will destroy protein activity. In addition, several types of detergents oxidize readily when exposed to the air or UV light, causing them to lose their properties and potency as solubilizing agents. We offer several high-purity, low peroxide–containing detergents that are packaged under nitrogen gas in clear glass ampules. These Thermo Scientific™ Surfact-Amps™ detergent solutions provide superior convenience, quality, and consistency for all detergent applications. Commonly used detergents for western blotting include Tween 20 and NP-40 detergent solutions.

BupH Phosphate Buffered Saline Packs (PBS)

Each pack of Thermo Scientific™ BupH™ Phosphate Buffered Saline yields 500 mL of 0.1 M phosphate, 0.15 M sodium chloride, pH 7.0, when dissolved in 500 mL deionized water (20 L total).

BupH Tris Buffered Saline (TBS)

Each pack of Thermo Scientific™ BupH™ Tris Buffered Saline yields 500 mL of 25 mM Tris, 0.15 M sodium chloride, pH 7.2, when dissolved in 500 mL deionized water (10 packs make 5 L total; 40 packs make 20 L total).

Tween 20 Surfact-Amps Detergent Solution

Thermo Scientific™ Tween™ 20 Surfact-Amps™ Detergent Solution is a highly purified Tween 20 detergent stabilized as a 10% solution and packaged under nitrogen in glass ampules or nonleaching, high-density polyethylene (HDPE) bottles, ensuring its stability and eliminating the accumulation of peroxides and degradation products.

Table 15. Wash buffer selection guide.

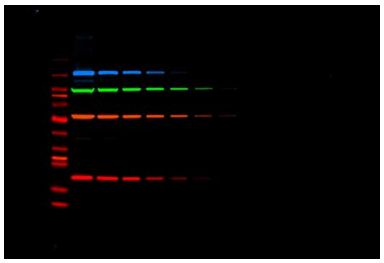
	TBS	PBS	TBST	PBST
Formulation	Both dry and liquid: • 25 mM Tris • 150 mM NaCl • pH 7.2	Dry: • 100 mM sodium phosphate • 150 mM NaCl • pH 7.2 Liquid: • 10 mM sodium phosphate • 150 mM NaCl • pH 7.5	• 25 mM Tris • 150 mM NaCl • 0.05% Tween 20 detergent • pH 7.5	• 10 mM sodium phosphate • 150 mM NaCl • 0.05% Tween 20 detergent • pH 7.5
Dry blend	Cat. No. 28376 (40 packs) Cat. No. 28379 (10 packs)	Cat. No. 28372 (40 packs)	NA	NA
Liquid concentrate	Cat. No. 28358 (500 mL)	Cat. No. 28348 (500 mL)	Cat. No. 28360 (500 mL)	Cat. No. 28352 (500 mL)
Application	• ELISA, western blotting, and other immunoassays • Use with HRP, AP, or fluorescent detection systems	• ELISA, western blotting, and other immunoassays • Crosslinking, biotinylation, and fluorescent labeling reactions requiring an amine-free buffer • Use with HRP or fluorescent detection systems	• ELISA, western blotting, and other immunoassays • Use with HRP or AP detection systems; can be used in fluorescent detection systems after blocking step	• ELISA, western blotting, and other immunoassays • Use with HRP detection systems; can be used in fluorescent detection systems after blocking step

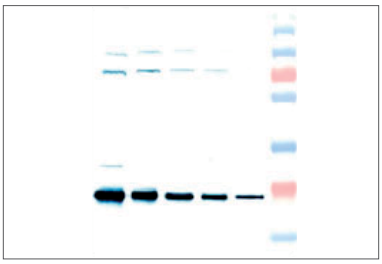
Learn more at **thermofisher.com/westernbuffers**

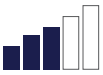
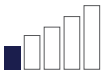
Overview of detection methods

With a variety of detection techniques to choose covering chemiluminescence, fluorescence, or chromogenic detection, you can select a technology that matches your experimental requirements and equipment available (Table 16).

Table 16. Comparison of western blot detection methods.





	Chemiluminescence	Fluorescence	Chromogenic
Major advantage	Highest sensitivity	Detect multiple targets at once	Easily visualize blots without equipment
Technology overview	Enhanced chemiluminescent (ECL) HRP and AP substrates providing pictogram- to femtogram-level detection	Simultaneous detection of multiple proteins on the same blot using a combination of primary antibodies and associated fluorophore-conjugated secondary antibodies	Direct visualization of your target protein using color detection reagents
Signal source	Indirect signal from enzymatic reaction	Direct signal from fluorophore	Indirect signal from enzymatic reaction
Signal duration	Limited (hours)	Extended (weeks to months)	Extended (weeks to months)
Sensitivity			
	Excellent	Good	Limited, best for high-abundance proteins
Consistency	Possible variation between blots, which can be mitigated by using high-duration substrates	High reproducibility between blots	Possible variation between blots
Detection	X-ray film and imaging instruments	Imaging instruments capable of exciting fluorophores and detecting the subsequent light emission	Visual—no instrumentation required
Other considerations	• Stripping and reprobing of blot possible • Long exposure times possible, as no excitation light source required to capture signal	• Care is needed to avoid fluorescence contamination • Antibody selection becomes more challenging as the degree of multiplexing increases (to avoid antibody cross-reactivity)	• Stripping and reprobing of blot not possible • Best method for high-abundance proteins and when imaging or film processing instrumentation is not available

Chemiluminescent western blot detection

Chemiluminescence is the western blot detection method of choice in many protein laboratories, as it provides high sensitivity and convenience for detection with film or digital imaging equipment. Chemiluminescent substrates are popular because they offer several advantages over other detection methods:

- Blots can be reprobed to optimize detection, or to visualize a second protein
- Capable of detecting and quantitating a wide range of protein concentrations
- Yield the greatest sensitivity of any available detection method

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 (Figure 21). This contrasts with chromogenic 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. There are a variety of chemiluminescent substrates available with different formats and sensitivities.

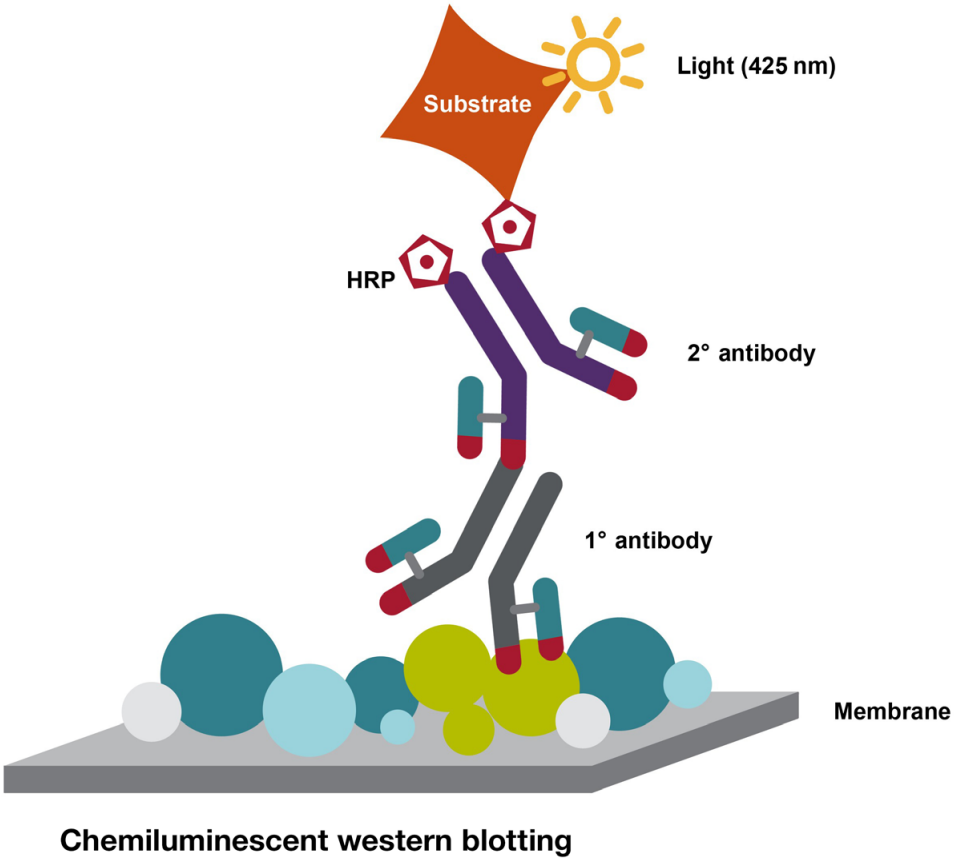


Figure 21. Overview of an enzyme–substrate reaction during chemiluminescent western blot detection.

Chemiluminescent substrates

The choice of substrate for chemiluminescent western blotting is determined by the reporter enzyme that is selected. Specifically, luminol- and acridan-based reagents are chemiluminescent horseradish peroxidase (HRP) substrates. For chemiluminescent detection of alkaline phosphatase (AP), acridan- and 1,2-dioxetane–based substrates are available (Figure 22). HRP substrates generally offer better detection sensitivity and buffer compatibility than AP substrates (Table 17).

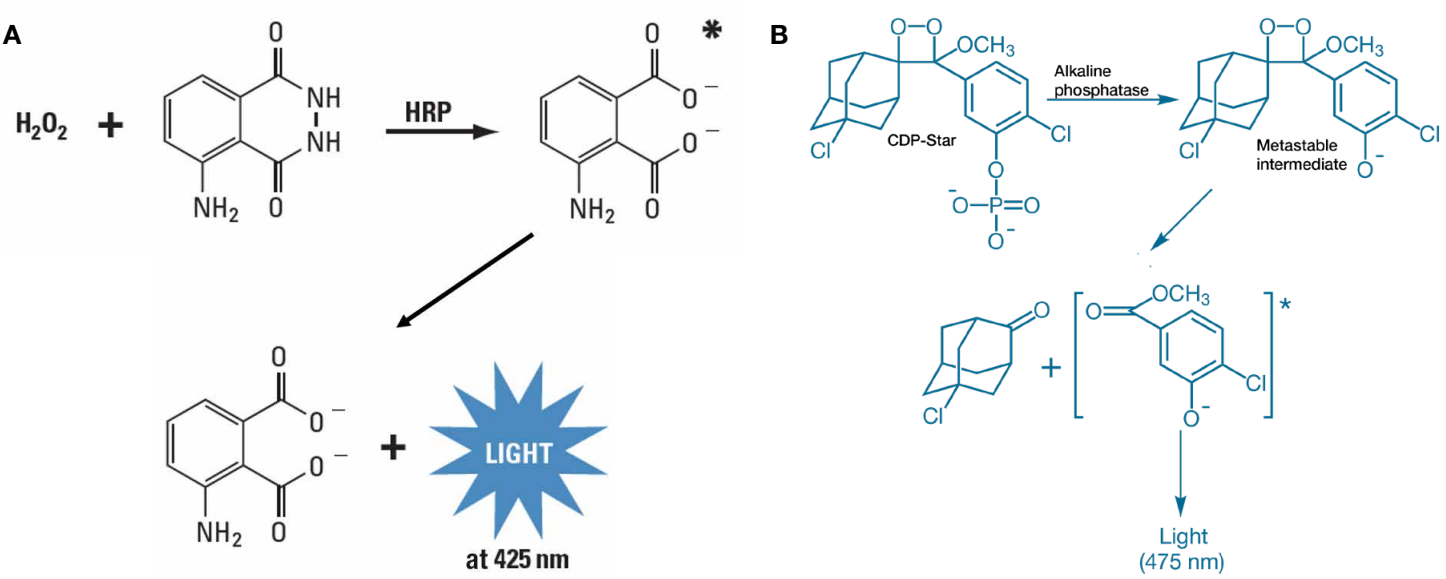
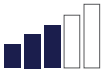


Figure 22. Chemiluminescent reaction of luminol and Invitrogen™ CDP-*Star*™ Substrate. (A) Chemiluminescence is a property of chemical reactions that emit light as a byproduct. Luminol is one of the most widely used chemiluminescent reagents. The oxidation of luminol by peroxide results in the creation of an excited-state product called 3-aminophthalate. This product decays to a lower-energy state by releasing photons of light. **(B)** Chemiluminescent reaction of CDP-*Star* Substrate with AP. CDP-*Star* Substrate is dephosphorylated by AP to yield meta-stable dioxetane phenolate anion intermediate that decomposes and emits light with a maximum intensity at a wavelength of 475 nm. Light emission occurs only during the enzyme–substrate reaction.

Table 17. Comparison of HRP and AP substrates for western blot detection.

	HRP substrates	AP substrates
Sensitivity		
	Femtogram sensitivity	Picogram sensitivity
Signal generation	Immediate	Gradually increases with signal maximum at ~30–60 minutes
Signal duration	Up to 24 hours	24–96 hours
Considerations	Compatible with common buffers such as TBS and PBS	Not compatible with phosphate buffers
When to use	Antibodies or probes conjugated to HRP	Antibodies or probes conjugated to AP

Chemiluminescent HRP substrates

Chemiluminescent substrates for HRP are typically two-component systems consisting of a stable peroxide solution and an enhanced luminol solution. In most cases, to make a working solution, equal volumes of the two components are mixed together. When incubated with a blot on which HRP-conjugated antibodies (or other probes) are bound, a chemical reaction emits light at 425 nm that can be captured with X-ray film or an imaging instrument that detects chemiluminescence. Although X-ray film provides qualitative and semiquantitative data and is useful to confirm the presence of target proteins, purpose-built western blot imaging instrumentation offers the advantages of quantitative analysis, simple exposure time adjustments, higher sensitivity, greater resolution, and a larger dynamic range than film. The choice of HRP chemiluminescent substrate should be based on abundance of the target protein of interest, abundance of sample containing the target protein, quality of the antibodies, and the level of sensitivity and type of instrumentation available for detection (Tables 18 and 19).

Table 18. Properties of Thermo Scientific™ HRP-based chemiluminescent substrates.

	Pierce ECL Western Blotting Substrate	SuperSignal West Pico PLUS Chemiluminescent Substrate	SuperSignal West Dura Extended Duration Substrate	SuperSignal West Atto Ultimate Sensitivity Substrate
Detection limit	Low- to mid-picogram	Low-picogram to high-femtogram	Mid-femtogram	Low-femtogram to high-attogram
Recommended antibody dilution	1°: 1:1,000–1:5,000 2°: 1:1,000–1:15,000	1°: 1:1,000–1:5,000 2°: 1:10,000–1:100,000	1°: 1:1,000–1:50,000 2°: 1:50,000–1:250,000	1°: 1:1,000–1:5,000 2°: 1:100,000–1:250,000
Advantages	Low cost, easy to switch from other entry-level ECL substrates	Superior sensitivity and intensity with longer duration than other entry-level ECL substrates	Good sensitivity with long duration and linearity for sensitive quantitation	Most sensitive substrate for HRP detection, with high signal-to-noise ratio
Select when	Target and sample are abundant	Routine western blots; working with new protein target when western blotting conditions are not yet optimized	Performing quantitative western blots or long duration is needed	Target is very low in abundance or sample is limited
Cat. No.	32106	34578	34075	A38554

Table 19. Choosing the correct HRP-based substrate for your application needs.

Conditions						
Sample	Target protein	Antibody	Recommended substrate	Primary	Secondary	Special notes
Sample is abundant	Target protein is abundant	Antibody quality is good	Pierce ECL Western Blotting Substrate	1:1,000	1:15,000	
Sample is abundant	Target protein abundance is unknown	Antibody quality is unknown	SuperSignal West Pico PLUS Chemiluminescent Substrate	1:1,000	1:100,000	If no bands are seen, the blot can be washed and re-imaged with SuperSignal West Atto substrate
Sample is abundant	Target protein abundance is low	Antibody quality is poor	SuperSignal West Atto Ultimate Sensitivity Substrate	1:1,000	1:100,000	
Sample is abundant	Target protein abundance is low	Antibody quality is good	SuperSignal West Atto Ultimate Sensitivity Substrate	1:2,000	1:250,000	
Sample is abundant	Target protein abundance is low	Antibody quality is limited	SuperSignal West Atto Ultimate Sensitivity Substrate	1:5,000	1:100,000	Assumes normal primary usage is 1:1,000
Sample is abundant	Target protein is abundant	Antibody quality is limited	SuperSignal West Atto Ultimate Sensitivity Substrate	1:20,000	1:250,000	Assumes normal primary usage is 1:1,000
Sample is limited	Target protein is abundant	Antibody quality is good	SuperSignal West Pico PLUS Chemiluminescent Substrate	1:1,000	1:100,000	Alternatively, could load less and use SuperSignal West Atto substrate at the same antibody concentrations
Sample is limited	Target protein abundance is unknown	Antibody quality is unknown	SuperSignal West Pico PLUS Chemiluminescent Substrate	1:1,000	1:100,000	If no bands are seen, the blot can be washed and re-imaged with SuperSignal West Atto substrate
Sample is limited	Target protein abundance is low	Antibody quality is good	SuperSignal West Atto Ultimate Sensitivity Substrate	1:1,000	1:250,000	
Sample is limited	Target protein abundance is low	Antibody quality is poor	SuperSignal West Atto substrate	1:1,000	1:100,000	

Chemiluminescent AP substrates

A variety of AP western blot detection substrates exist that can be used as an alternative to the more popular HRP-based systems. With AP-based detection, reaction rates remain linear, and sensitivity can be improved by allowing the reaction to proceed for longer periods of time. The activity of AP is not affected by exposure to antibacterial agents, such as sodium azide or thimerosal, so it may be stored for long periods of time in nonsterile environments. Optimal enzymatic activity occurs at pH 9.0–9.6; these enzymes are activated by divalent cations and are not compatible with phosphate buffers and divalent cation chelators such as EDTA. For AP-based western blot detection we offer CDP-*Star* Substrate, which delivers picogram-level sensitivity and is compatible with both traditional X-ray film and western blot imaging instrumentation (Table 20). Choose from a stand-alone substrate, Invitrogen™ Novex™ AP Chemiluminescent Substrate, or a complete kit such as the Invitrogen™ WesternBreeze™ Chemiluminescent Kit that contains all solutions necessary for your application.

Table 20. Properties of Invitrogen™ AP-based chemiluminescent substrates.

	Novex AP Chemiluminescent Substrate (CDP-*Star*)	CDP-*Star* Substrate (0.25 mM Ready-to-Use) with Nitro-Block-II Enhancer	CSPD Substrate (0.25 mM Ready-to-Use)	CSPD Substrate (0.25 mM Ready-to-Use) with Nitro-Block Enhancer
Detection limit	Mid- to low-picogram	Mid- to low-picogram	High-picogram	High-picogram
Exposure ranges	1 second to 15 minutes	1 second to 15 minutes	2 minutes to 2 hours	2 minutes to 2 hours
Recommended membrane	PVDF, nylon	Nitrocellulose, PVDF	PVDF, nylon	Nitrocellulose, PVDF
Cat. No.	WP20002	T2218	T2142	T2217

Did you know

Tris buffers are recommended when using AP-based western detection because phosphate buffers (e.g., PBS) can function as a substrate for alkaline phosphatase.

Watch a how-to video **here**.

Learn more at **thermofisher.com/chemisubstrates**

Pierce ECL Western Blotting Substrate

Thermo Scientific™ Pierce™ ECL Western Blotting Substrate is a value-priced, entry-level peroxidase substrate for enhanced chemiluminescence (ECL) that directly replaces costlier products without the need to re-optimize conditions. Pierce ECL Western Blotting Substrate provides reliability and performance equivalent to other standard ECL substrates for detection of HRP enzyme activity (Figures 23 and 24). Because the luminol and peroxide reagent formulations are identical to other commercially available substrate products, one can switch to Pierce ECL substrate without needing to optimize probing conditions or incubation protocols.

