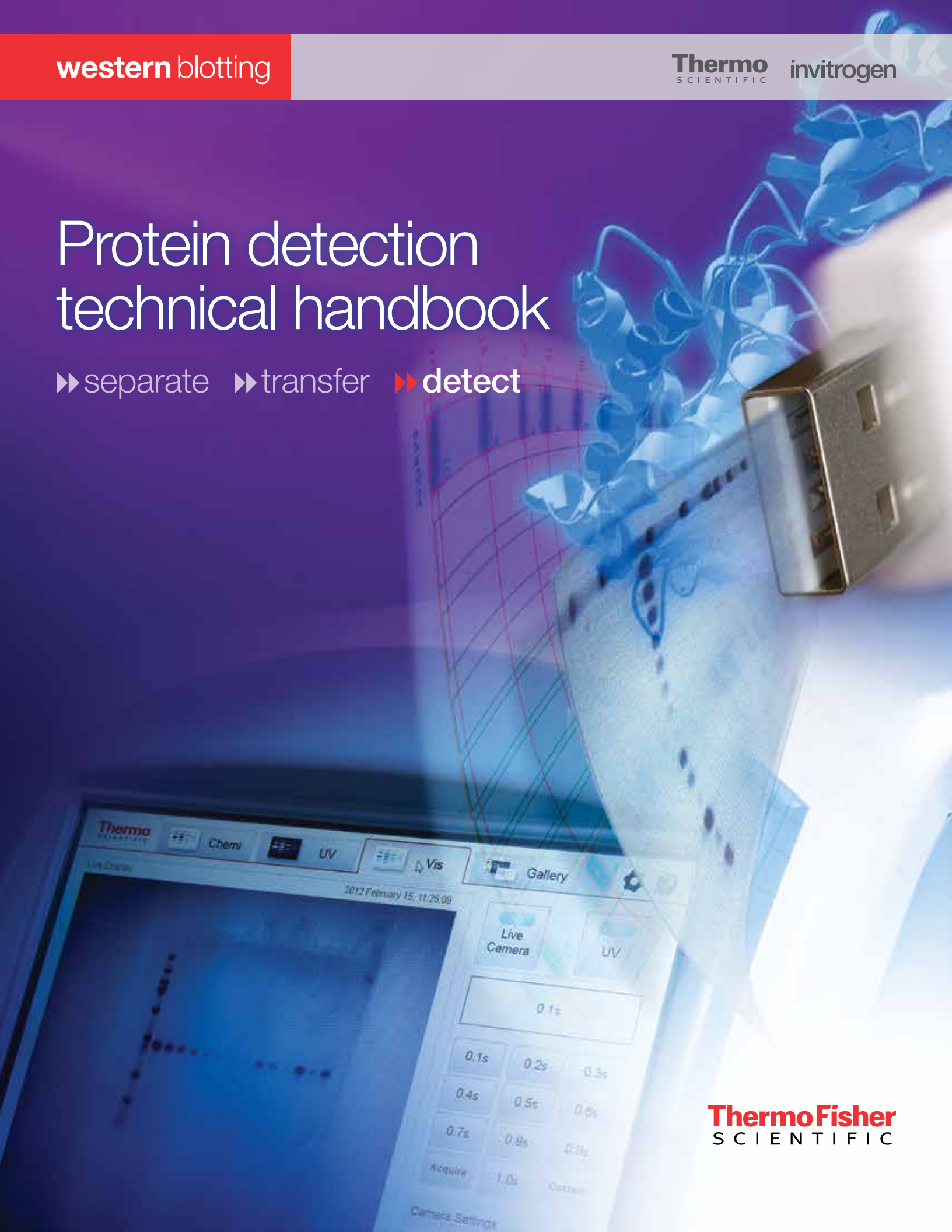
western blotting

Thermo SCIENTIFIC invitrogen

Protein detection technical handbook

separate transfer **detect**



ThermoFisher SCIENTIFIC

Comprehensive protein detection solutions designed to drive your success

The last step in the Western blot workflow, after the separation of proteins by PAGE and their transfer from gel to membrane, is detection. In this step, primary antibodies specific to the protein of interest bind the protein on the membrane. With a variety of detection techniques to choose from (chemiluminescence, fluorescence or chromogenic), you can select a technology to match your experimental requirements and the instruments you have available. Quick visualization or precise quantitation, single-probe detection or multiplexing — we offer a range of reagents and kits for Western blot detection and subsequent analysis.