Features:

- **Economical**—about half the cost of other ECL substrates
- **No optimization required**—switching to Pierce ECL substrate from other entry-level ECL substrates does not require optimization or protocol changes

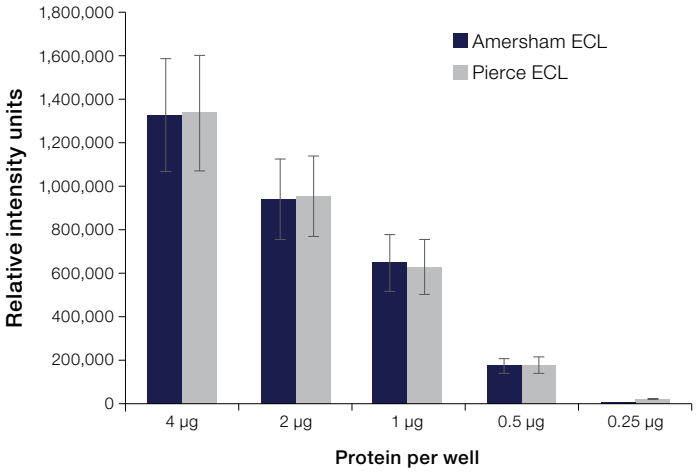


Figure 23. Signal intensity of Pierce ECL Western Blotting Substrate is comparable to Cytiva Amersham ECL Western Blotting Detection Reagent. HeLa cell lysate was separated by SDS-PAGE and transferred to nitrocellulose membrane to detect β-actin. The signal was detected and analyzed using Kodak™ 1D Image Analysis Software. Error bars represent ±20% difference in relative intensity units.

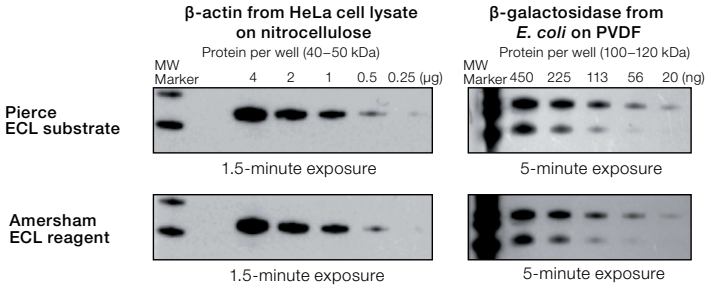


Figure 24. Pierce ECL Western Blotting Substrate for detection of proteins on nitrocellulose or PVDF membranes. β-actin and β-galactosidase protein in HeLa cell and *E. coli* lysates, respectively, were detected by western blotting. The membranes were blocked with 5% nonfat milk and probed with primary antibody at 1 µg/mL. The membranes were washed, then incubated with 0.2 µg/mL of HRP-conjugated goat anti–mouse IgG and washed again. Working solutions of the Pierce ECL Western Blotting Substrate and Cytiva Amersham ECL Western Blotting Detection Reagent were prepared according to the manufacturers' instructions and added to replicate membranes for one minute. The membranes were removed from the substrates, placed in plastic sheet protectors, and exposed to CL-XPosure Film and developed.

SuperSignal West Pico PLUS Chemiluminescent Substrate

Thermo Scientific™ SuperSignal™ West Pico PLUS Chemiluminescent Substrate is an enhanced chemiluminescent HRP substrate that enables low-picogram to high-femtogram levels of protein detection by western blot analysis. The intensity of the light emission combined with the exceptional duration allows for the acquisition of multiple exposures to more easily obtain publication-quality blot images. SuperSignal West Pico PLUS substrate is compatible with numerous membranes, blocking reagents, and a wide range of antibody dilutions, making it an ideal choice for most western blotting applications (Figures 25–27).

SuperSignal West Pico PLUS Chemiluminescent Substrate characteristics:

- **Sensitivity:** pictogram to femtogram sensitivity
- **Stability:** 8 hr working solution stability; 1-year kit stability at room temperature
- **Compatibility:** nitrocellulose and PVDF membranes
- **Signal duration:** 6–24 hours
- **Recommended primary antibody concentration:** 1:1,000–1:5,000 dilution (0.2–1.0 µg/mL)
- **Recommended secondary antibody concentration:** 1:20,000–100,000 dilution (10–50 ng/mL)

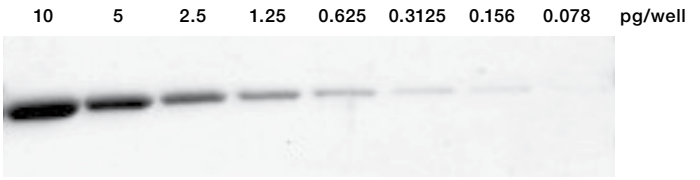


Figure 25. SuperSignal West Pico PLUS provides low-picogram to high-femtogram detection. TurboGFP-His-HA-Flag was diluted in electrophoresis reducing sample buffer. Lane 1 contained 10 pg of the purified protein with serial dilutions prepared 1:1 and applied at 10 µL/well. After electrophoresis, proteins were transferred to nitrocellulose membrane using the Power Blotter System and Pierce 1-Step Transfer Buffer. The membrane was blocked with SuperBlock Blocking Buffer. The membrane was then incubated with anti-His antibody at 1 µg/mL, followed by incubation with HRP-conjugated goat anti–mouse IgG at 100 ng/mL. SuperSignal West Pico PLUS substrate was used for detection. A 30-second exposure was acquired on the Thermo Scientific™ MyECL™ Imager, and the image was inverted and contrasted (black = 55,000, white = 65,535, gamma = 1.0).

Figure 26. Performance of SuperSignal West Pico PLUS substrate compared to another chemiluminescent substrate. Detection of the indicated targets was performed using 2-fold serial dilutions of HEK293 or HeLa cell lysates, starting with the amount indicated in parentheses above. Following separation by SDS-PAGE, proteins were transferred to either PVDF or nitrocellulose membranes using the Power Blotter System and Pierce 1-Step Transfer Buffer. The membranes were blocked with 5% nonfat dry milk dissolved in Thermo Scientific™ Pierce™ 20X TBS Tween™ 20 Buffer, and incubated with antibodies against beta-catenin, eIF4E, RSK2, or ezrin, followed by incubation with an Invitrogen™ goat anti–mouse IgG (H+L) secondary antibody (HRP conjugate), at a concentration of 20 ng/mL. Chemiluminescent detection and substrate comparison was performed following a 5-minute incubation with either SuperSignal West Pico PLUS or Bio-Rad Clarity™ substrates. Signal was captured using film.

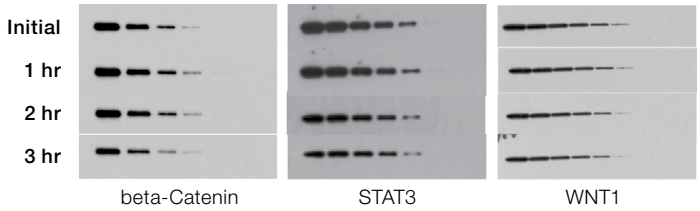


Figure 27. SuperSignal West Pico PLUS substrate produces robust signals over a long duration of time. Detection of the indicated targets was performed using 2-fold serial dilutions of HEK293 or HeLa cell lysates, starting at 4 µg/well or 20 µg/well, respectively. Following separation by SDS-PAGE, proteins were transferred to either Thermo Scientific™ PVDF or nitrocellulose membranes using the Power Blotter System and Pierce 1-Step Transfer Buffer. The membranes were blocked with 5% nonfat dry milk dissolved in Pierce 20X TBS Tween 20 Buffer, and incubated with Invitrogen™ antibodies against beta-catenin, STAT3, or WNT1 followed by incubation with goat anti–mouse IgG (H+L) secondary antibody (HRP conjugate) at a concentration of 20 ng/mL. Chemiluminescent detection was performed following a 5-minute incubation with SuperSignal West Pico PLUS substrate. Signal was captured on film at the indicated time points after addition of substrate.

SuperSignal West Dura Extended Duration Substrate

Thermo Scientific™ SuperSignal™ West Dura Extended Duration Substrate is an enhanced chemiluminescence HRP substrate with stable light output for mid-femtogram–level detection. It is ideal for quantitative western blotting.

Features:

- **24-hour signal duration**—light emission is stable for 10 times longer than with typical ECL substrates; acquire multiple exposures to obtain publication-quality blot images (Figures 28 and 29)
- **Great sensitivity**—provides detection of mid-femtogram levels of target proteins
- **High intensity**—immediate, stable signal generation provides easy detection via film or cooled CCD imaging systems
- **Stable reagent**—24-hour working solution stability; one-year kit stability at room temperature
- **Less antibody usage**—works best with dilute antibody concentrations

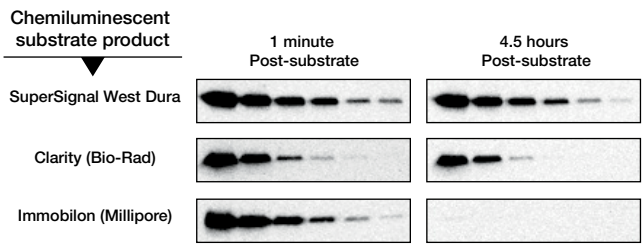


Figure 28. Better sensitivity and signal duration with SuperSignal West Dura Extended Duration Substrate. A431 cell lysate was serially diluted two-fold in electrophoresis reducing sample buffer and separated by gel electrophoresis. Lane 1 contained 5 µg of A431 lysate. After electrophoresis, proteins were transferred to a nitrocellulose membrane using the Power Blotter System and Pierce 1-Step Transfer Buffer. Membranes were blocked with 5% milk in TBST buffer. The membranes were incubated with Invitrogen™ Mouse beta-Catenin Antibody at 0.3 µg/mL and then with goat anti–mouse IgG, HRP conjugate, at 20 ng/mL. Identical blots were incubated in either SuperSignal West Dura substrate, Clarity™ Western ECL Substrate, or Immobilon™ Western Chemiluminescent HRP Substrate according to respective manufacturer's instructions. Thirty-second exposures of the resulting blots were simultaneously acquired on the MyECL Imager with the following settings: black = 65,296, white = 65,535, gamma = 1.0).

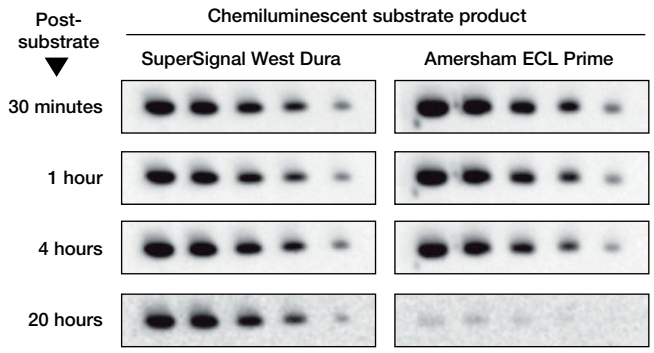


Figure 29. Longer signal duration with SuperSignal West Dura Extended Duration Substrate. Detection of Hsp86 in HeLa cell lysate on nitrocellulose membranes. Lane 1 contained 10 µg total protein (total 10 µL/well). Two-fold serial dilutions were then prepared and loaded at 10 µL/well (lanes 2–5). After electrophoresis and transfer to nitrocellulose membranes, blots were blocked with StartingBlock (TBS) Blocking Buffer, probed with Invitrogen™ Rabbit Anti-Hsp86 Antibody at 1:2,000 dilution, followed by goat anti–rabbit IgG HRP secondary antibody at 6.6 ng/mL. Finally, respective blots were incubated with SuperSignal West Dura substrate or Cytiva Amersham ECL Prime Western Blotting Detection Reagent per product instructions. At various time points following substrate incubation, the two blots were imaged using a CCD camera imager. (Identical imaging exposure parameters were used for both blots at each time point.)

SuperSignal West Atto Ultimate Sensitivity Substrate

Thermo Scientific™ SuperSignal™ West Atto Ultimate Sensitivity Substrate is an ultrasensitive ECL substrate that enables protein detection at high-attogram levels by western blot analysis with the HRP enzyme. It provides higher-level sensitivity and better signal-to-noise ratios than other commercially available high-performance HRP substrates (Figure 30). It is the ideal choice for detection of very low-abundance targets or when using precious samples that require maximum levels of sensitivity.

Features:

- **Sensitivity**—low-femtogram to high-attogram
- **Stability**—48 hours for working solution, one year for kit at 4°C
- **Compatibility**—nitrocellulose and PVDF membranes
- **Signal duration**—up to 6 hours
- **Recommended primary antibody concentration**—1:1,000–1:5,000 dilution (0.2–1.0 µg/mL)
- **Recommended secondary antibody concentration**—1:100,000–1:250,000 dilution (4–10 ng/mL)

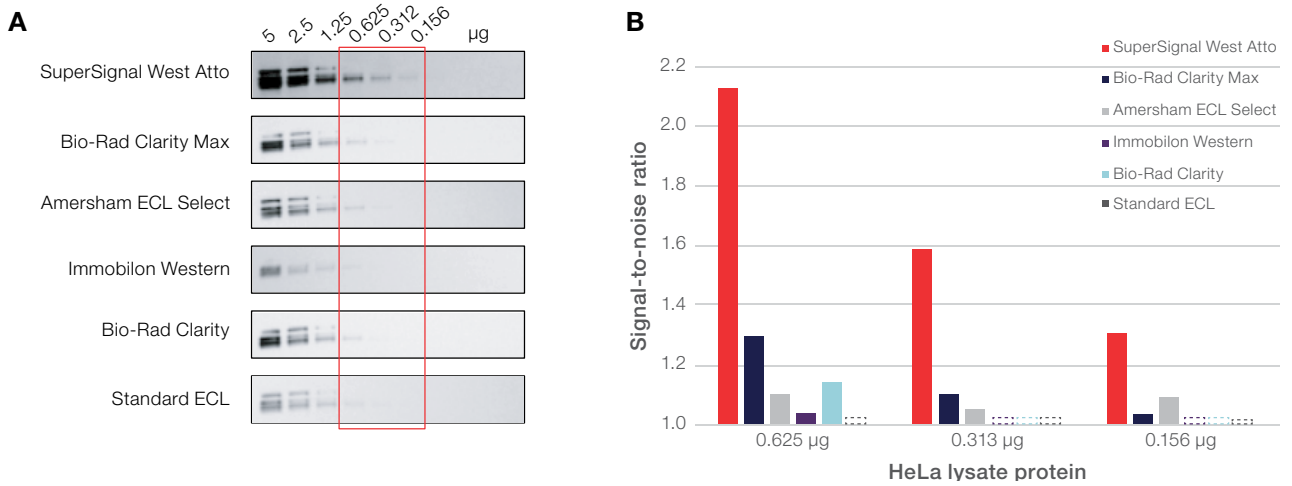


Figure 30. Chemiluminescent substrate product comparison with SuperSignal West Atto substrate. (A) Akt (pan) detection in HeLa lysate was performed using SuperSignal West Atto, Bio-Rad Clarity Max, Amersham ECL Select, Millipore Immobilon Western, Bio-Rad Clarity, and standard ECL substrates. The blots were developed using Akt (pan) monoclonal antibody, followed by HRP-conjugated goat anti–rabbit IgG secondary antibody. Blots were incubated with respective substrates per product instructions. Images were captured using the iBright Imaging System. **(B)** The signal-to-noise ratios for the western blot bands in the boxed area for SuperSignal West Atto, Bio-Rad Clarity Max, Amersham ECL Select, Millipore Immobilon Western, Bio-Rad Clarity, and standard ECL substrates are shown in bar graph form. SuperSignal West Atto substrate provided the best signal-to-noise ratios across all protein loads. The standard ECL substrate didn't have detectable signal above background noise after 0.625 µg. Clarity and Immobilon substrates didn't have detectable signal above background noise after 0.313 µg.

SuperSignal West Atto substrate allows use of very dilute samples. p23 in whole-cell HeLa cell lysates was detected with SuperSignal West Atto, Bio-Rad Clarity, or Millipore Immobilon substrate. Only 1.25 µg of cell lysate was needed to get a strong signal with SuperSignal West Atto substrate, whereas 20 µg of cell lysate was required to get the same level of detection with the other two substrates (Figure 31).

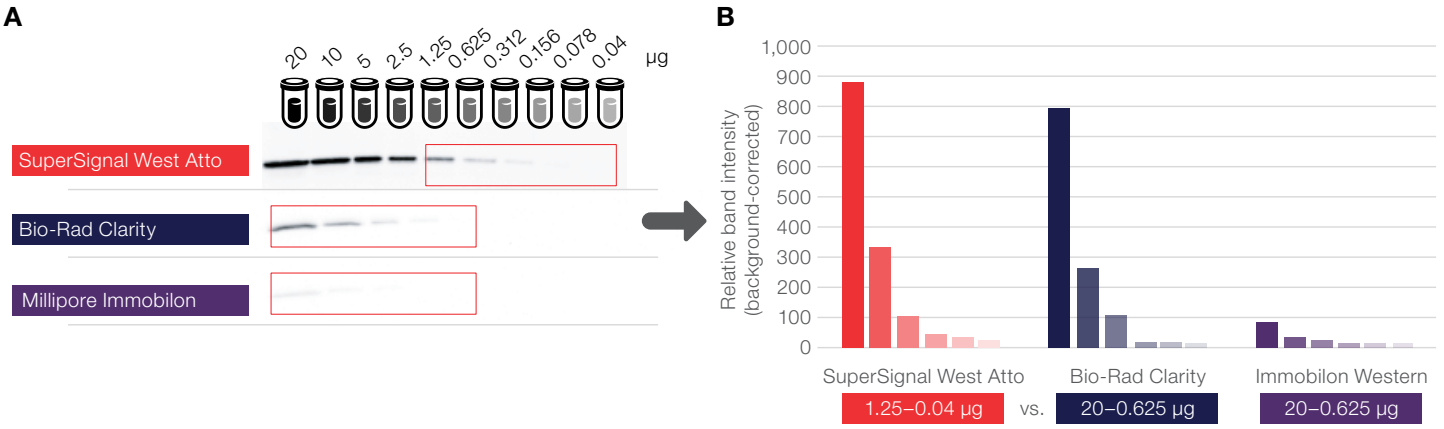


Figure 31. SuperSignal West Atto substrate allows use of very dilute samples. HeLa cell lysates were separated on Novex Tris-glycine gels and transferred to nitrocellulose membranes using the Power Blotter System with Power Blotter Select Transfer Stacks. The blots were incubated with mouse anti-p23, followed by an HRP-conjugated goat anti–mouse IgG secondary antibody. Finally, respective blots were incubated with SuperSignal West Atto substrate, Clarity Western ECL Substrate (Bio-Rad), or Immobilon Western Chemiluminescent HRP Substrate (EMD Millipore) per product instructions. All blots were simultaneously acquired using the iBright FL Imaging System.

Did you know

A speckled background on film can be caused by aggregate formation in the HRP conjugate. Filtering the conjugate through a 0.2 µm filter may help, or use a new HRP conjugate.

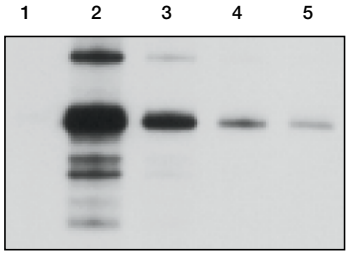
WesternBreeze AP chemiluminescent kits

Invitrogen™ WesternBreeze™ chemiluminescent kits detect proteins that have been immobilized on membranes (nitrocellulose or PVDF) following western transfer or bound directly from solution (dot blots). Detection is accomplished with a ready-to-use CDP-*Star* chemiluminescent substrate for alkaline phosphatase. Protein bands can be captured either by X-ray film or with a compatible imaging system (Figures 32 and 33).

The WesternBreeze chemiluminescent kits include blocking solutions, primary antibody diluent, ready-to-use secondary antibody solution (anti-mouse, anti-rabbit or anti-goat), ready-to-use chemiluminescent substrate, wash solutions, incubation trays, pre-cut filter papers, and a polyester sheet for even substrate development on the membrane. Each kit contains complete reagents for 20 blots.

Features:

- **Good signal-to-noise ratio**—high specificity, clean background
- **High sensitivity**—femtogram levels detectable
- **Long signal duration**—up to 5 days
- **Fast**—results in less than 3 hours



Blot A: Detected with the WesternBreeze Chemiluminescent Kit, 10-second exposure.

Figure 32. Human IgG detected with WesternBreeze kits. Human IgG (h-IgG) was separated on an Invitrogen™ NuPAGE™ 4–12% Bis-Tris gel (with MES SDS buffer) and transferred to a nitrocellulose membrane. The blot was probed with a 1:500 dilution of rabbit anti–human IgG and developed with the WesternBreeze Chemiluminescent Kit (anti-rabbit). Lane 1: 3 µL Invitrogen™ MultiMark™ Multi-Colored Standard (no longer available); lane 2: 10 ng of h-IgG; lane 3: 1 ng of h-IgG; lane 4: 100 pg of h-IgG; lane 5: 10 pg of h-IgG.

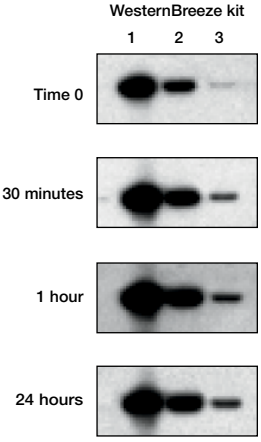


Figure 33. Signals achieved with the WesternBreeze chemiluminescent kits. A 53 kDa protein containing a V5 epitope (Invitrogen™ Positope™ Control Protein) was separated on a NuPAGE 4–12% Bis-Tris gel (with MES SDS buffer) then transferred to a PVDF membrane. The blot was probed with a 1:5,000 dilution of mouse anti-V5 primary antibody. Detection was performed using anti-mouse versions of the WesternBreeze detection system with film exposures taken over time. Exposures were two minutes in duration. Time 0 refers to an exposure taken less than 5 minutes after excess substrate removal. Subsequent time points are number of minutes or hours after Time 0. Lanes 1–3 contained 20 ng, 2 ng, and 200 pg, respectively, of Positope Control Protein.

Novex AP Chemiluminescent Substrate (CDP-*Star*)

Invitrogen™ Novex™ AP Chemiluminescent Substrate is a nonradioactive ready-to-use CDP-*Star* solution for chemiluminescence-based immunodetection of AP on western blot or dot blot membranes. This reagent provides detection sensitivities superior to that of precipitating chromogenic substrates. Low-picogram levels of detection can be achieved using either X-ray film or imaging equipment.

Features:

- **Good signal-to-noise ratio**—high specificity, clean background
- **High sensitivity**—low-picogram to femtogram levels detectable
- **Long signal duration**—up to 5 days
- **Fast**—results in less than 3 hours

Did you know

Colorimetric or chromogenic substrates cannot be stripped using stripping buffers because a colored precipitate is formed during the detection process.

CSPD Substrate

Invitrogen™ CSPD™ Substrate is a chemiluminescent AP substrate, ready to use for protein or nucleic acid blotting on PVDF and nitrocellulose membranes (with Nitro-Block™ enhancer). The CSPD chemiluminescent substrate lets you detect AP and AP-labeled molecules with a high level of sensitivity, speed, and ease.

This versatile chemiluminescent substrate exhibits high sensitivity in membrane-based applications such as Southern, northern, and western blotting. Maximum light levels are reached in approximately 10 minutes, and glow emission persists for several hours.

Features:

- Chemiluminescent substrates provide highly sensitive replacements for the widely used fluorogenic substrate methylumbelliferyl phosphate (MUP), and the colorimetric substrate p-nitrophenyl phosphate (pNPP)
- Low background luminescence coupled with high-intensity light output enables detection of AP labels with exceptional sensitivity and signal-to-noise ratio

Chromogenic western blot detection

Chromogenic or precipitating substrates have been used widely for many years and offer 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 (Figure 34). The resulting colored band or spot requires no special equipment for processing or visualizing. Chromogenic blotting substrates are available in a variety of specifications and formats. The appropriate substrate choice depends on the enzyme label and desired sensitivity.

The low sensitivity of chromogenic substrates makes it difficult to detect proteins of low abundance. Though the reaction can be allowed to develop for several hours or even overnight, this also allows background signal to develop as well. Where chromogenic substrates fail in terms of sensitivity, they are ideal for applications where protein abundance is high. Because the product of the enzyme–substrate reaction is a colored precipitate, the signal is stable, and therefore chromogenic substrates do not typically have issues with false-negative results (ghost bands) that can occur with chemiluminescent substrates. An added advantage of chromogenic detection is that the reaction can be stopped when the desired signal has been achieved, which minimizes saturation of signal. However, unlike with chemiluminescent blotting assays, the colored precipitate cannot be easily stripped off for reprobing. The performance of a particular substrate may vary dramatically when obtained from different suppliers. This is 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.

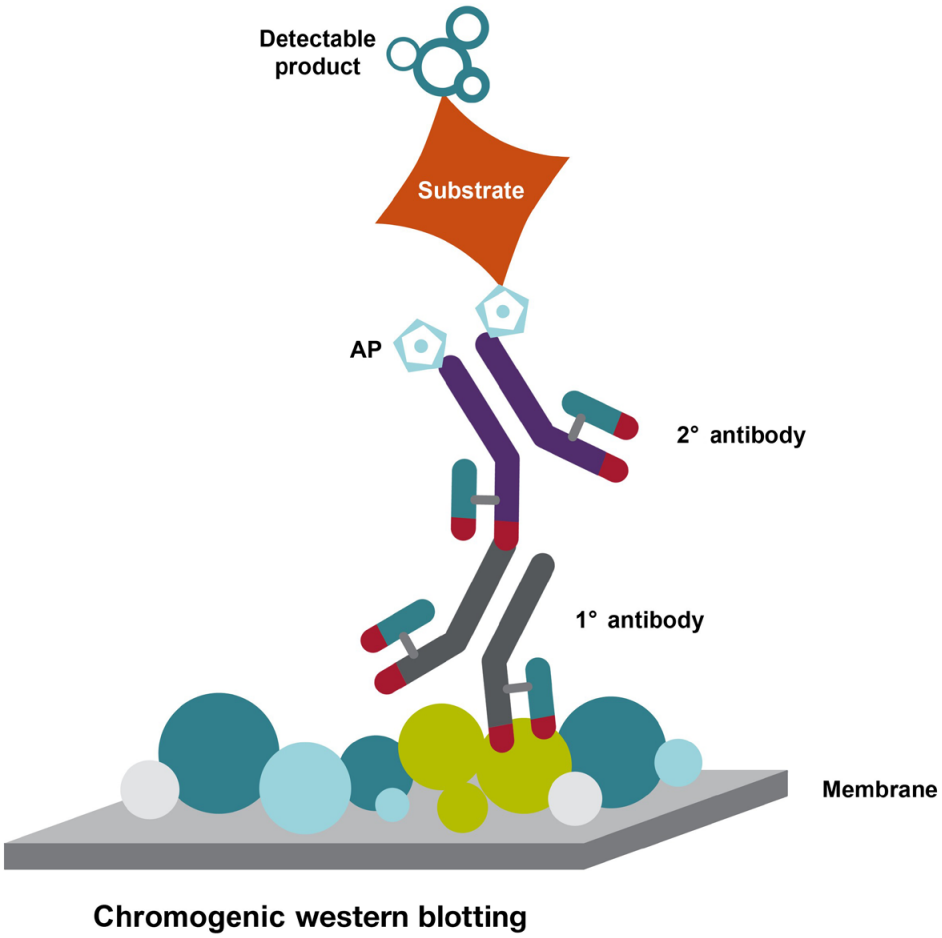
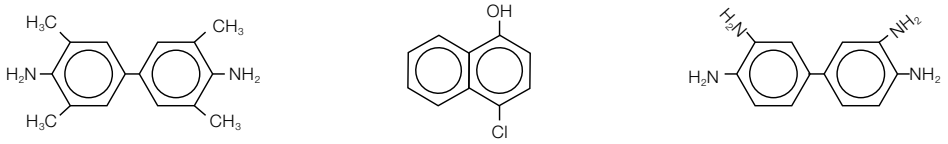


Figure 34. Overview of an enzyme–substrate reaction during chromogenic western blot detection.

Chromogenic HRP substrates

There are numerous options for chromogenic HRP substrates, but all require the presence or addition of peroxide for colorimetric detection (Figure 35). Because of its extremely short shelf life at the desired concentration, hydrogen peroxide traditionally was added to a buffer, along with the substrate, immediately prior to use. As a result, these substrates typically had a useful shelf life of only a few hours. Many commercially available precipitating HRP substrates are supplied with, or come prepared in, stable peroxidase substrate buffer. The stabilized peroxide in these solutions is generally concentrated and less corrosive than the traditional 30% stock solution of hydrogen peroxide. Since 30% hydrogen peroxide and dilution solutions of hydrogen peroxide are not stable, reagents prepared with stabilized peroxide will provide more consistent results. TMB 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™ 1-Step™ Ultra TMB-Blotting Solution is a single-component peroxidase substrate for western blotting and immunohistochemistry (Table 21). Precipitating the product results in dark blue bands where the enzyme is located. 1-Step Ultra TMB-Blotting Solution is well suited for applications that require a high signal-to-noise ratio. DAB yields a brown precipitate in the presence of HRP and peroxide. The brown, insoluble product can be readily chelated with osmium tetroxide. 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. Chloronaphthol (4-chloro-1-naphthol, 4-CN) can be used for chromogenic detection of HRP in western blotting and immunohistochemistry. 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. The individual benefits of 4-CN and DAB are often combined into a single substrate mixture, such as the CN/DAB solution used in the Thermo Scientific™ CN/DAB Substrate Kit. CN/DAB substrate has excellent sensitivity, yielding a dark black precipitate that photographs well. CN/DAB substrate works well in western blotting and dot blotting applications.



TMB
MW: 240.4

4-CN
MW: 178.6

DAB
MW: 214.1

Figure 35. Chromogenic substrates for western blotting with HRP.

Table 21. Properties of Thermo Scientific™ HRP-based chromogenic substrates.

	Metal Enhanced DAB Substrate Kit	1-Step Ultra TMB-Blotting Solution	Pierce CN/DAB Substrate Kit	1-Step Chloronaphthol Substrate Solution
Detection limit	17 pg	20 pg	500 pg	5 ng
Color	Brown-black	Dark blue	Black	Blue-purple
Format	2-component reagent kit	Ready-to-use solution	Ready-to-use solution	2-component reagent kit
Recommended antibody dilutions (from 1 mg/mL stock)	1°: 1:1,000 2°: 1:5,000–1:50,000	1°: 1:1,000 2°: 1:5,000–1:10,000	1°: 1:500 2°: 1:2,000–1:20,000	1°: 1:500 2°: 1:2,000–1:20,000
Cat. No.	34065	37574	34000	34012

Chromogenic AP substrates

Nitro blue tetrazolium (NBT) 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. 5-bromo-4-chloro-3-indolyl phosphate (BCIP) hydrolysis by AP results in a blue-purple precipitate that can be deposited on nitrocellulose or nylon membranes. BCIP can be used as a chromogenic substrate for both immunoblotting and immunohistochemical studies. An ideal system for blotting applications with AP is the combination of NBT and BCIP. Together, they yield an intense black purple precipitate that provides much greater sensitivity than either substrate alone (Table 22 and Figure 36).

This reaction proceeds at a steady rate, allowing accurate control of its relative sensitivity. NBT/BCIP characteristically produces sharp band resolution with little background staining of the membrane.

Table 22. Properties of Thermo Scientific™ AP-based chromogenic substrate.

	1-Step NBT/BCIP Substrate Solution
Detection limit	30 pg
Color	Black-purple
Format	Ready-to-use solution
Recommended antibody dilutions (from 1 mg/mL stock)	1°: 1:500 2°: 1:5,000–1:50,000
Cat. No.	34042

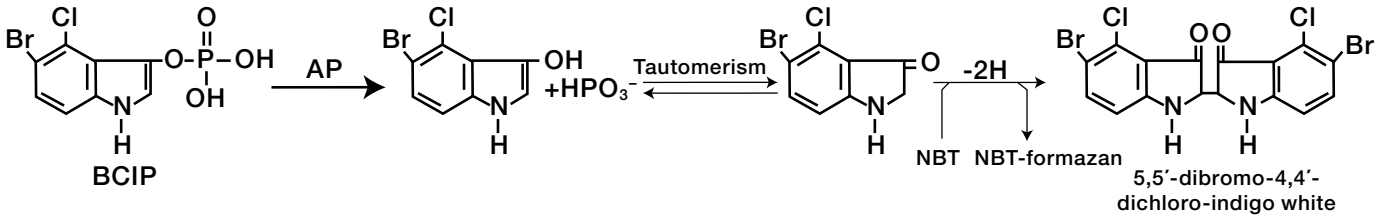


Figure 36. Chemical reaction of NBT and BCIP substrates with AP. BCIP is hydrolyzed by alkaline phosphatase to form an intermediate that undergoes dimerization to produce an indigo dye. The NBT is reduced to the NBT-formazan by the two reducing equivalents generated by the dimerization. This reaction proceeds at a steady rate, allowing accurate control of the relative sensitivity and control of the development of the reaction.

Pierce Metal Enhanced DAB Substrate Kit

Thermo Scientific™ Pierce™ Metal Enhanced DAB Substrate Kit uses cobalt and nickel chloride in a special formulation of diaminobenzidine peroxidase substrate that yields intense color for immunohistochemical staining using HRP.

Features:

- **Superior sensitivity**—50 times more sensitive than the traditional DAB method and 30 times more sensitive than other metal-intensified versions of DAB
- **Low background, high intensity**—get a crisp, dark brown-black precipitate that is more intense than the dull brown precipitate when using DAB without enhancement; even with the increased intensity, background is almost nonexistent
- **Only two components**—simply mix the two liquid components and your working solution is ready to use
- **6-hour stability**—the innovative working solution is stable for more than 6 hours at room temperature, while other DAB substrates must be used immediately

1-Step Ultra TMB-Blotting Solution

Thermo Scientific™ 1-Step Ultra TMB-Blotting Solution is an enhanced single-component HRP substrate for western blotting. This precipitating, colorimetric western blot substrate for HRP provides high sensitivity, increased signal-to-noise ratio, and low background compared to other chromogenic substrates (Figure 37). The blotting solution contains soluble TMB (3,3',5,5'-tetramethylbenzidine), which reacts very quickly with HRP enzyme to produce an insoluble dark blue precipitate that does not fade or flake. The substrate is compatible with both nitrocellulose and PVDF membranes. The blotting solution is supplied ready to use with no mixing required.

Features:

- **Fast**—protein bands visible in less than one minute
- **Fade-resistant**—protein bands stable after membrane drying
- **Sensitive**—detection limit similar to Thermo Scientific™ Pierce™ ECL Western Blotting Substrate
- **Chromogenic**—no special equipment needed for visualization; produces dark blue bands
- **Ready to use**—no organic solvents are required to dissolve; no dilution necessary for use

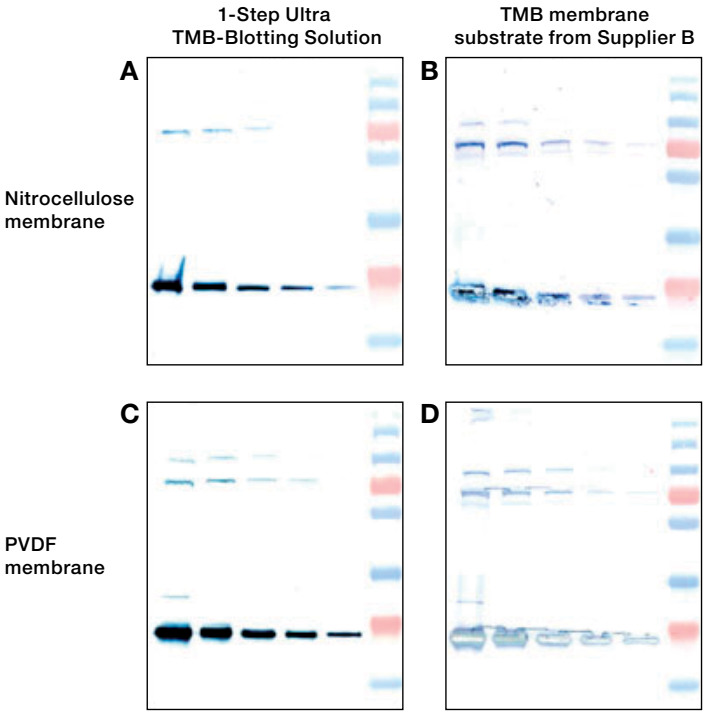


Figure 37. Detection with 1-Step Ultra TMB-Blotting Solution and another commercial TMB substrate. Serial dilutions of HepG2 cell lysates (15, 7.5, 3.45, 1.88, 0.94 µg) were prepared and separated by electrophoresis. The proteins were transferred to **(A, B)** nitrocellulose membranes and **(C, D)** PVDF membranes. The membranes were blocked with Pierce Clear Milk Blocking Buffer at 1X. After blocking, the membranes were incubated with Invitrogen™ Mouse PLK-1 Monoclonal Antibody and Rabbit Cyclophilin B Polyclonal Antibody. The membranes were washed and then incubated with 0.2 µg/mL of Invitrogen™ Goat Anti–Mouse IgG (H+L) Secondary Antibody, HRP Conjugate, and Goat Anti–Rabbit IgG (H+L) Secondary Antibody, HRP Conjugate, and then washed again. The membranes were placed in **(A, C)** 10 mL of 1-Step Ultra TMB-Blotting Solution, and **(B, D)** TMB substrate from Supplier B. The color development was stopped at **(C, D)** 3 minutes and **(A, B)** 5 minutes by rinsing the membranes with ultrapure water.

Pierce CN/DAB Substrate Kit

Thermo Scientific™ Pierce™ CN/DAB Substrate Kit includes substrate and peroxide solutions for combined chloronaphthol- and diaminobenzidine-based detection of HRP activity in western blot and tissue staining methods. This kit combines two popular chromogenic peroxidase substrate compounds (4-CN and DAB) in a single, stable, 10X stock solution. This CN/DAB solution produces an intense dark black precipitate at sites of bound HRP-conjugated antibodies on probed blots and tissue samples. The kit includes the 10X solution of CN/DAB and accompanying 1X stable peroxide substrate buffer.

Features:

- **HRP substrate**—for detection of HRP activity on solid media via the combined action of 4-CN and DAB
- **Chromogenic**—no special equipment needed for visualization; yields intense black bands that are easy to photograph
- **Easy-to-use kit**—substrate stock solution and stable peroxide substrate buffer mean that there's no dry powders to measure and dissolve
- **Stable**—store refrigerated at 4°C for at least one year

1-Step Chloronaphthol Solution

Thermo Scientific™ 1-Step Chloronaphthol Solution is a solution of 4-chloro-1-naphthol (4-CN) for the chromogenic detection of HRP activity in western blot and tissue staining methods. Chloronaphthol reacts with HRP to form a distinct blue to blue-purple product to localize and visualize the activity of peroxidase-conjugated antibodies on blots or fixed-tissue samples. The HRP substrate is not as sensitive or stable as other chromogenic substrates, such as TMB and DAB. However, the blue-purple precipitate photographs well, and its alcohol solubility is a property that can be utilized in certain double-staining applications.

Features:

- **Ready to use**—no ethanol or methanol required to dissolve; no dilution necessary for use
- **Complete**—does not require buffer or addition of hydrogen peroxide
- **Pre-filtered**—does not need to be filtered before use like powders or tablets after they are dissolved
- **Stable**—store refrigerated at 4°C for at least one year

Pierce NBT and BCIP substrates

Thermo Scientific™ NBT and BCIP substrates are powders and ready-made solutions of nitro blue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) for chromogenic blot and IHC staining with an alkaline phosphatase probe, yielding an intense, insoluble black purple precipitate. The NBT and BCIP powders are available individually and in two convenient, ready-to-use Thermo Scientific™ 1-Step™ NBT/BCIP substrate solutions (with and without a levamisole suppressor of endogenous phosphatase activity).