For a complete listing of all available products and more, visit **thermofisher.com/western**

Contents

Introduction

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 chemiluminescence, fluorescence 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

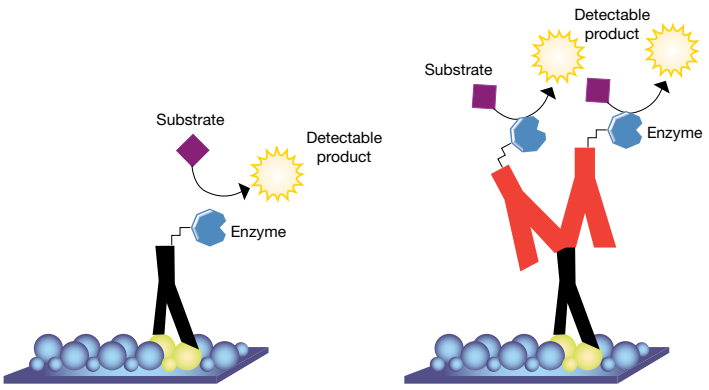
Signal-to-noise ratio compares the level of desired or relevant signal to the level of background noise or non-relevant signal; the higher the ratio, the better the result. In Western blotting, the signal is the density of the specific probed protein band of interest; the noise is the density of the 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, please see page 74.

Direct versus indirect detection

The antibody that recognizes the target antigen 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 antigen 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 antigen. Thus, the antigen 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 1).

Indirect methods can offer increased sensitivity through the signal amplification that occurs as multiple secondary antibodies bind to a single primary antibody. In addition, a given secondary antibody can be used with 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.



Direct method

Advantages:
- Quicker since only one antibody is used
- No concern for 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 antigen 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

Indirect detection

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 secondary antibody allows change of detection method

Disadvantages:
- Secondary antibodies may produce nonspecific staining
- Additional steps required compared to the direct method

Figure 1. Comparison of direct and indirect Western blot detection methods.

Detection systems: chromogenic, chemiluminescence and fluorescence

Another consideration in Western blotting concerns the choice of detection method. This decision will be based on a number of factors including equipment available and sensitivity needs. Table 1 covers a number of the parameters that could affect your choice of Western blot detection system. A more thorough discussion of these methods can be found starting on page 28.

Table 1. Comparison of Western blotting detection systems.

Detection type	Chromo-genic	Chemilumi-nescence	Fluores-cence	Long-wave-length fluo-rescence
Sensitivity	++	+++++	++++	+++
Signal duration	Months	Hours	Months/ Years	Years
Multiplexing	No	No	Yes	Yes
Means of visualization	Visual; no instrument required	X-ray film, CCD imagers	Fluores-cent digital imager, NIR imag-ing system (depen-dent on fluor used)	Fluorescent digital imager, UV transillumi-nator, UV gel documenta-tion systems

Did you know?

Over 30 years ago, **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.

Probing the Western blot membrane: block, antibody incubation and wash steps

Introduction

Traditionally, probing a Western blot prior to visualization involved a series of manual steps, many were individually short but collectively required significant hands-on time. Today, instruments are becoming 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 2).

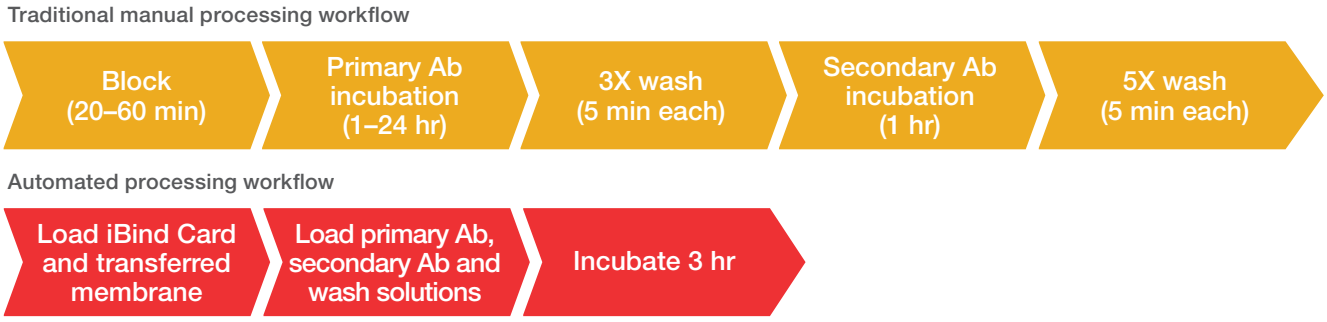


Figure 2. Comparison of traditional manual versus automated Western blot processing workflows. Traditional manual Western blot processing workflow requires more than 10 hands-on steps and at least four hours before final detection can occur. Automated Western blot processing using the iBind and iBind Flex Western Systems require only a 15- to 30-minute setup time and no additional hands-on steps before you can move to final detection.