Features:

- Best for immunoblotting applications
- Sensitive with low background and minimal assay-to-assay variability
- Choose individual NBT and BCIP substrate powders, or preformulated, ready-to-use 1-Step NBT/BCIP substrate solutions, with or without levamisole suppressor

WesternBreeze AP chromogenic kits

The Invitrogen™ WesternBreeze™ chromogenic kits yield high-sensitivity results with extremely low background without the need for any additional equipment—just watch the signal develop over a short period of time. The kit uses the chromogenic phosphatase substrate BCIP and electron-transfer agent NBT to produce a dark blue precipitate at the precise site of enzymatic activity on the blot.

The WesternBreeze kit has been optimized to provide picogram sensitivity. Each kit includes ready-to-use and easy-to-dilute blockers, primary antibody diluent, wash solution, conjugated secondary antibody solution (either AP-conjugated anti-mouse, anti-rabbit, or anti-goat), substrate, and two convenient incubation trays.

Features:

- Clear background
- High sensitivity, low picogram levels detectable
- High specificity
- One simple protocol, no optimization required

Fluorescent western blot detection

Fluorescence-based western blot detection differs from other detection systems in that the signal produced is not a product of an enzyme reaction, but rather a transient light emission resulting from the excitation and subsequent release of photons as the excited fluorophore returns back to its normal state (Figure 38). Fluorescence-based western detection can be more quantitative than enyzme-based chemiluminesence detection because the signal is typically more stable over time.

Fluorescence-based western blotting is growing in popularity because it offers increased time savings over chemiluminescent detection and reduced chemical waste compared to both chemiluminescent and chromogenic detection systems. Historically, the instrumentation available for fluorescent detection has not been able to offer the sensitivity required by many researchers or was prohibitively expensive. However, with the advancements in imaging technology, new fluorescent probe development, and the reduced cost of both, fluorescent detection systems are quickly replacing chromogenic and chemiluminescent detection methods in many laboratories. While the detection limits are still not as low as chemiluminescent detection, fluorescent detection has the unique advantage of allowing multiple targets to be assayed for on the same blot at the same time without the need to strip and reprobe (known as multiplexing).

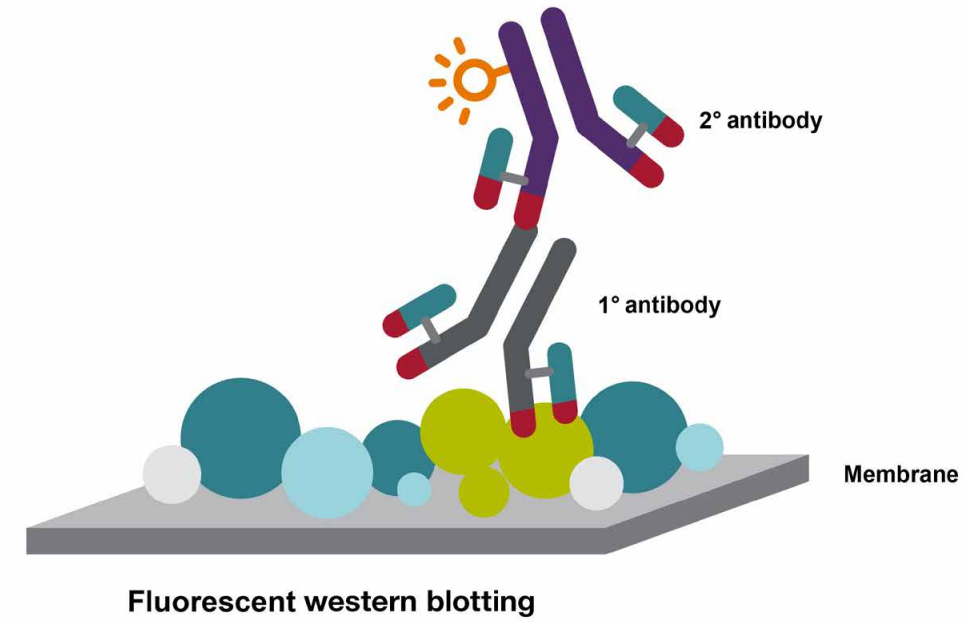


Figure 38. Overview of fluorescent western blot detection.

Watch a how-to video **here**.

Alexa Fluor and Alexa Fluor Plus secondary antibodies

The wide range of Invitrogen™ Alexa Fluor™ and Alexa Fluor™ Plus secondary antibodies for western blotting provides multiple options of nonoverlapping spectra to better facilitate multiplex analysis. Multiple targets can be detected independently in the same lane and blot with clearly distinguishable colors. Alexa Fluor Plus secondary antibodies combine enhanced sensitivity and low background for higher signal-to-noise ratios and better multiplexing results.

Features:

- Detection of low-abundance targets; up to 5.8 times higher signal-to-noise ratio in fluorescent western blotting
- High cross-adsorption to minimize cross-reactivity for low background and better multiplexing results
- Improved sensitivity and range of linear detection to provide more detail (Figure 39)
- Increased photostability and superior brightness

Alexa Fluor Plus secondary antibodies are available for key target species and are labeled with a wide range of superior dyes across the color spectrum (488–800 nm) for multiplex western blotting applications (Table 23).

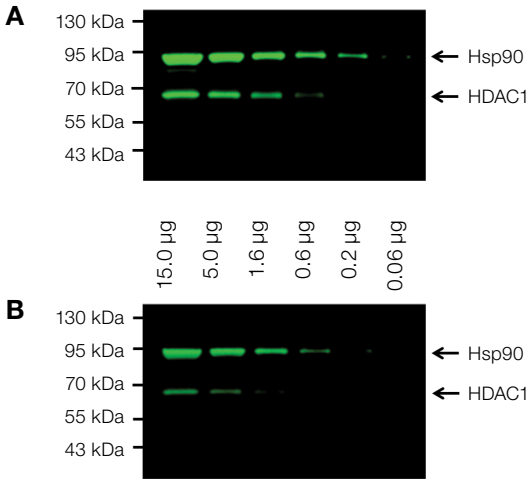


Figure 39. Comparison of detection sensitivity. (A) Invitrogen™ Alexa Fluor™ Plus 800 Secondary Antibody shows more bands at higher dilutions, demonstrating greater sensitivity and range of detection compared to **(B)** another supplier's fluorophore-conjugated secondary antibody.

Table 23. Alexa Fluor Plus secondary antibodies are available in a wide range of target species and wavelengths.

	Alexa Fluor Plus 405	Alexa Fluor Plus 488	Alexa Fluor Plus 555	Alexa Fluor Plus 594	Alexa Fluor Plus 647	Alexa Fluor Plus 680	Alexa Fluor Plus 800
Goat anti-rabbit	A48254	A32731	A32732	A32740	A32733	A32734	A32735
Goat anti-mouse	A48255	A32723	A32727	A32742	A32728	A32729	A32730
Goat anti-chicken	A48260	A32931	A32932	A32759	A32933	A32934	A32935
Goat anti-rat	A48261	A48262	A48263	A48264	A48265	—	—
Goat anti-human	A48275	A48276	A48277	A48278	A48279	—	—
Donkey anti-goat	A48259	A32814	A32816	A32758	A32849	A32860	A32930
Donkey anti-rabbit	A48258	A32790	A32794	A32754	A32795	A32802	A32808
Donkey anti-mouse	A48257	A32766	A32773	A32744	A32787	A32788	A32789

Did you know

All Alexa Fluor Plus secondary antibodies are cross-adsorbed against potential cross-reactive species. Cross-adsorption or pre-adsorption is a purification step to increase specificity of the antibody resulting in less background staining and cross-reactivity. The secondary antibody solution is passed through a column matrix containing immobilized serum proteins from potentially cross-reactive species. Only the nonspecific-binding secondary antibodies are captured in the column, and the highly specific secondary antibodies flow through. Further passages through additional columns result in highly cross-adsorbed preparations of secondary antibody. The benefits of these extra steps are apparent in multicolor-staining experiments where there is potential cross-reactivity with other primary antibodies.

Chemiluminescence and fluorescence detection data capture

With chemiluminescence-based detection methods, the chemical reaction (HRP- or AP-driven) produces light that can be captured with X-ray film or a capable western blot imaging instrument. When capturing chemiluminescent signal with X-ray film, it is necessary to expose several pieces of film for different time periods in an attempt to obtain the proper balance between signal and background. The goal is to time the exposure so that the desired signal is clearly visible while the background remains low. This is difficult to accomplish since the process cannot be observed and stopped when the desired endpoint is reached.

If the film is underexposed, the signal will not be visible. If the film is overexposed, the signal may be saturated, lost in the background, or once-distinguishable bands may become blurred together. All situations make post-signal capture data analysis challenging. Although X-ray film provides qualitative and semi-quantitative data and is useful to confirm the presence of target proteins, purpose-built western blot imaging instruments can offer higher sensitivity and broader dynamic range than film, which makes it possible to detect more subtle differences between samples. Furthermore, you don't have to spend time in the darkroom exposing your blot to film and developing the film thereafter. With western blot imaging instruments, the data are captured digitally, which negates the need to scan X-ray film to perform post-capture analysis. Furthermore, many of these systems offer auto-exposure algorithms to help best optimize signal-to-noise ratio.

Although X-ray film is inexpensive, consider the expenses of maintaining a darkroom and the associated developing equipment. Modern western blot imaging instruments may cost more up-front than the typical expense of X-ray film, but these instruments can often capture signal for multiple applications (such as colorimetric protein gel stains) in addition to chemiluminescent western blot signals, which adds value to the overall investment.

Several manufacturers offer fluorescence western blot imaging systems capable of capturing fluorescence signals, most of which use either filter-based or laser-based technologies to deliver the appropriate excitation wavelength and then record the emission light output. The majority of the available instrumentation is CCD camera–based, but scanning systems with photoavalanche diode–based sensors are also available. Captured images are saved digitally. The number of commercially available fluorescently labeled antibodies with different excitation and emission spectra continues to increase, which provides multiple options to the end user based on preference or the selected instrument's imaging capability.

When used with an imager equipped with the appropriate filters or lasers, fluorophores with nonoverlapping spectra enable multiplex analysis, in which multiple targets can be detected and independently distinguished in the same blot. This is a unique attribute compared to the popular chemiluminescence-based detection, which would otherwise require multiple rounds of stripping and reprobing the blot in order to visualize signal from each protein of interest. With each round of stripping and reprobing, one risks stripping protein from the blot, which would make the comparison between different proteins of interest challenging. Thus, multiplexing helps make research more efficient and productive. For example, one can visualize a protein of interest simultaneously with a loading control protein (Figure 40), differentiate proteins of similar molecular weights (Figure 41), and evaluate complex biological pathways (Figure 42). In multiplexing terminology, probing for 2 proteins of interest is known as a 2-plex experiment, probing for 3 proteins of interest is known as a 3-plex experiment, and so on. With the Invitrogen™ iBright™ FL1500 Imaging System, one can perform up to a 4-plex fluorescent western blot with the appropriate experimental setup (Figure 43).

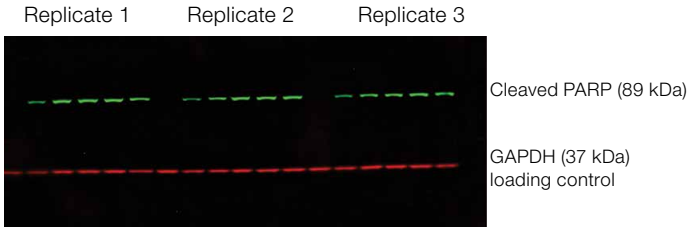


Figure 40. Simultaneous detection of protein of interest and loading control protein. The signal of each protein is captured in a different fluorescence channel, which enables the detection of two proteins on a single blot without stripping and reprobing. A composite image is shown, overlaying the signals from each probe.

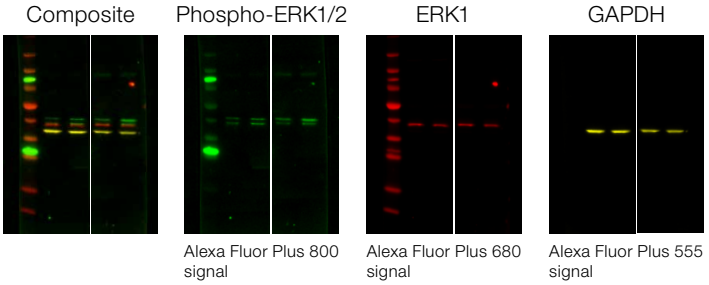


Figure 41. Detection of targets of similar molecular weights. Fluorescent multiplexing allows for clear distinction of multiple targets on the same blot, even when they are of similar molecular weights. A composite image is shown along with images showing the single-color signals of individual proteins. Visualizing the individual signals can sometimes enable assessment of details that may be harder to see in a composite.

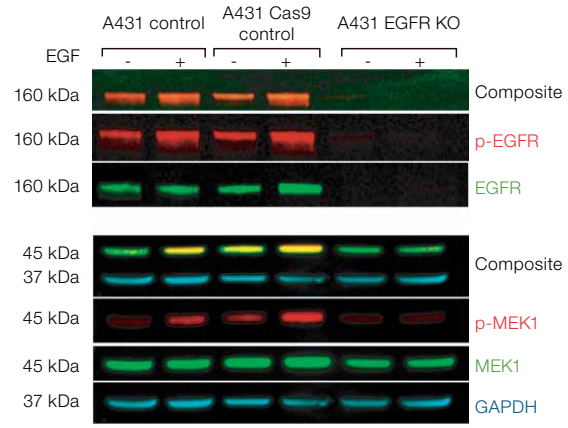


Figure 42. Evaluation of complex biological signaling pathways. Western blot analysis of the phosphorylation of EGFR and its downstream target, MEK1, after EGF treatment of A431 EGFR knockout (KO) cells.

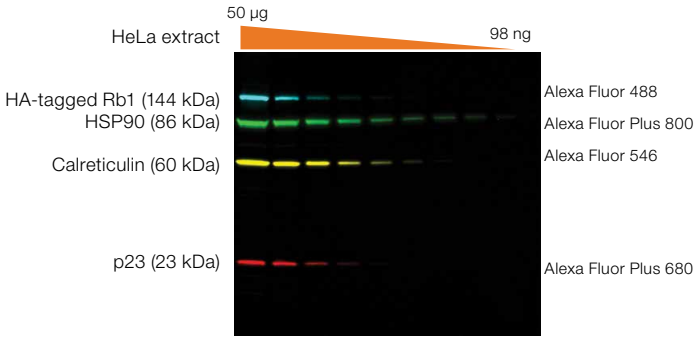


Figure 43. A 4-plex western blot imaged on an iBright Imaging System.

iBright Imaging Systems

Experience an easier time capturing and analyzing data from gels and western blots with Invitrogen™ iBright™ Imaging Systems. Designed with a streamlined, intuitive interface and workflows, iBright Imaging Systems are easy to use for researchers of all experience levels.

There are three models in the iBright Imaging Systems family: the iBright™ CL750 Imaging System, the iBright™ CL1500 Imaging System, and the iBright™ FL1500 Imaging System. The iBright CL750 Imaging System offers the core essential western blot and gel imaging functions and makes the transition from the darkroom and film easy. The iBright CL1500 Imaging System expands application support and has many of the high-performance specifications of our premier iBright FL1500 model. The iBright FL1500 Imaging System features maximum application support, including fluorescent western blot imaging with up to four fluorescence channels at a time.

Features:

- **Push-button, optimized exposure**—Smart Exposure™ acquisition technology for the rapid determination of optimal exposure times helps minimize the need to repeat exposures to get the desired signal
- **Powerful 9.1 megapixel (MP) camera**—capture crystal-clear images with robust imaging potential
- **Advanced automated features**—automatic sample rotation, automatic zoom, automatic focus, and automatic onboard data analysis provide a smooth imaging experience
- **Five-channel fluorescent blotting**—multiplex with the five fluorescence channels of the iBright FL1500 model; capture up to four proteins in a single blot for more meaningful and representative experiments
- **Compliance support**—all models offered with 21 CFR Part 11 compliance support software packages to set up and control security, audit, and e-signature settings

View overview video:

Capture your data faster and more easily than ever before.
Learn more at **thermofisher.com/ibright**

iBright Imaging Systems offer up to five imaging modes to support your multiple applications (Table 24). Efficiently and easily capture data from protein gels, nucleic acid gels, chemiluminescent western blots, fluorescent western blots, and more (Figure 44).

Table 24. Imaging modes available on iBright Imaging Systems.

Imaging mode	What kind of signal can be captured?
Protein gel	Colorimetric staining of gels (e.g., Coomassie, silver) and membranes (e.g., Ponceau S, Thermo Scientific™ Pierce™ Reversible Protein Stain), fluorescent staining of gels (e.g., Invitrogen™ SYPRO™ Ruby stain)
Nucleic acid gel	Ethidium bromide and Invitrogen™ SYBR™ stains
Chemiluminescent blot	Chemiluminescence using all popular HRP and AP substrates (e.g., Thermo Scientific™ SuperSignal™ and Invitrogen™ WesternBreeze™ substrates)
Fluorescent blot	Fluorescence with popular RGB (visible range) and near-IR fluorophores (e.g., Invitrogen™ Alexa Fluor™ and Alexa Fluor™ Plus conjugates)
Universal	Custom mode to image samples containing multiple signals, such as chemiluminescence, fluorescence, colorimetric stains, and/or visible images; image display is similar to fluorescent blot mode and allows one to assign false color to any sample

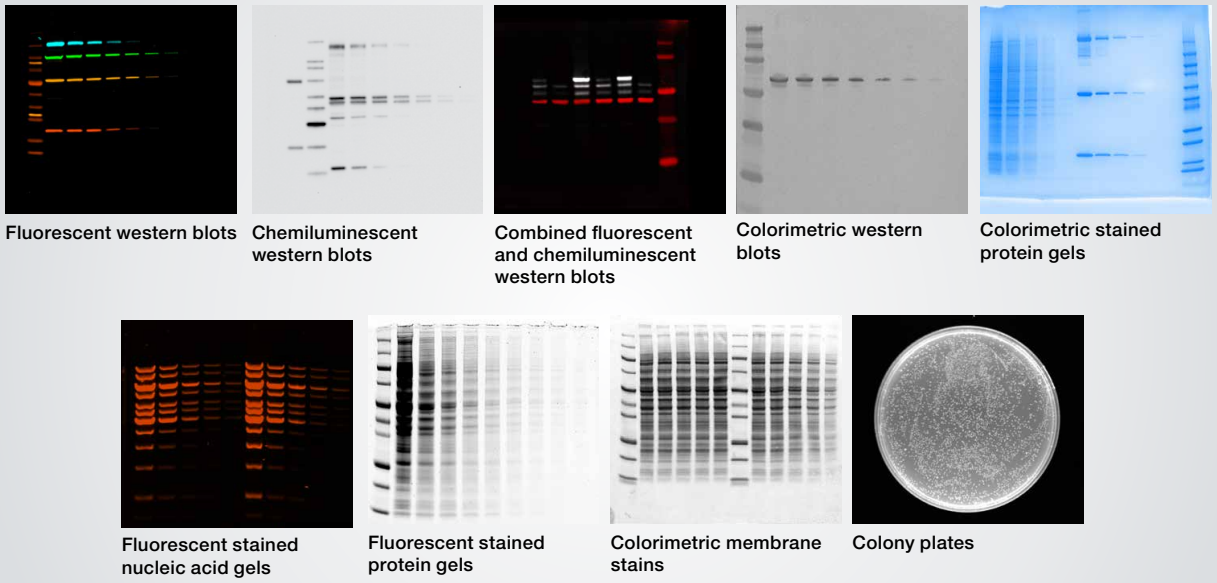


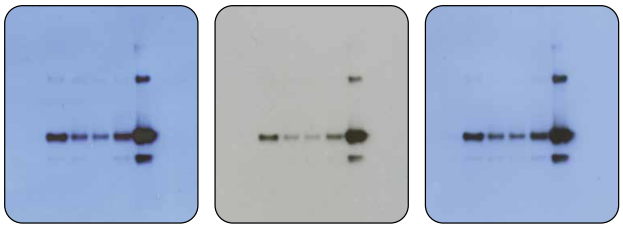
Figure 44. Example imaging applications. Images pictured for fluorescent stained nucleic acid gels and colorimetric stained protein gels shown in pseudocolor (false color applied). Data is captured in grayscale.

CL-XPosure Film

Thermo Scientific™ CL-XPosure™ Film is an economically priced clear–blue X-ray film for detection and documentation of chemiluminescent western blots and other chemiluminescent protein or nucleic acid assay methods. CL-XPosure Film is an excellent choice for ECL-type substrates for HRP or AP. Convenient sizes are offered for standard film cassettes and film developing equipment. The film also works well for chemiluminescent or isotopic DNA and RNA methods, including Southern and northern blotting and electrophoretic mobility shift assays (EMSAs).

Features:

- **High performance**—as sensitive as other commercially available autoradiography films (Figure 45)
- **Affordable**—costs up to 75% less than other brands of comparable quality X-ray film
- **Convenient**—available in five sheet sizes in packages containing 100 non-interleaved sheets



CL-XPosure Film | Kodak™ BioMax™ MR-1 Film | Kodak™ X-Omat™ Blue (XB) Film

Figure 45. CL-XPosure Film vs. Kodak Film. Three types of X-ray film (2 blue, 1 gray) were tested using identical western blotting conditions. The results showed no appreciable difference between any of these films. The only significant difference is the cost per sheet of film.

Did you know

Western blot X-ray film can be developed entirely by hand.

Normalization and quantitation in western blot detection

As the amount of protein loaded on an electrophoresis gel can vary greatly from sample to sample, leading journals have developed protein normalizing guidelines for submitting quantitative western blot data.

One common method of protein normalization relies on measuring the abundance of a stably expressed housekeeping protein that is endogenous to all the samples in an experiment. Proteins such as α-tubulin, β-actin, or glyceraldehyde 3-phosphate dehydrogenase (GAPDH) are commonly used as loading controls for protein normalization, but there are potential drawbacks, as the expression of housekeeping proteins can vary with experimental conditions and they often have saturated western blotting signals. These drawbacks can be minimized with optimization, but this optimization can be time-consuming. One must first confirm that the signal obtained for the housekeeping protein is linear over a wide concentration range, such that it can be used as a reliable reference for normalization, and that it is unaffected by the conditions of the experiment. The use of antibodies to detect housekeeping proteins may require the extra steps of stripping and reprobing the blot to detect the experimental targets, which adds another layer of time investment.

An alternative protein normalization method is total target protein normalization for posttranslationally modified proteins. Targets with posttranslational modifications (e.g., phosphorylation, glycosylation, acetylation, and ubiquitination) are normalized to their respective total protein using a pan antibody that recognizes the target regardless of modification state. There are multiple drawbacks to using this method to normalize for posttranslationally modified target protein. The pan or total target protein antibody must recognize all posttranslationally modified versions of the target as well as the unmodified target. In addition, the expression of the total protein target used for normalization must be constant across all experimental conditions. Further, the linear dynamic range for this method is often narrow.

Total protein normalization (TPN) is a better alternative to using housekeeping proteins or total target protein normalization for posttranslationally modified proteins. It avoids the variability and inaccuracy of using housekeeping proteins, the time-consuming effort to optimize conditions, and the cost of the immunodetection reagents to detect the normalization targets.

For TPN, the total protein on a blot is labeled or stained to enable the relative amount of protein loaded in each lane to be compared and normalized to a reference lane. Ponceau S and several reversible fluorescent dyes are available to stain the total protein on a blot for TPN. A shortcoming of these dyes is that they must be removed before the western blotting antibody detection steps, requiring the blots to be imaged after staining, followed by removal of the dye before moving to the immunoblotting steps. The finished western blot must be imaged again at the end of the process before moving on to normalization of the results. These staining, destaining, and multiple imaging steps are time-consuming.

Other than using blot stains like Ponceau S for TPN, an alternative method has become available. However, this method requires the purchase and use of a specialized gel that may yield very different results from your current optimized system for separating your target proteins. The method requires a specific gel imager to capture the image and requires the total protein on the blot to be imaged before the immunoblotting step, as the chemiluminescent signal from the target proteins interferes with the total protein signal on the blot.

Invitrogen™ No-Stain™ Protein Labeling Reagent offers the benefits of TPN and also allows you to use your gel of choice. Labeling the blotted proteins on a membrane after protein transfer is easy (Figure 46). Prepare a working solution of No-Stain Protein Labeling Reagent by adding No-Stain Activator and No-Stain Derivatizer to 1X No-Stain Labeling Buffer, and incubate with the membrane for 10 minutes.

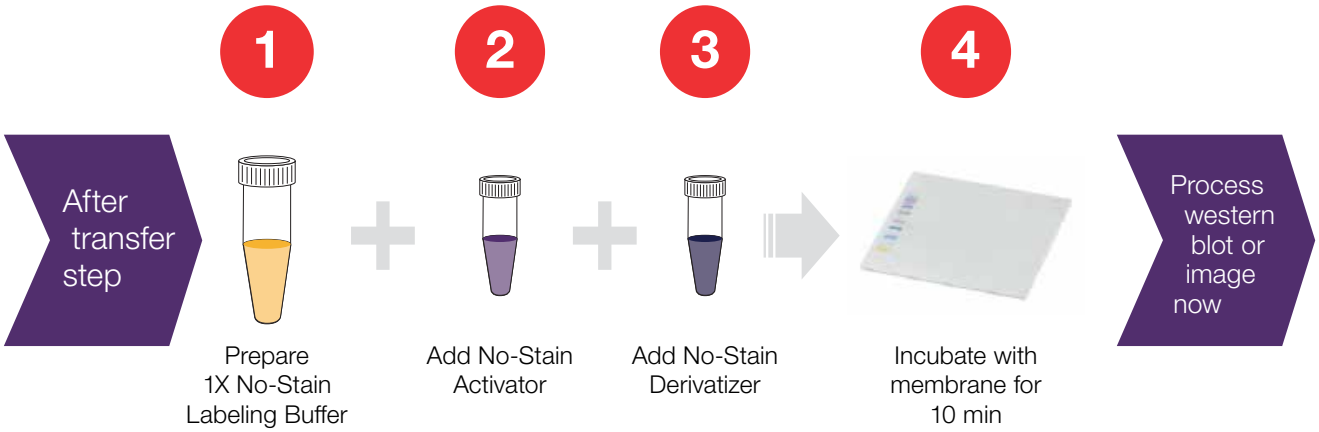


Figure 46. Labeling a protein blot with No-Stain Protein Labeling Reagent after transfer.

Since it forms covalent bonds with a portion of the lysines in the total-protein population, the No-Stain reagent signal is stable, and it can be imaged at the same time as the fully processed immunoblot. It can be used with PVDF or nitrocellulose membranes and is compatible with downstream antibody detection using chemiluminescence or fluorescence methods.

View how-to video:

No-Stain Protein Labeling Reagent

The use of housekeeping proteins for normalization of western blots has its drawbacks, as the expression of housekeeping proteins can vary with experimental conditions, and these proteins can often have oversaturated western blotting signals that interfere with quantitative analysis. Total protein normalization using Invitrogen™ No-Stain™ Protein Labeling Reagent avoids the need for housekeeping protein detection, thereby overcoming the variability and inaccuracy of using housekeeping proteins for normalization.

Features:

- **Flexible-use labeling format**—use with any protein gel to perform total protein labeling of the membrane post-transfer, or use it as a fast protein stain after electrophoresis of gels you do not intend to transfer
- **Easy-to-use and rapid protocol**—mix and incubate with the post-transfer membrane (PVDF or nitrocellulose) or gel to label lysine residues; the reaction time is only 10 min
- **Flexible visualization**—visualize with UV or blue LED transilluminators, or by using imaging systems with fluorescence (~488 nm) light sources, including iBright Imaging Systems
- **Accurate total protein normalization**—broad linear range for protein detection of 1–80 µg (total protein load; Figure 47); protein bands are detected down to 20 ng and signal is compatible with antibody detection using chemiluminescence or fluorescence methods

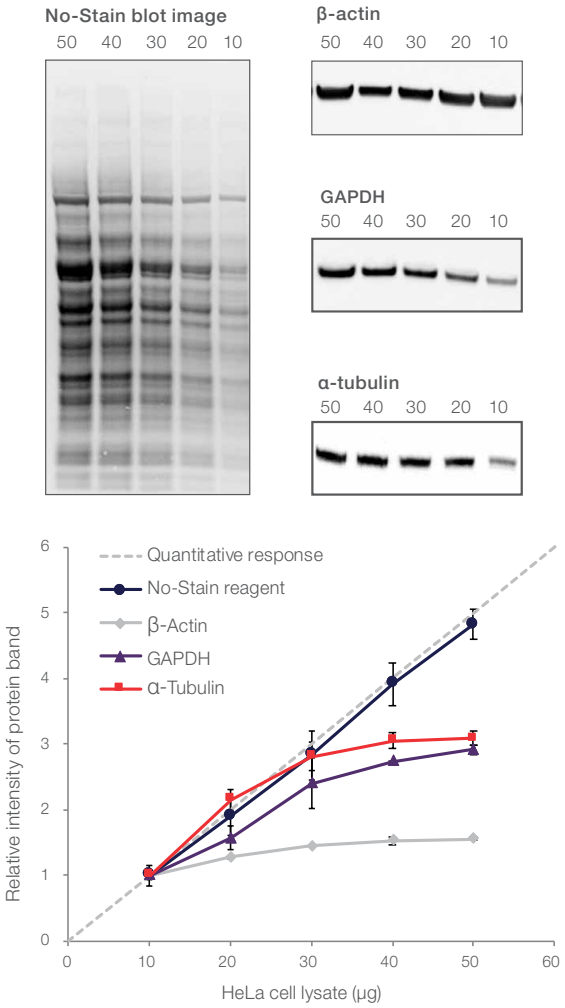


Figure 47. Total protein normalization with No-Stain Protein Labeling Reagent. Bolt 4–12% Bis-Tris Plus gels were loaded with HeLa cell lysate ranging from 10 to 50 µg. Proteins from the gels were transferred onto PVDF membranes. The PVDF membranes were washed with ultrapure water and labeled with No-Stain labeling solution. The membranes were then washed with ultrapure water, followed by immunoblotting for β-actin, GAPDH, and α-tubulin antibodies, followed by goat anti-mouse Alexa Fluor Plus 680 antibody. The blot was imaged and analyzed with the iBright FL1500 Imaging System. The linear regression value for the entire concentration range using the No-Stain reagent was R^2 = 0.9990, whereas the R^2 values for β-actin, GAPDH, and α-tubulin were 0.8851, 0.9438, and 0.8332, respectively.

Learn more at **thermofisher.com/no-stain**

Post-detection and signal enhancement

Stripping and reprobing western blots

One of the major advantages offered by chemiluminescent and fluorescent detection methods is the ability to strip reagents from the blot and then reprobe the same blot under modified or new conditions. This is a useful technique when optimizing antibody concentrations, signal-to-noise ratios, or when multiple detection experiments are to be performed on the same blot (Table 25). With chemiluminescence and fluorescence, all of the reagents can be removed (stripped) from the membrane, in contrast to chromogenic detection where a colored precipitate is deposited on the membrane.

Stripping western blot membranes

The key to stripping a membrane is to use conditions that allow the release of antibody from the target protein, without releasing a significant amount of protein from the membrane. Various protocols have been developed to accomplish this purpose, and they generally include some combination of detergent, reducing agent, heat, and low pH. During the stripping procedure, some amount of protein is inevitably lost from the membrane, making it important to minimize this loss by stripping the antibody under gentle conditions. Because each antibody–target pair has unique characteristics, there is no guaranteed method to remove every antibody while preserving the target protein.

Testing and reprobing stripped blots

After any stripping procedure, test the blot to ensure that all of the detection reagents have been removed. To do this, wash the membrane several times, block, incubate with secondary antibody, and then reincubate with the detection substrate. If the primary antibody was effectively removed by the stripping procedure, no secondary antibody should bind to the membrane and no signal should 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 may be necessary to alter the composition of the stripping buffer or change methods. A variety of Thermo Scientific™ Restore™ stripping buffers are available to make the optimization process easier (Table 26).

Table 25. Advantages to restripping and reprobing western blots.

Conserves sample	When the protein mixture is rare or valuable, reprobing conserves the sample and allows the membrane to be reanalyzed 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.
Saves money	By reusing the same blot, you save money on the costs of membrane, buffers, and protein sample.
Assay optimization is easier	The signal intensity of high-sensitivity chemiluminescent substrates often requires antibody concentration optimization to achieve the highest-quality blot. Optimization is achieved easily by stripping the membrane and reprobing with a different antibody concentration.
Quickly 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 to run new samples.
Correct mistakes	Immunoblotting requires many steps, providing ample opportunity for mistakes to occur. By stripping the membrane, the blot can be reused.

Table 26. Stripping buffer selection guide.

	Restore Western Blot Stripping Buffer	Restore PLUS Western Blot Stripping Buffer	Restore Fluorescent Western Blot Stripping Buffer
Features	Gentle, odor-free	Robust yet gentle, odor-free	Optimized for NIR fluorescent blotting
Ready to use	Yes	Yes	Yes
Membrane	Nitrocellulose and PVDF	Nitrocellulose and PVDF	Use with low-fluorescence PVDF membrane
Time of incubation	15–30 min at room temperature	5–15 min at room temperature	15 min at room temperature
Select when …	Primary antibody is susceptible to stripping buffers	Using high-affinity primary antibody	Using NIR-labeled antibody
Applications	• Detect different targets • Optimize antibody concentrations	• Designed for use with antibodies that are difficult to remove from western blots, require longer incubation times, or incubation temperatures greater than 22°C	• Gentle and highly effective reagent for quickly removing primary and NIR dye-labeled secondary antibodies from western blots

Restore Western Blot Stripping Buffer

Thermo Scientific™ Restore™ Western Blot Stripping Buffer safely and effectively removes primary and secondary antibodies from nitrocellulose and PVDF membranes to allow chemiluminescent western blots to be reprobed. By stripping and reprobing, there is no need to waste rare or costly samples by running multiple gels in order to probe for different targets (Figures 48–50). The procedure with Restore stripping buffer only takes 15 to 30 minutes, depending on the affinity of the primary antibody.

Features:

- **Saves time**—no need to rerun gels and blots
- **Saves costly sample**—reprobe the membrane using the same target sample
- **Effective**—formulation is more efficient at stripping antibodies than homemade buffers
- **Gentle**—does not damage the target protein during stripping, allowing efficient reprobing
- **Odor-free**—no mercaptans means no acrid odors
- **Economical**—less expensive than other commercial stripping buffers

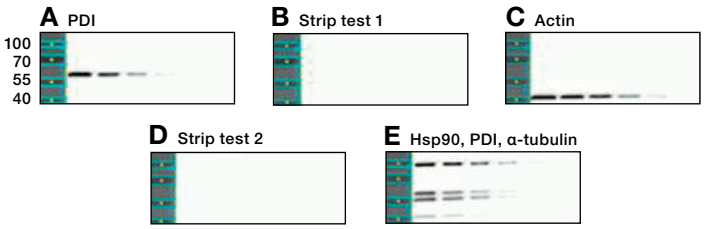


Figure 48. Stripping and reprobing blots for similar molecular weight targets with Restore Western Blot Stripping Buffer. A431 cell lysate was diluted to 125 µg/mL in electrophoresis reducing sample buffer, and 2-fold serial dilutions were made. 10 µL of each dilution (1,250 ng to 39 ng of total protein) was separated by SDS-PAGE and transferred to a 0.45 µm nitrocellulose membrane. The membrane was blocked with 5% nonfat dry milk in 1X Thermo Scientific™ Pierce™ PBS Tween™ 20 Buffer and analyzed by western blot using SuperSignal West Dura Extended Duration Substrate and imaged. **(A)** The first target was detected by probing with Invitrogen™ PDI Monoclonal Antibody at 0.33 µg/mL, followed by Invitrogen™ Goat Anti–Mouse IgG HRP Conjugate at 6.7 ng/mL and imaged. **(B)** Next, the blot was stripped in Restore Western Blot Stripping Buffer for 15 minutes at 37°C, washed in 1X Pierce PBS Tween 20 Buffer, incubated with substrate, and imaged to check for stripping efficiency. **(C)** The second target was detected by reblocking the membrane and probing with Invitrogen™ Actin Monoclonal Antibody at 0.5 µg/mL, followed by the anti–mouse IgG HRP conjugate at 6.7 ng/mL and imaged. **(D)** The blot was stripped again and then **(E)** probed for multiple targets (α-tubulin at 0.2 µg/mL, PDI at 0.33 µg/mL, and hsp90 at 0.14 µg/mL), and imaged as described above.

View how-to video:

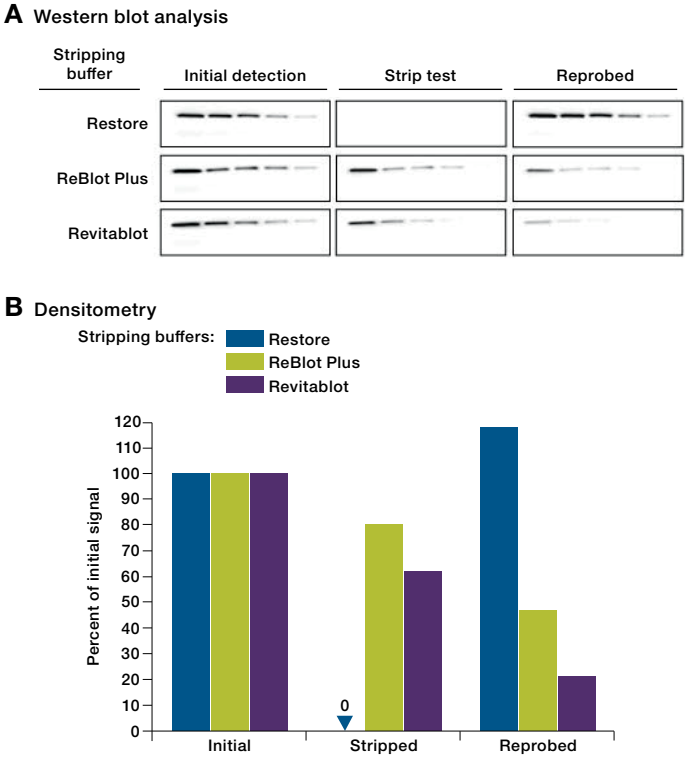


Figure 49. Western blot stripping and reprobing performance of three stripping buffers on nitrocellulose. HeLa cell lysate was diluted to 1 mg/mL in electrophoresis reducing sample buffer, and 1:1 serial dilutions were made. Three sets of 10 µL per dilution (10 µg to 0.31 µg of total protein) were separated by SDS-PAGE and transferred to 0.45 µm nitrocellulose membrane. **(A)** The membrane was blocked with 5% nonfat dry milk in 1X Pierce TBS Tween 20 Buffer and analyzed by western blot using SuperSignal West Dura Extended Duration Substrate and imaged. The membrane was probed with Invitrogen™ Hsp90 Polyclonal Antibody at 0.5 µg/mL followed by Invitrogen™ Goat Anti–Rabbit IgG, HRP Conjugate, at 5.7 ng/mL, and imaged. Following the initial detection, the blot was cut into three strips to separate the serial dilution sets, and each part of the blot was stripped, according to manufacturer's instructions, in either Restore Western Blot Stripping Buffer (15 minutes at 37°C), ReBlot Plus Stripping Solution (MilliporeSigma; 15 minutes at room temperature), or Revitablot™ Western Blot Stripping Buffer (Rockland Immunochemicals Inc.; 15 minutes at room temperature). After the stripping procedure, the membrane strips were washed in 1X PBS Tween 20 buffer and incubated with the substrate and imaged. The membrane strips were reblocked, and the western blot procedure was repeated as described above. **(B)** Densitometry analysis shows that Restore Western Blot Stripping Buffer permitted complete signal removal and maintained nearly identical levels of detection between the initial and reprobed western blot analysis.