For ordering information refer to page 78.

Automated probing systems

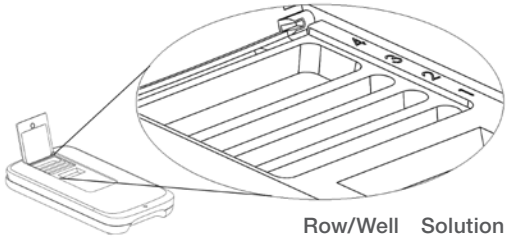
Watch our iBind video at **thermofisher.com/ibind**

iBind Western Systems

The Invitrogen™ iBind™ Western Systems are non-powered 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, with subsequent steps proceeding 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 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 2 compares the two systems.

▸▸ Learn more at **thermofisher.com/ibind**



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

Figure 3. Sample loading wells for the iBind Western System.

Table 2. Characteristics of iBind Western Systems.

	iBind Western Device	iBind Flex Western Device
Mini blot (single)	Yes	Yes
Mini blot (dual)	No	Yes
Midi blot	No	Yes
Vertically cut strips	No	Yes

Did you know?

More than 90% of the cost of manual Western blot methods is due to the amount of primary antibody used. For example, to process a single mini blot using a 10mL solution of diluted primary antibody at a 1:1,000 concentration can cost approximately $30 (USD).

iBind Flex Western System

The Invitrogen™ iBind™ Flex Western System offers flexible blot processing to optimize antibody use, easily change blot formats, and reduce hands-on time. Simply load primary and secondary antibody solutions and wash solutions into the device, then walk away, because the system automatically performs all immunodetection steps using SLF technology, a simple form of capillary action. In less than three hours, the blot is ready for final detection. The iBind Flex Western System delivers flexible solutions with automated convenience.

Features:

- **Flexibility** — process up to one midi blot, two mini blots or six vertically cut strips using the same or different conditions
- **Compatibility** — use nitrocellulose or PVDF membranes, directly labeled primary or secondary antibody detection (AP, HRP or fluorescent labeled)
- **Cost savings** — use up to 80% less primary antibody than with traditional tray-based incubation steps for Western blotting

The iBind Flex Western System comes with interchangeable wells, which allow you to run multiple membrane formats and even run different primary and secondary antibody conditions in the same device at the same time.

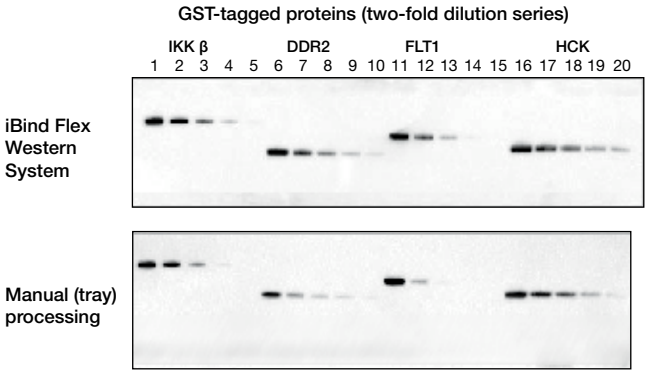
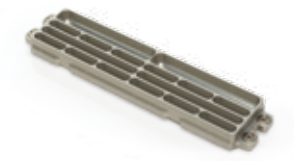


Figure 4. Comparison of Western blots processed manually vs. with the iBind Flex Western System. Samples containing GST-tagged recombinant proteins were separated on Invitrogen™ NuPAGE™ 4–12%, 20-well midi gels in MOPS SDS running buffer and then transferred to nitrocellulose membranes using the Invitrogen™ iBlot™ Dry Blotting System. Blots were probed with identical concentrations of the same pair of primary and secondary antibodies. The primary antibody was rabbit anti-GST diluted 1:500 (8µL in 4mL iBind Flex Solution for the iBind system, 40µL in 20mL for manual tray incubation). The secondary antibody was goat anti-rabbit HRP diluted 1:600 (6.7µL in 4mL iBind Flex Solution for the iBind system, 33.3µL in 20mL for manual tray incubation). For final detection, blots were incubated for five minutes in SuperSignal West Dura Extended Duration Substrate for visualization with an imaging system.
Lanes 1–5: IKK β (80ng, 40ng, 20ng, 10ng, 5ng)
Lanes 6–10: DDR2 (120ng, 60ng, 30ng, 15ng, 7.5ng)
Lanes 11–15: FLT1 (40ng, 20ng, 10ng, 5ng, 2.5ng)
Lanes 16–20: HCK (360ng, 180ng, 90ng, 45ng, 22.5ng)

Midi blots	Mini blots	Vertically cut strips
		
Using same antibody conditions	Using same or different antibody conditions	Using same or different antibody conditions

For ordering information refer to page 78.