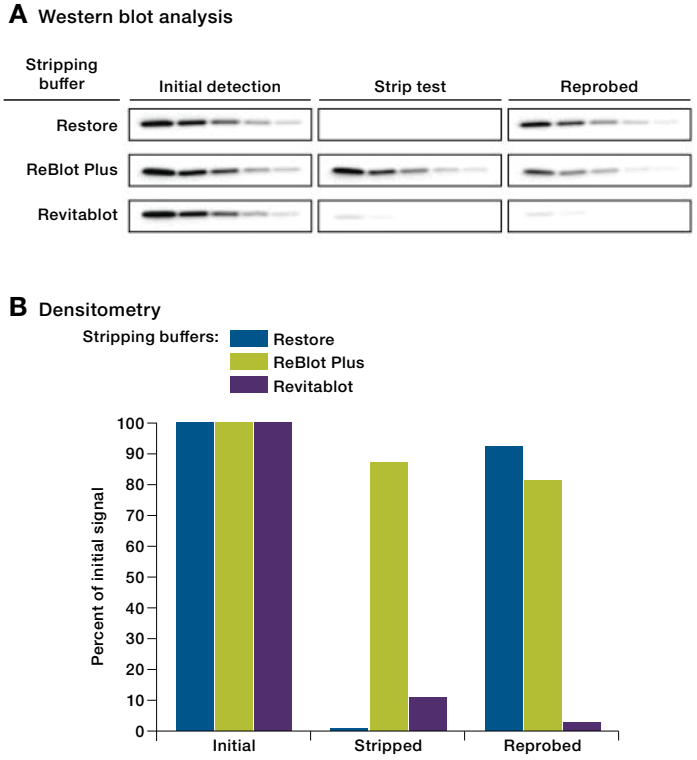


Figure 50. Western blot stripping and reprobing performance of three stripping buffers on PVDF. HeLa cell lysate was diluted to 1 mg/mL in electrophoresis reducing sample buffer, and 1:1 serial dilutions were made. Three sets of 10 µL per dilution (10 µg to 0.31 µg of total protein) were separated by SDS-PAGE and transferred to 0.45 µm PVDF membrane. **(A)** The membrane were as blocked with 5% nonfat dry milk in 1X Pierce TBS Tween 20 Buffer and analyzed by western blot using SuperSignal West Dura Extended Duration Substrate and imaged. The membrane was probed with Invitrogen™ Cyclophilin B Polyclonal Antibody at 0.2 µg/mL followed by Goat Anti–Rabbit IgG, HRP Conjugate, at 5.7 ng/mL, and imaged. Following the initial detection, the blot was cut into three strips to separate the serial dilution sets, and each part of the blot was stripped, according to manufacturer's instructions, in either Restore Western Blot Stripping Buffer (15 minutes at 37°C), ReBlot Plus Stripping Solution (MilliporeSigma; 15 minutes at room temperature), or Revitablot Western Blot Stripping Buffer (Rockland Immunochemicals Inc.; 15 minutes at room temperature). After the stripping procedure, the membrane strips were washed in 1X PBS Tween 20 buffer and incubated with the substrate and imaged. The membrane strips were reblocked, and the western blot procedure was repeated as described above. **(B)** Densitometry analysis shows that Restore Western Blot Stripping Buffer permitted complete signal removal and maintained nearly identical levels of detection between the initial and reprobed western blot analysis.

Restore PLUS Western Blot Stripping Buffer

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 chemiluminescent western blots and require longer incubation times or incubation temperatures greater than 22°C. Thermo Scientific™ Restore™ PLUS Western Blot Stripping Buffer was developed to reduce incubation times while keeping incubations at room temperature using gentler formulations. High-affinity antibodies can be quickly and effectively stripped from western blots without removing transferred proteins, allowing multiple reprobes of the target.

Features:

- **Ready and easy to use**—no dilution necessary; no offensive odors; store at room temperature
- **Compatible**—use on nitrocellulose and PVDF membranes, whether still wet or already dry; works with practically any blocking buffer, enzyme conjugate, and chemiluminescent substrate
- **Cost effective**—save valuable time and samples; strip blots effectively the first time
- **Robust yet gentle**—transferred proteins remain functional; strip the same blot up to 5 times
- **Flexible**—strip and reprobe to optimize antibody concentrations or to detect a new target protein with different antibodies (Figure 51)

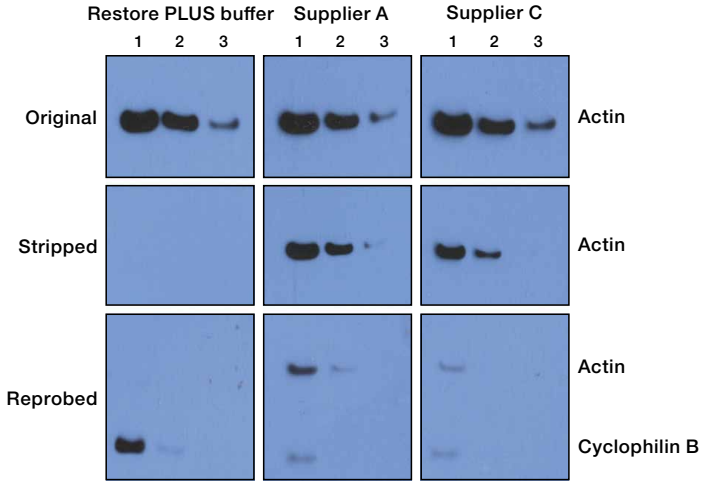


Figure 51. Restore PLUS Western Blot Stripping Buffer is most effective for reprobing with different antibodies. HeLa cell lysate was probed for actin and detected with Pierce ECL substrate ("Original"). Blots were then stripped with either Restore PLUS Western Blot Stripping Buffer or other suppliers' stripping buffers. The blots were then reblocked and reprobed for cyclophilin B and detected with Pierce ECL substrate.

Watch a how-to video **here**.

Restore Fluorescent Western Blot Stripping Buffer

Thermo Scientific™ Restore™ Fluorescent Western Blot Stripping Buffer is a gentle and highly effective reagent for quickly removing primary antibodies and NIR dye-labeled secondary antibodies from western blots, allowing the same blot to be reprobed with different primary antibodies to detect alternative targets (Figure 52). Restore Fluorescent Western Blot Stripping Buffer is for use with low-fluorescence PVDF membranes only.

Features:

- **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

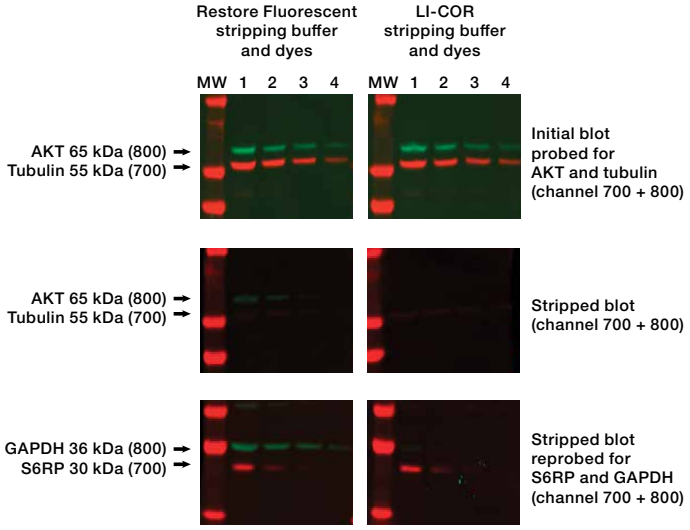


Figure 52. Effective stripping and reprobing of NIR fluorescent western blots on PVDF membranes. A549 whole cell lysates (20, 10, 5, and 2.5 µg of protein in lanes 1, 2, 3, and 4, respectively) were transferred to PVDF membranes. **Top panels:** The blots were probed with AKT and tubulin antibodies and detected using Invitrogen™ DyLight™ 680 Goat Anti–Mouse IgG and DyLight™ 800 Goat Anti–Rabbit IgG, or IRDye™ 680 Goat Anti–Mouse IgG and IRDye™ 800CW Goat Anti–Rabbit IgG (LI-COR). An imaging system capable of fluorescence-based data capture (channel 700 and 800) was used for imaging. **Middle panels:** Blots were stripped with Restore Fluorescent Western Blot Stripping Buffer or NewBlot™ PVDF 5X Stripping Buffer (LI-COR) 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 (S6RP) and mouse anti-GAPDH antibodies. Targets were detected using DyLight 800 Goat Anti–Mouse IgG and DyLight 680 Goat Anti–Rabbit IgG, or IRDye 800CW Goat Anti–Mouse IgG and IRDye 680 Goat Anti–Rabbit IgG and imaged as described above.

Learn more at **thermofisher.com/westernbuffers**

Signal enhancements

There are many ways to increase the sensitivity of a western blot. Some methods are as simple as switching detection substrates or blocking buffers in the immunodetection protocols, while others are more time-consuming, such as optimizing antibody titer.

A number of post-transfer membrane treatments also exist that can improve signal intensity. Some of these methods increase sensitivity, decrease background, or both; others conserve the amount of antibody needed downstream while maintaining signal intensity.

SuperSignal Western Blot Enhancer

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

Thermo Scientific™ SuperSignal™ Western Blot Enhancer contains a membrane treatment reagent and a primary antibody diluent that increases both signal intensity and sensitivity 3- to 10-fold compared to a detection performed without it.

When a protein is difficult to detect because of low abundance or poor immunoreactivity, use of SuperSignal Western Blot Enhancer can help significantly reduce background and enhance detection.

It minimizes the routine problem of overexposing blots, thereby reducing the need to experiment with multiple exposure times to acquire the “perfect blot”. This kit works with both PVDF and nitrocellulose membranes and is compatible with fluorescence, chromogenic, and chemiluminescent detection (Figures 53–55).

Features:

- **Increase sensitivity**—achieve 3- to 10-fold increase in signal intensity and sensitivity
- **Improve specificity**—helps improve signal-to-noise ratio for poor-quality and low-affinity antibodies
- **Reduce background**—helps improve clarity for cleaner western blots
- **Membrane compatibility**—provides effective signal enhancement with PVDF and nitrocellulose membranes
- **Substrate compatibility**—verified for use with chromogenic, chemiluminescent, and fluorescent detection methods

Learn more at **thermofisher.com/supersignalenhancer**

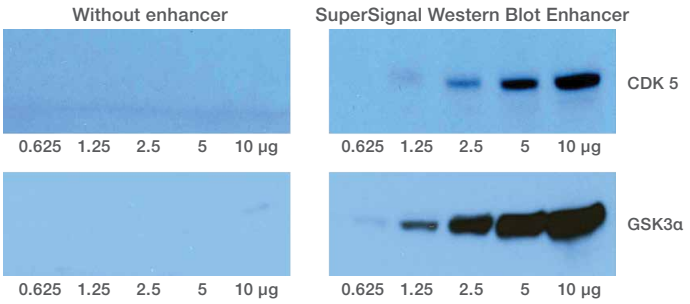


Figure 53. SuperSignal Western Blot Enhancer improves the lower detection limit for chemiluminescent substrates. Cell lysates were separated by electrophoresis, transferred to nitrocellulose, and western blotting was performed using the conventional method **(left)** or using the SuperSignal Western Blot Enhancer protocol **(right)**.

	Top panel	Bottom panel
Blocking buffer	5% milk in Tris-buffered saline with 0.05% Tween™ 20 surfactant	Thermo Scientific™ Blocker™ BLOTTO in TBS
Primary antibody	Mouse anti-CDK5 at 1 µg/mL	Rabbit anti-GSK3α at 1 µg/mL
Secondary antibody	HRP-conjugated goat anti–mouse IgG at 0.1 µg/mL	HRP-conjugated goat ant–rabbit IgG at 0.1 µg/mL
Substrate	Thermo Scientific™ Pierce™ ECL Western Blotting Substrate	Thermo Scientific™ SuperSignal™ West Pico PLUS substrate
Detection	X-ray film with 1 min exposure time	X-ray film with 10 min exposure time

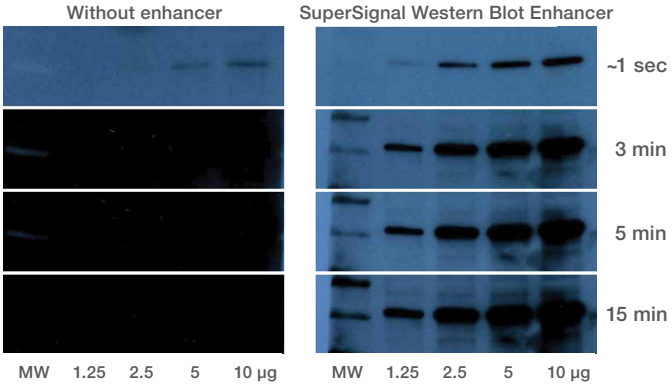


Figure 54. SuperSignal Western Blot Enhancer reduces background to enable detection of low-abundance targets. K562 cell lysate was loaded into Tris-glycine SDS-PAGE gels at 1.25, 2.5, 5, and 10 µg per lane. After electrophoresis, the proteins were transferred to nitrocellulose membranes.

Blocking buffer	Thermo Scientific™ SuperBlock™ (TBS) Blocking Buffer
Primary antibody	Mouse anti-ERK1 primary antibody at 1 µg/mL
Secondary antibody	HRP-conjugated goat anti–mouse IgG at 0.08 µg/mL
Substrate	Pierce ECL substrate
Detection	X-ray film

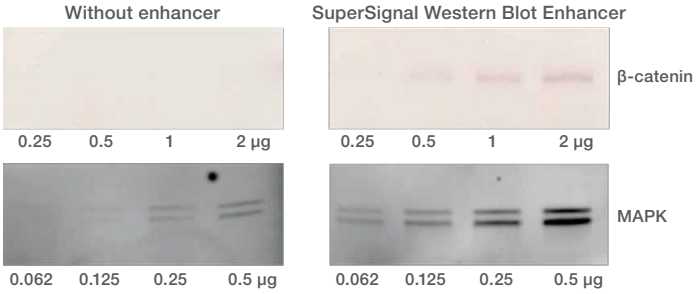


Figure 55. SuperSignal Western Blot Enhancer is compatible with chromogenic and fluorescent detection methods. HeLa cell lysate was separated by electrophoresis, transferred to nitrocellulose **(top)** or Thermo Scientific™ Low-Fluorescence PVDF Membrane **(bottom)**, and western blotting was performed using the conventional method **(left)** or using the SuperSignal Western Blot Enhancer protocol **(right)**.

	Top panel	Bottom panel
Blocking buffer	SuperBlock buffer in TBS	Blocker BLOTTO in TBS
Primary antibody	Rabbit anti–β-catenin at 0.2 µg/mL	Rabbit anti–MAP kinase at 1 µg/mL
Secondary antibody	AP-conjugated goat anti–rabbit IgG at 0.04 µg/mL	Invitrogen™ DyLight™ 488–conjugated goat anti–rabbit IgG at 0.1 µg/mL
Substrate	Thermo Scientific™ Pierce™ 1-Step NBT/BCIP Substrate Solution	–
Detection	Visual	Typhoon™ 9410 imager

Recommended products

SuperSignal West Pico PLUS Chemiluminescent Substrate and **Pierce ECL Western Blotting Substrate** are recommended for use with the SuperSignal Western Blot Enhancer. For chromogenic detection, we recommend **Pierce 1-Step NBT/BCIP Substrate Solution**.

Invitrogen™ goat anti-mouse or **goat anti-rabbit HRP-conjugated secondary antibodies** are recommended for your western blot detection.

Specialty detection reagents

Clean-Blot IP Detection Reagent

Thermo Scientific™ Clean-Blot™ IP Detection Reagent is an HRP conjugate that is optimized for post-immunoprecipitation western blot detection of primary antibodies without interference from denatured immunoprecipitation (IP) antibody fragments.

The Clean-Blot IP Reagent allows trouble-free western blot detection of target proteins following IP assays. It works by specifically binding to functional primary antibodies (whole IgG) without also binding to fragments of the IP antibodies, which usually accompany the immunoprecipitated protein through electrophoresis and membrane transfer. The Clean-Blot IP Reagent and Kit eliminate detection interference from both heavy-chain (approximately 50 kDa) and light-chain (25 kDa) IgG fragments of antibodies used for the initial immunoprecipitation assay.

Features:

- **Universal**—bind and detect most IgG isotypes and subclasses of primary antibodies that are commonly used for western blotting (Table 27)
- **Compatible**—effective with IP assays performed using protein A, protein G, or anti-IgG agarose beads and any blocking buffer
- **Cost-effective**—eliminates the cost and extra work associated with covalently immobilizing IP antibodies as a means of overcoming western blot interference
- **Flexible**—HRP reagent for detection with chemiluminescent, fluorescent, or colorimetric substrates
- **Easy to use**—no need to change the western blotting protocol; simply replace conventional secondary HRP conjugate with the Clean-Blot IP Detection Reagent (Figure 56)
- **Unobstructed detection**—clear western blot results for immunoprecipitation assays without significant interference from denatured IgG bands (Figure 57)

Table 27. Primary antibodies recognized by Clean-Blot IP Detection Reagent.

Species	Isotype*
Bovine	IgG2
Goat	IgG2
Human	IgG1, IgG2, IgG4
Mouse	IgG2a, IgG2b, IgG3
Rat	IgG2c
Sheep	IgG2

* Clean-Blot IP Detection Reagent recognizes polyclonal and monoclonal antibodies. To determine specific antibody compatibility, perform a dot-blot analysis.

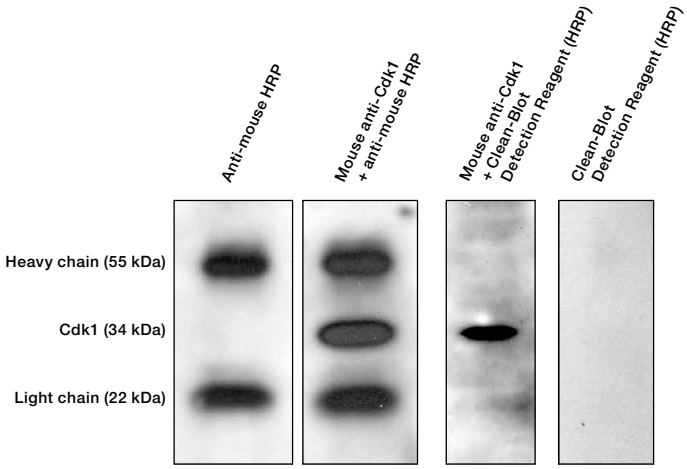


Figure 56. Easily distinguish your target protein on a western blot with Clean-Blot Detection Reagent (HRP). Mouse liver extract (50 µg) total protein was separated on a Bio-Rad Criterion™ Gel, transferred to PVDF membrane, and blocked with 5% milk in TBST. The membrane was probed with mouse monoclonal anti-Cdk1 (LabVision, 0.2 µg/mL) and goat anti-mouse HRP (0.16 µg/mL) or Clean-Blot Detection Reagent (HRP) (0.2 µg/mL). SuperSignal West Pico substrate was used for detection of Cdk1 protein.

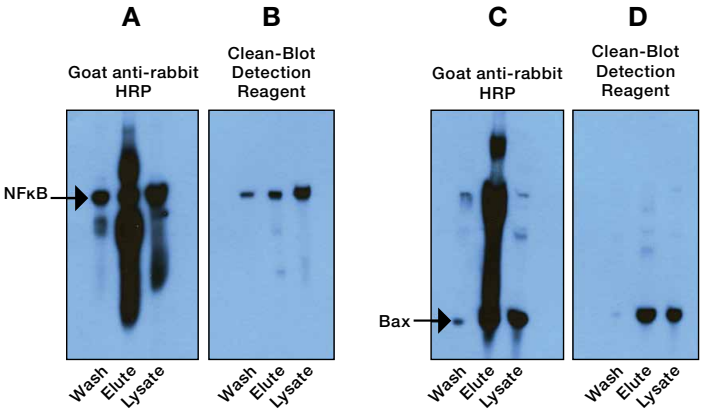


Figure 57. Reveal your target protein with Clean-Blot Detection Reagent (HRP). To demonstrate unmasking of the target protein, we performed IP and western blot experiments. NF-κB and Bax were immunoprecipitated from A549 lysate using protein A/G agarose resin and **(A and B)** rabbit anti–NF-κB and **(C and D)** rabbit anti-Bax. **(A and C)** Detection was performed with goat anti-rabbit HRP, which masked the target. **(B and D)** Detection was performed with the Clean-Blot Detection Reagent (HRP), revealing the target protein.

SuperSignal West HisProbe Kit

The Thermo Scientific™ SuperSignal™ West HisProbe™ Kit is a chemiluminescent system that uses HisProbe-HRP conjugate to overcome the limitations of anti-histidine antibodies and other detection strategies. HisProbe-HRP conjugate is more specific for polyhistidine tags, reducing background problems. Unlike anti-His antibodies, HisProbe-HRP conjugate can recognize polyhistidine tags independent of adjacent tags.

Features:

- **Fast**—His-tag detection is achieved with a one-step probe incubation that eliminates the need to run a lengthy two-step, primary–secondary antibody reaction protocol
- **Economical**—no need to purchase expensive primary and secondary antibodies; considerably less costly than 6xHis-tag antibodies
- **High specificity**—HisProbe-HRP can recognize His-tags independent of adjacent tags
- **Less steric hindrance**—HisProbe-HRP conjugate is smaller than antibodies and detects His-tagged fusion proteins that are often undetectable when using some anti-His-tag antibodies

Appendix

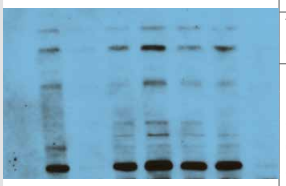
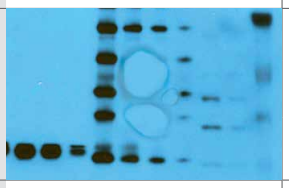
Protocol and recipe quick references

- **General procedure for chemiluminescent western blotting**
- **General procedure for fluorescent western blotting**
- **Protein electrophoresis and western blot recipes**
- **Power Blotter and Power Blotter XL transfer systems**
- **iBlot 2 Dry Blotting System**
- **iBind Western Device**
- **iBind Flex Western Device**
- **iBright CL1500 and FL1500 Imaging Systems**

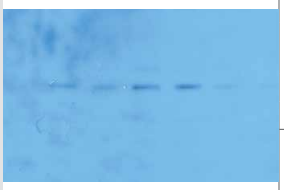
If you find that these procedures aren't right for your specific protein of interest, try our BlotBuilder Product Selection Tool to get a set of recommended products with a personalized western blot protocol. Visit **thermofisher.com/blotbuilder**

Troubleshooting tips

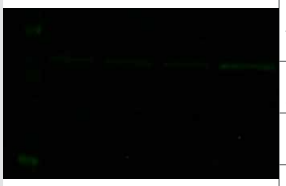
General western blotting

Observation		Cause	Solution
Nonspecific or diffuse bands		Antibody concentration too high	Reduce concentrations of antibodies, particularly of primary antibody.
		Too much protein loaded on gel	Reduce the amount of sample loaded on gel.
		Signal from chemiluminescent substrate too strong	Reduce the length of time the blot is exposed to film.
			Reduce the concentration of the substrate.
			Shorten incubation time of membrane with substrate.
			Completely remove substrate after incubation period.
			Decrease the concentration of antibodies, particularly HRP- and AP-conjugated antibodies.
Partially developed areas or blank areas		Incomplete transfer	Ensure sufficient contact between the gel and membrane during transfer by using a gel roller across the transfer stack.
			Wet and activate membrane according to manufacturer's instructions.
			Always wear clean gloves or use forceps when handling membrane.
High background		Antibody concentration too high	Decrease concentration of primary and/or secondary antibody.
		Incompatible blocking buffer	Do not use milk with avidin–biotin system. Milk contains biotin, which will result in high background.
			When probing for phosphoproteins, avoid phosphate-based buffers like PBS and phosphoprotein-containing blockers like milk or casein. Instead, block with BSA in Tris-buffered saline.
			Test for cross-reactivity in blocking buffer by blocking a clean piece of membrane, incubating with antibodies, and then detecting with the substrate of choice.
			When using an alkaline phosphatase (AP) conjugate, a blocking buffer in Tris-buffered saline (TBS) should be selected because phosphate-buffered saline (PBS) interferes with AP activity.
			Try a different blocking buffer. Use our blocking buffer selection guide at **thermofisher.com/blockingbuffers** to find the most compatible blocking buffer for your experiment.
		Insufficient blocking of nonspecific sites	Increase the concentration of protein in the blocking buffer.
			Optimize blocking time and/or temperature. Block for at least 1 hour at room temperature (RT) or overnight at 4°C.
			Adding Tween 20 detergent to the blocking buffer can help minimize background. However, too much detergent can interfere with antibody binding. A final concentration of 0.05% often works well. For ease of use, choose a blocking buffer that already contains 0.05% Tween 20 detergent, such as Thermo Scientific™ StartingBlock™ T20 Blocking Buffer (Cat. No. 37543 or 37539) or SuperBlock™ T20 Blocking Buffer (Cat. No. 37536 or 37516).
			Prepare antibody dilutions in a blocking buffer that contains 0.05% Tween 20 detergent.
			Use Thermo Scientific™ SuperSignal™ Western Blot Enhancer (Cat. No. 46640) to reduce background and enhance detection of low-abundance and weakly immunoreactive target proteins.
		Insufficient washing	Increase the number of washes and/or the volume of buffer used.
			Add Tween 20 detergent to the wash buffer to a final concentration of 0.05%. If the concentration of Tween 20 detergent is too high, it can strip proteins off the membrane.
		Membrane handled improperly	Wet and activate membrane according to manufacturer's instructions.
			Always wear clean gloves or use forceps when handling membrane.
			Cover the membrane with liquid at all times to prevent drying.
			Use agitation during all incubations.
			Handle membrane carefully—damage to the membrane can cause nonspecific binding.

General western blotting (continued)

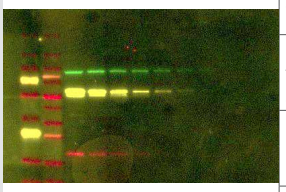
Observation		Cause	Solution
High background (continued)		Contamination of equipment or materials	Prepare fresh buffers and filter them before use.
			Use only clean and contaminant-free electrophoresis equipment, blotting equipment, and incubation trays.
		Signal from chemiluminescent substrate too strong	Reduce the length of time the blot is exposed to film.
			Reduce the concentration of the substrate.
			Shorten incubation time of membrane with substrate.
			Completely remove substrate after incubation period.
			Decrease the concentration of antibodies, particularly HRP- and AP-conjugated antibodies.
Weak signal or no signal		Incomplete or inefficient transfer	After transfer, stain the gel with a total protein stain to determine transfer efficiency, such as Ponceau S or the Thermo Scientific™ Pierce™ Reversible Protein Stain Kit (Cat. No. 24585 or 24580).
			Ensure sufficient contact between the gel and membrane during transfer by using a gel roller across the transfer stack.
			Ensure that the stack is placed in the transfer apparatus in the proper orientation such that proteins will migrate onto the membrane.
			Wet and activate the membrane according to the manufacturer's instructions.
			Use a positive control, such as prestained molecular weight markers, to quickly assess whether transfer has occurred.
			Use molecular weight markers compatible with a western imaging substrate, such as the Invitrogen™ iBright™ Prestained Protein Ladder (Cat. No. LC5615) or Invitrogen™ MagicMark™ XP Western Protein Standard (Cat. No. LC5602), as a positive control.
			Increase transfer time and/or voltage.
			Make sure sample preparation conditions have not destroyed the antigenicity of the sample. (Some proteins cannot be run under reducing conditions.)
		Insufficient binding to membrane	For low molecular weight (MW) targets, add 20% methanol to the transfer buffer to help binding and prevent proteins from passing through the membrane.
			Reduce transfer time. Low MW proteins may pass through the membrane.
			For high MW targets, add 0.01–0.05% SDS to the transfer buffer to facilitate the movement of proteins from the gel to the membrane.
			Change membrane type (nitrocellulose vs. PVDF).
			Change to membrane with smaller pore size.
		Antibody concentration too low	Increase antibody concentrations. Antibody may have poor affinity for the target protein.
			Antibody may have lost activity. Perform a dot blot to determine activity.
		Insufficient target protein present	Load more protein onto the gel.
		Protein masked by blocking buffer	Decrease concentration of protein in blocking buffer.
			Try a different blocking buffer. Use our blocking buffer selection guide at **thermofisher.com/blockingbuffers** to find the most compatible blocking buffer for your experiment.
		Buffer contains sodium azide	Sodium azide inhibits HRP. Do not use it with HRP-conjugated antibodies.
		Signal from chemiluminescent substrate too weak	Increase incubation time of membrane with substrate.
			Increase film exposure time.
			Ensure that the substrate is not expired.
			When you have minimal protein, use Thermo Scientific™ SuperSignal™ West Atto Ultimate Sensitivity Substrate (Cat. No. A38555) to maximize your western blot signals.
		Membrane has been stripped and reprobed	Avoid repeated stripping of the same membrane.
			Shorten incubation time in stripping buffer to prevent loss of target protein.
		Digestion of protein on membrane	Blocking buffer may contain a substance that has proteolytic activity (e.g., gelatin); try changing the blocking buffer to a purified blocking protein such as casein.
		Protein degradation from prolonged blot storage	Prepare new blot.

Fluorescent western blotting

Observation		Cause	Solution
Nonspecific or diffuse bands		Poor antibody specificity for the target of interest	Evaluate additional primary antibodies.
			Use only primary antibodies validated* for western blots.
		Poor sample integrity	Sample degradation due to overheating or protease activity results in target breakdown and low target recognition by the antibody. For example, do not boil SDS-PAGE samples in SDS sample buffer, but rather heat them at 70°C for 10 minutes to avoid proteolysis.
		Antibody cross-reactivity in multiplex detection	Choose primary antibodies raised in distantly related host species.
			Use highly cross-adsorbed secondary antibodies.
			Reduce the amount of the secondary antibody used, to remain within the optimal performance range.
		Fluorescent bleed-through from another channel when multiplexing (appearance of an unexpected band)	Avoid spectrally close conjugates, especially when the signal is very strong.
			Ensure that your fluorescent dyes can be distinctly detected on your imaging instrument.
			If available, use the autoexposure feature on your imaging instrument to determine the optimal exposure time(s) for each channel.
Weak or no signal		Insufficient amount of primary antibody	Increase primary antibody concentration.
			Ensure that the primary antibody has a good titer and is specific for the target protein.
			For a low-abundance target in a cell or tissue lysate, increase the amount of primary antibody or the amount of sample loaded on the gel.
			Extend the incubation time to overnight at 4°C, or 3–6 hours at room temperature.
			Try using an antibody enhancer.
		Lost activity of antibody	Ensure the antibody was stored appropriately.
			Check the expiration date of the antibody.
			Avoid multiple uses of prediluted antibodies.
		Imaging exposure time is too short	Increase exposure time.
			If available, use the autoexposure feature on your imaging instrument to determine the optimal exposure time(s).
		Incorrect instrument settings	Ensure the correct excitation and emission ranges are selected for the intended fluorophore.
		Use of detergent	Too much detergent or the nature of the detergent can result in washing away the signal—decrease or eliminate detergent.
		Blocking buffer blocks target protein	Some blocking solutions can mask the blot and reduce the availability of the target protein to the antibody, especially if the blocking step is >1 hour.
			Dilute the primary antibody in wash buffer.
			Evaluate another blocking buffer.
		Quantity of sample loaded on the gel	Too much lysate can overcrowd your specific target and reduce the antibody sensitivity.
			Too little lysate leads to insufficient availability of the target of interest.
			Perform serial dilutions of the lysate or sample to determine the optimal amount of protein to load.
		Poor transfer of protein, or loss of the protein after transfer	Check transfer conditions to confirm protein transfer.
			Reoptimization may be required when probing for a new protein.

* The use or any variation of the word "validation" refers only to research use antibodies that were subject to functional testing to confirm that the antibody can be used with the research techniques indicated. It does not ensure that the product(s) was validated for clinical or diagnostic uses.

Fluorescent western blotting (continued)

Observation		Cause	Solution
Background issues (high, uneven, or speckled)		High background due to membrane contamination	Handle the membrane using clean forceps and clean incubation trays or dishes.
			Determine the best blocking buffer for your application—primary antibodies will react differently in different blocking buffers. Blocking buffers like normal animal sera or milk may result in cross-reactivity. Use our blocking buffer selection guide at **thermofisher.com/blockingbuffers** to find the most compatible blocking buffer for your experiment.
		Artifacts from overloading the protein marker or ladder	Load less of the molecular weight marker onto the gel.
		Nonoptimal wash or diluent solutions	Use a wash buffer with 0.1–0.2% Tween 20 detergent.
			Prepare the secondary antibody dilution with 0.05% Tween 20 detergent.
			Increase the number or duration of wash steps.
		High background from an excess of secondary antibody	Optimize the secondary antibody dilution depending on the dye being used, following the vendor-recommended dilution and adapting accordingly.
		Blotchy or uneven background due to the membrane drying out	Ensure good coverage of the whole blot during all incubation steps.
			Ensure consistent agitation during every incubation step.
		Incorrect choice of membrane	The nature of the membrane can affect the background; for example, PVDF membranes can autofluoresce and cause high background, so use low-fluorescence PVDF membranes.
		Speckles and fingerprints on the membrane	Use clean forceps to handle the membrane, and avoid directly touching the membrane; particulates and contaminants from unclean tools may fluoresce.
			Use clean incubation trays or dishes—rinsing with methanol followed by water will help dissolve residual dried dyes from previous uses.
			Clean transfer devices and dusty consumables if using a wet transfer method, as they can introduce speckles.
			Clean the imager surface with ethanol to remove dust, lint, and residue before capturing the image.

Ordering information

Pre-transfer

Product	Quantity	Cat. No.
Membranes		
Ready-to-use sandwiches		
Nitrocellulose/Filter Paper Sandwiches, 0.2 µm pore size, 8.5 x 13.5 cm	16/pack	**LC2009**
Nitrocellulose/Filter Paper Sandwiches, 0.45 µm pore size, 8.5 x 13.5 cm	16/pack	**LC2006**
Power Blotter Select Transfer Stacks, nitrocellulose, mini	10 stacks	**PB3210**
Power Blotter Select Transfer Stacks, nitrocellulose, mini	40 stacks	**PB3240**
Power Blotter Select Transfer Stacks, PVDF, mini	10 stacks	**PB5210**
Power Blotter Select Transfer Stacks, PVDF, mini	40 stacks	**PB5240**
Power Blotter Select Transfer Stacks, nitrocellulose, regular size	10 stacks	**PB3310**
Power Blotter Select Transfer Stacks, nitrocellulose, regular size	40 stacks	**PB3340**
Power Blotter Select Transfer Stacks, PVDF, regular size	10 stacks	**PB5310**
Power Blotter Select Transfer Stacks, PVDF, regular size	40 stacks	**PB5340**
Power Blotter Pre-cut Membranes and Filters, nitrocellulose, mini	20 stacks	**PB7220**
Power Blotter Pre-cut Membranes and Filters, PVDF, mini	20 stacks	**PB9220**
Power Blotter Pre-cut Membranes and Filters, nitrocellulose, regular size	20 stacks	**PB7320**
Power Blotter Pre-cut Membranes and Filters, PVDF, regular size	20 stacks	**PB9320**
iBlot 2 Transfer Stacks, Nitrocellulose, Mini	10 stacks	**IB23002**
iBlot 2 Transfer Stacks, Nitrocellulose, Regular	10 stacks	**IB23001**
iBlot 2 Transfer Stacks, PVDF, Mini	10 stacks	**IB24002**
iBlot 2 Transfer Stacks, PVDF, Regular	10 stacks	**IB24001**
Pre-cut nitrocellulose membranes		
Nitrocellulose/Filter Paper Sandwiches, 0.2 µm pore size, 8.3 x 7.3 cm	20/pack	**LC2000**
Nitrocellulose/Filter Paper Sandwiches, 0.45 µm pore size, 8.3 x 7.3 cm	20/pack	**LC2001**
SureLock Tandem Midi Pre-cut Membranes and Filters, 0.2 µm, nitrocellulose, 8.6 x 14 cm	20/pack	**STM2007**
SureLock Tandem Midi Pre-cut Membranes and Filters, 0.45 µm, nitrocellulose, 8.6 x 14 cm	20/pack	**STM2008**
Nitrocellulose Membranes, 0.2 µm, 8 x 12 cm	25 sheets	**77012**
Nitrocellulose Membranes, 0.2 µm, 7.9 x 10.5 cm	15 sheets	**88013**
Nitrocellulose Membranes, 0.2 µm, 8 x 8 cm	15 sheets	**88024**
Nitrocellulose Membranes, 0.45 µm, 8 x 12 cm	25 sheets	**77010**
Nitrocellulose Membranes, 0.45 µm, 7.9 x 10.5 cm	15 sheets	**88014**
Nitrocellulose Membranes, 0.45 µm, 8 x 8 cm	15 sheets	**88025**
Nitrocellulose rolls		
Nitrocellulose Membrane, 0.45 µm, 30 cm x 3.5 m	1 roll	**88018**

Pre-transfer

Product	Quantity	Cat. No.
Pre-cut PVDF membranes		
Invitrolon PVDF/Filter Paper Sandwiches, 0.45 µm pore size, 8.3 x 7.3 cm (for mini gels)	20/pack	**LC2005**
Invitrolon PVDF/Filter Paper Sandwiches, 0.45 µm pore size, 8.5 x 13.5 cm	16/pack	**LC2007**
SureLock Tandem Midi Pre-cut Membranes and Filters, 0.45 µm, PVDF, 8.6 x 14 cm	20/pack	**STM2006**
Low-Fluorescence PVDF Transfer Membranes, 0.2 µm, 7 x 8.4 cm	10 sheets	**22860**
PVDF/Filter Paper Sandwiches, 0.2 µm pore size	20/pack	**LC2002**
PVDF Transfer Membranes, 0.45 µm, 10 x 10 cm	10 sheets	**88585**
Tropifluor PVDF Membranes, 0.45 µm pore size, 15 x 15 cm	5 sheets	**T2234**
PVDF rolls		
PVDF Transfer Membrane, 0.2 µm, 26.5 cm x 3.75 m	1 roll	**88520**
PVDF Transfer Membrane, 0.45 µm, 26.5 cm x 3.75 m	1 roll	**88518**
Filter paper for blotting		
Western Blotting Filter Papers, 8 x 10.5 cm	100 sheets	**88600**
Western Blotting Filter Papers, 7 x 8.4 cm	100 sheets	**84783**
Western Blotting Filter Papers, 8 x 13.5 cm	100 sheets	**84784**
Western Blotting Filter Papers, Extra Thick, 7 x 8.4 cm	50 sheets	**88605**
Western Blotting Filter Papers, Extra Thick, 8.5 x 9 cm	50 sheets	**88610**
Western Blotting Filter Papers, Extra Thick, 8 x 13.5 cm	50 sheets	**88615**
Western Blotting Filter Papers, Extra Thick, 20 x 20 cm	50 sheets	**88620**
Blotting Filter Papers, 2.5 mm thickness, 8.6 x 13.5 cm	50 each	**LC2008**
Blotting Filter Papers, 2.5 mm thickness, 7.5 x 8.4 cm	50 sheets	**LC2010**
Protein ladders		
PageRuler Plus Prestained Protein Ladder, 10 to 250 kDa	2 x 250 µL	**26619**
PageRuler Plus Prestained Protein Ladder, 10 to 250 kDa	10 x 250 µL	**26620**
PageRuler Prestained Protein Ladder, 10 to 180 kDa	2 x 250 µL	**26616**
PageRuler Prestained Protein Ladder, 10 to 180 kDa	10 x 250 µL	**26617**
iBright Prestained Protein Ladder, 11 to 250 kDa	2 x 250 µL	**LC5615**
HiMark Pre-stained Protein Standard, 30 to 460 kDa	250 µL	**LC5699**
Spectra Multicolor Broad Range Protein Ladder, 10 to 260 kDa	10 x 250 µL	**26623**
Spectra Multicolor Broad Range Protein Ladder, 10 to 260 kDa	2 x 250 µL	**26634**
Spectra Multicolor High Range Protein Ladder, 40 to 300 kDa	2 x 250 µL	**26625**
PageRuler Unstained Protein Ladder, 10 to 200 kDa	2 x 250 µL	**26614**

Pre-transfer

Product	Quantity	Cat. No.
PageRuler Unstained Low Range Protein Ladder, 3.4 to 100 kDa	2 x 250 µL	**26632**
NativeMark Unstained Protein Standard, 20 to 1,200 kDa	5 x 50 µL	**LC0725**
MagicMark XP Western Protein Standard, 20 to 220 kDa	250 µL	**LC5602**
MagicMark XP Western Protein Standard, 20 to 220 kDa	50 µL	**LC5603**
BenchMark Fluorescent Protein Standard, 11 to 155 kDa	125 µL	**LC5928**
PageRuler Prestained NIR Protein Ladder, 11 to 250 kDa	2 x 250 µL	**26635**
BenchMark His-Tagged Protein Standard, 10 to 160 kDa	125 µL	**LC5606**
IEF Marker 3–10, pI 3 to 10	500 µL	**39212-01**
Transfer buffers		
Bolt Transfer Buffer (20X)	125 mL	**BT0006**
Bolt Transfer Buffer (20X)	1 L	**BT00061**
BupH Tris-Glycine Buffer Packs	40 packs	**28380**
Novex Tris-Glycine Transfer Buffer (25X)	500 mL	**LC3675**
NuPAGE Transfer Buffer (20X)	125 mL	**NP0006**
NuPAGE Transfer Buffer (20X)	1 L	**NP0006-1**
Power Blotter 1-Step Transfer Buffer (5X)	1 L	**PB7300**
Power Blotter 1-Step Transfer Buffer (5X)	250 mL	**PB7100**
Pierce 10X Tris-Glycine Buffer	1 L	**28363**
Pierce 10X Western Blot Transfer Buffer, Methanol-Free	5 L	**35040**
Accessories		
Blotting Roller	1 unit	**LC2100**
Western Blot Roller	1 unit	**84747**

Transfer

Product	Quantity	Cat. No.
Wet transfer systems		
Mini Blot Module	1 unit	**B1000**
Mini Gel Tank and Blot Module Set	1 unit	**NW2000**
SureLock Tandem Midi Blot Module	1 module	**STM2001**
SureLock Tandem Transfer Tray	1 tray	**STM3001**
XCell II Blot Module	1 unit	**EI9051**
XCell *SureLock* Mini-Cell and XCell II Blot Module	1 unit	**EI0002**
PowerEase Touch 350W Power Supply (115 VAC)	1 unit	**PS0350**
PowerEase Touch 120W Power Supply (115 VAC)	1 unit	**PS0120**
PowerEase Touch 350W Power Supply (230 VAC)	1 unit	**PS0351**
PowerEase Touch 120W Power Supply (230 VAC)	1 unit	**PS0121**
Bolt Western Pack A (Nitrocellulose)	1 kit	**B1000A**
Bolt Western Pack B (PVDF)	1 kit	**B1000B**
Semi-dry transfer systems		
Power Blotter Station	1 unit	**PB0010**
Power Blotter Cassette	1 cassette	**PB0002**
Power Blotter Cassette XL	1 cassette	**PB0003**
Power Blotter System	1 system	**PB0012**
Power Blotter XL System	1 system	**PB0013**
Power Blotter Welcome Pack	1 kit	**PB0112**
Power Blotter XL Welcome Pack	1 kit	**PB0113**
Dry transfer systems		
iBlot 2 Gel Transfer Device	1 device	**IB21001**
iBlot 2 Electrode Replacement Kit	1 kit	**IB28001**

Post-transfer

Product	Quantity	Cat. No.
Reversible protein stain kits		
Pierce Reversible Protein Stain Kit for Nitrocellulose Membranes	1.5 L	**24580**
Pierce Reversible Protein Stain Kit for PVDF Membranes	1.75 L	**24585**
Signal enhancement		
SuperSignal Western Blot Enhancer	500 mL	**46640**
SuperSignal Western Blot Enhancer	50 mL	**46641**

Block, wash, probe

Product	Quantity	Cat. No.
Automated		
Bandmate Automated Western Blot Processor	1 system	**BW1000**
iBind Flex Western Starter Kit	1 kit	**SLF2000S**
iBind Flex Western Device	1 device	**SLF2000**
iBind Flex Cards	10 cards	**SLF2010**
iBind Flex Fluorescent Detection (FD) Solution Kit	1 kit	**SLF2019**
iBind Flex Solution Kit	1 kit	**SLF2020**
iBind Western Starter Kit	1 kit	**SLF1000S**
iBind Western Device	1 device	**SLF1000**
iBind Cards	10 cards	**SLF1010**
iBind Fluorescent Detection (FD) Solution Kit	1 kit	**SLF1019**
iBind Solution Kit	1 kit	**SLF1020**
Blocking solutions		
Blocker BSA (10X) in PBS	200 mL	**37525**
Blocker BSA (10X) in TBS	125 mL	**37520**
Blocker Casein in TBS	100 mL	**37583**
Blocker Casein in PBS	100 mL	**37582**
Pierce Protein-Free (PBS) Blocking Buffer	1 L	**37572**
Pierce Protein-Free (PBS) Blocking Buffer	100 mL	**37584**
Pierce Protein-Free (TBS) Blocking Buffer	1 L	**37570**
Pierce Protein-Free (TBS) Blocking Buffer	100 mL	**37585**
Pierce Protein-Free T20 (PBS) Blocking Buffer	1 L	**37573**
Pierce Protein-Free T20 (TBS) Blocking Buffer	1 L	**37571**
StartingBlock (PBS) Blocking Buffer	1 L	**37538**
StartingBlock (PBS) Blocking Buffer	100 mL	**37578**
StartingBlock (TBS) Blocking Buffer	1 L	**37542**
StartingBlock (TBS) Blocking Buffer	100 mL	**37579**
StartingBlock T20 (PBS) Blocking Buffer	1 L	**37539**
StartingBlock T20 (TBS) Blocking Buffer	1 L	**37543**
Blocker FL Fluorescent Bocking Buffer (10X)	100 mL	**37565**

Block, wash, probe

Product	Quantity	Cat. No.
Secondary antibodies and solutions		
Stabilized Goat Anti–Mouse IgG (H+L) Secondary Antibody, HRP, 0.01 mg/mL	2 mL	**32430**
Stabilized Goat Anti–Rabbit IgG (H+L) Secondary Antibody, HRP, 0.01 mg/mL	2 mL	**32460**
Pierce High Sensitivity Streptavidin-HRP	0.5 mL	**21130**
Pierce High Sensitivity Streptavidin-HRP	5 mL	**21132**
Pierce High Sensitivity Streptavidin-HRP, Prediluted	2 mL	**21134**
Pierce High Sensitivity NeutrAvidin-HRP	0.5 mL	**31030**
Pierce High Sensitivity NeutrAvidin-HRP	5 mL	**31032**
Goat Anti–Mouse IgG (H+L) Secondary Antibody, HRP	2 mL	**31430**
Goat Anti–Rabbit IgG (H+L) Secondary Antibody, HRP	2 mL	**31460**
Goat Anti–Mouse IgG (H+L) Poly-HRP Secondary Antibody, HRP	0.5 mL	**32230**
Goat Anti–Rabbit IgG (H+L) Poly-HRP Secondary Antibody, HRP	0.5 mL	**32260**
Ethylene Glycol (50% solution)	200 mL	**29810**
Guardian Peroxidase Conjugate Stabilizer/Diluent	200 mL	**37548**
Guardian Peroxidase Conjugate Stabilizer/Diluent	1 L	**37552**
HRP-Conjugate Diluent/Stabilizer	1 L	**00-2018**
Pierce Stable Peroxide Substrate Buffer (10X)	100 mL	**34062**
Poly-HRP Dilution Buffer	1 L	**N501**
Poly-HRP Dilution Buffer	100 mL	**N500**
Wash buffers		
Pierce Fast Wash Buffer, 10X	1 L	**37577**
Wash Buffer (25X)	100 mL	**WB01**
Wash Buffer (25X)	1 L	**WB02**
BupH Phosphate Buffered Saline Packs	40 packs	**28372**
BupH Tris Buffered Saline Packs	40 packs	**28376**
BupH Tris Buffered Saline Packs	10 packs	**28379**
Tween 20 Surfact-Amps Detergent Solution	50 mL	**85113**
PBS (Phosphate-Buffered Saline) Tablets	100 tablets	**00-3002**
PBS (Phosphate-Buffered Saline) (10X), pH 7.4, RNase-free	500 mL	**AM9624**
PBS (Phosphate-Buffered Saline) (10X), pH 7.4, RNase-free	1 L	**AM9625**
Pierce 20X PBS Tween 20 Buffer	500 mL	**28352**
Pierce 20X Phosphate Buffered Saline	500 mL	**28348**
Pierce 20X TBS Buffer	500 mL	**28358**
Pierce 20X TBS Tween 20 Buffer	500 mL	**28360**
TBS Buffer	1 pack	**R017R.0000**
WesternBreeze Wash Solution (16X)	2 x 100 mL	**WB7003**

For more Invitrogen primary and secondary antibodies for western blot analysis, go to **thermofisher.com/antibodies**

Detect

Product	Quantity	Cat. No.
Chromogenic HRP substrates		
Metal Enhanced DAB Substrate	275 mL	**34065**
1-Step Ultra TMB-Blotting Solution	250 mL	**37574**
Pierce CN/DAB Substrate Kit	275 mL	**34000**
Chromogenic AP substrates		
1-Step NBT/BCIP Substrate Solution	250 mL	**34042**
1-Step NBT/BCIP plus Suppressor Substrate Solution	100 mL	**34070**
BCIP Substrate Powder (5-bromo-4-chloro-3-indolyl phosphate p-toluidine)	1 g	**34040**
NBT Substrate Powder (nitro-blue tetrazolium chloride)	1 g	**34035**
WesternBreeze Chromogenic Kit, anti-goat	1 kit	**WB7107**
WesternBreeze Chromogenic Kit, anti-mouse	1 kit	**WB7103**
WesternBreeze Chromogenic Kit, anti-rabbit	1 kit	**WB7105**
Chemiluminescent HRP substrates		
Novex ECL Chemiluminescent Substrate Reagent Kit	2 x 125 mL	**WP20005**
Pierce ECL Western Blotting Substrate	500 mL	**32106**
Pierce ECL Western Blotting Substrate	50 mL	**32109**
Pierce ECL Western Blotting Substrate	250 mL	**32209**
SuperSignal West Dura Extended Duration Substrate	100 mL	**34075**
SuperSignal West Dura Extended Duration Substrate	200 mL	**34076**
SuperSignal West Dura Extended Duration Substrate	20 mL	**37071**
SuperSignal West Pico PLUS Chemiluminescent Substrate	1 L	**34578**
SuperSignal West Pico PLUS Chemiluminescent Substrate	500 mL	**34580**
SuperSignal West Pico PLUS Chemiluminescent Substrate	200 mL	**34577**
SuperSignal West Pico PLUS Chemiluminescent Substrate	20 mL	**34579**
SuperSignal West Atto Ultimate Sensitivity Substrate	100 mL	**A38555**
SuperSignal West Atto Ultimate Sensitivity Substrate	200 mL	**A38556**
SuperSignal Western Blot Substrate Bundle, Pico PLUS + Trial Size Femto Combo Kit	500 mL + 20 mL	**A43840**
SuperSignal Western Blot Substrate Bundle, Pico PLUS + Trial Size Atto Combo Kit	500 mL + 20 mL	**A45915**
SuperSignal Western Blot Substrate Bundle, Atto + Trial Size Pico PLUS Combo Kit	100 mL + 20 mL	**A45917**
SuperSignal Western Blot Substrate Trial Pack, Atto + Pico PLUS Combo Kit	20 mL + 20 mL	**A45918**

Product	Quantity	Cat. No.
Chemiluminescent AP substrates		
WesternBreeze Chemiluminescent Kit, anti-goat	1 kit	**WB7108**
WesternBreeze Chemiluminescent Kit, anti-mouse	1 kit	**WB7104**
WesternBreeze Chemiluminescent Kit, anti-rabbit	1 kit	**WB7106**
CDP-*Star* Substrate (0.25 mM Ready-To-Use)	50 mL	**T2145**
CDP-*Star* Substrate (0.25 mM Ready-To-Use)	100 mL	**T2146**
CDP-*Star* Substrate (0.25 mM Ready-To-Use)	250 mL	**T2147**
CDP-*Star* Substrate (0.25 mM Ready-To-Use) with Nitro-Block-II Enhancer	100 mL	**T2218**
CDP-*Star* Substrate (0.4 mM Ready-To-Use) with Emerald-II Enhancer, Size A	100 mL	**T2216**
CDP-*Star* Substrate (0.4 mM Ready-To-Use) with Sapphire-II Enhancer, Size A	100 mL	**T2214**
CDP-*Star* Substrate (12.5 mM Concentrate)	1 mL	**T2304**
CDP-*Star* Substrate (12.5 mM Concentrate)	2 mL	**T2305**
CDP-*Star* Substrate (12.5 mM Concentrate)	5 mL	**T2306**
CDP-*Star* Substrate (12.5 mM Concentrate)	10 mL	**T2307**
CDP-*Star* Substrate (12.5 mM Concentrate)	20 mL	**T2308**
CDP-*Star* Substrate (12.5 mM Concentrate)	50 mL	**T2309**
CDP-*Star* Substrate (12.5 mM Concentrate)	200 mL	**T2310**
CSPD Substrate (0.25 mM Ready-To-Use)	50 mL	**T2141**
CSPD Substrate (0.25 mM Ready-To-Use)	100 mL	**T2142**
CSPD Substrate (0.25 mM Ready-To-Use)	250 mL	**T2143**
CSPD Substrate (0.25 mM Ready-To-Use) with Nitro-Block Enhancer	100 mL	**T2217**
CSPD Substrate (0.4 mM Ready-To-Use) with Emerald-II Enhancer, Size A	100 mL	**T2212**
CSPD Substrate (0.4 mM Ready-To-Use) with Sapphire-II Enhancer, Size B	100 mL	**T2210**
CSPD Substrate (25 mM Concentrate)	0.5 mL	**T2040**
CSPD Substrate (25 mM Concentrate)	2 mL	**T2041**
CSPD Substrate (25 mM Concentrate)	5 mL	**T2042**
CSPD Substrate (25 mM Concentrate)	10 mL	**T2043**
CSPD Substrate (25 mM Concentrate)	25 mL	**T2044**
CSPD Substrate (25 mM Concentrate)	1 mL	**T2098**
CSPD Substrate (25 mM Concentrate)	100 mL	**T2138**
Luminescence Enhancer For Membrane Blotting Assays, Nitro-Block Enhancer	20 mL	**T2184**
Novex AP Chemiluminescent Substrate	100 mL	**WP20002**
Novex AP Mouse Chemiluminescent Detection Kit	1 kit	**SLF1021**
Novex AP Rabbit Chemiluminescent Detection Kit	1 kit	**SLF1022**

Product	Quantity	Cat. No.
Chemiluminescent and fluorescent detection instruments		
iBright FL1500 Imaging System	1 system	**A44114**
iBright CL1500 Imaging System	1 system	**A44115**
iBright CL750 Imaging System	1 system	**A44116**
iBright SAE Software for 21 CFR Part 11, single license	1 license	**A49208**
X-ray film		
CL-XPosure Film, 5 x 7 in. (13 x 18 cm)	100 sheets	**34090**
CL-XPosure Film, 7 x 9.5 in. (18 x 24 cm)	100 sheets	**34089**
CL-XPosure Film, 8 x 10 in. (20 x 25 cm)	100 sheets	**34091**

Product	Quantity	Cat. No.
Specialty western kits		
SuperSignal West HisProbe Kit	685 mL	**15168**
HisProbe-HRP Conjugate	2 mg	**15165**
Clean-Blot IP Detection Kit (HRP)	25 blots	**21232**
Clean-Blot IP Detection Reagent (HRP)	2.5 mL	**21230**
SuperSignal Western Blot Enhancer	500 mL	**46640**
SuperSignal Western Blot Enhancer	50 mL	**46641**
Normalization and quantitation		
No-Stain Protein Labeling Reagent	40 reactions	**A44449**
No-Stain Protein Labeling Reagent, Trial Size	10 reactions	**A44717**

Product	Quantity	Cat. No.
Blot stripping and reprobing		
Restore Fluorescent Western Blot Stripping Buffer	20 mL	**62299**
Restore Fluorescent Western Blot Stripping Buffer	100 mL	**62300**
Restore PLUS Western Blot Stripping Buffer	30 mL	**46428**
Restore PLUS Western Blot Stripping Buffer	500 mL	**46430**
Restore Western Blot Stripping Buffer	500 mL	**21059**
Restore Western Blot Stripping Buffer	5 L	**21063**
Restore Western Blot Stripping Buffer, Trial Size	30 mL	**21062**

Recommended references for western blotting

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Additional reading

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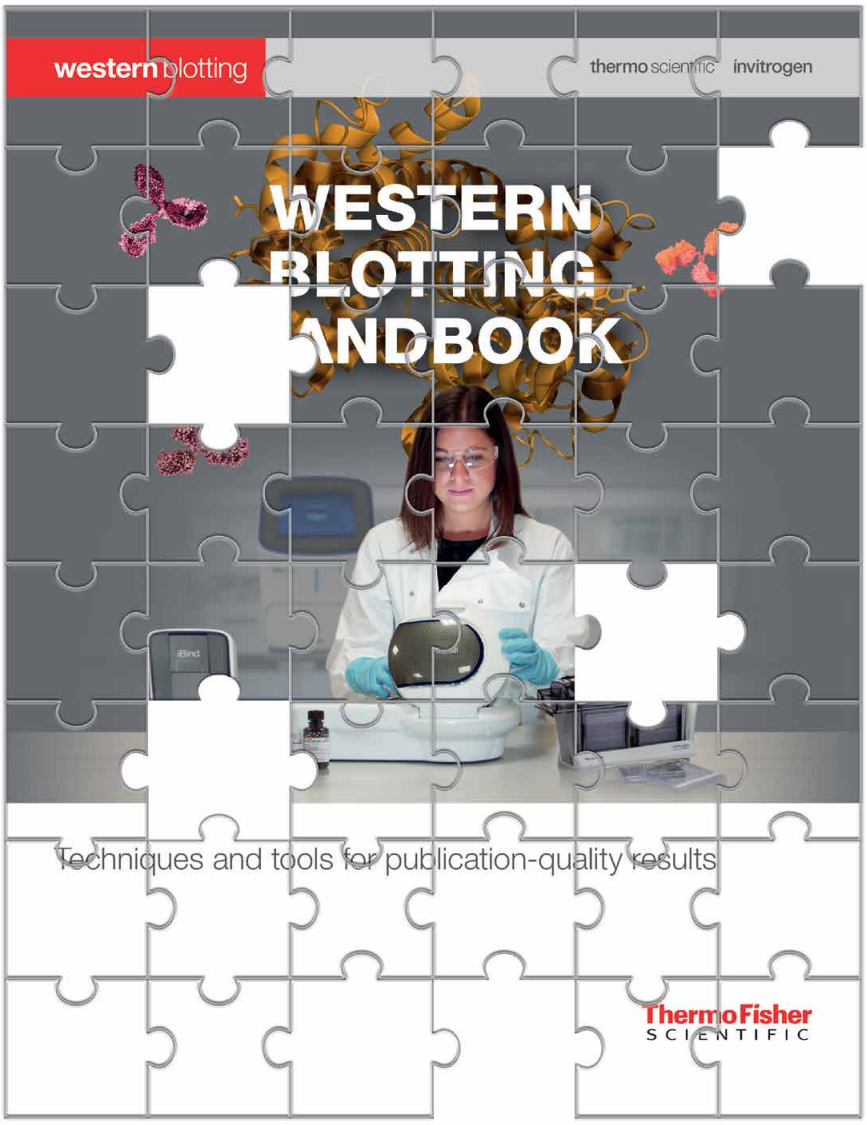

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