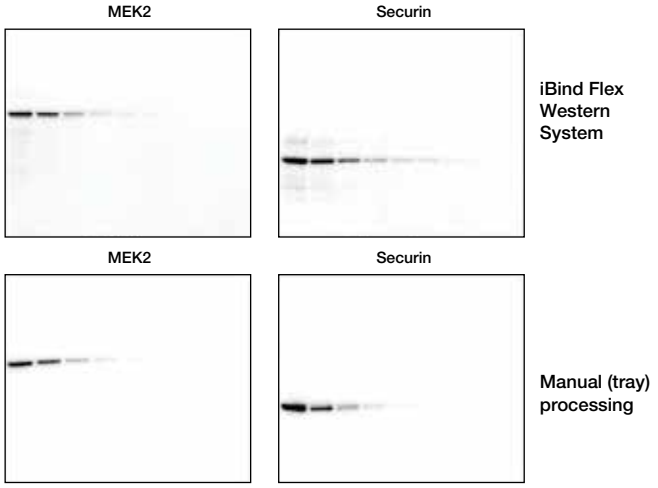


Figure 5. Better Western blot results using less primary antibody. Comparison of mini blots processed manually (probing and washing steps performed in a tray) vs. with the iBind Flex Western Device. Blots were produced by separating samples on Invitrogen™ Bolt™ 4–12% Bis-Tris 10-well mini gels with MES SDS running buffer and then transferred to nitrocellulose membrane using the Invitrogen™ iBlot™ device. Each gel contained 10 lanes loaded with two-fold dilution series of 293 cell extracts (30µg to 0.06µg). After immunodetection using the conditions described below, blots were incubated for five minutes in SuperSignal West Dura substrate and the signal documented with an imager.

- MEK2 (45kDa): rabbit anti-MEK2 antibody applied at 1:1,000 for iBind blot processing (2µL in 2mL of iBind Flex Solution), and 1:1,000 for manual blot processing (10µL in 10mL buffer).
- Securin (428kDa): rabbit anti-Securin antibody applied at 1:1,000 for iBind blot processing (2µL in 2mL of iBind Flex Solution), and 1:1,000 for manual blot processing (10µL in 10mL buffer).
- Secondary Antibody (both targets): goat anti-rabbit IgG at 1:600 for iBind blot processing (3.33µL in 2mL of iBind Flex Solution), and at 1:1,800 for manual blot processing (5.55µL in 10mL).

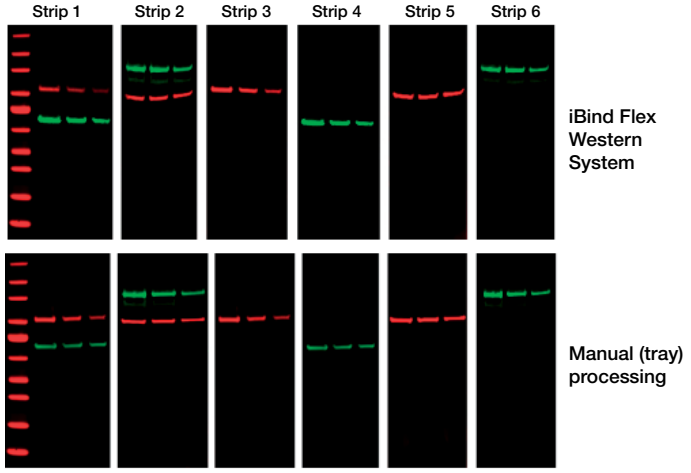


Figure 6. Excellent Western blot results with vertically cut strips and fluorescence detection. Comparison of mini blots processed manually (probing and washing steps performed in a tray) vs. with the iBind Flex Western Device. Blots were produced by separating samples on Bolt Bis-Tris Plus 4–12% 10-well mini gels with MES SDS running buffer. They were then transferred to nitrocellulose membranes using the iBlot 2 Gel Transfer Device, cutting each into three-lane strips. Final imaging was performed using the Odyssey™ CLx Infrared Imaging System.
Samples and lanes were as follows:

- Lane 1 Strip 1: Thermo Scientific™ PageRuler™ Prestained NIR Protein Ladder (3µL)
- Strip 1: Phosphorylated AKT cell extract (15µg, 7.5µg, 3.75µg) and Elk-1 Fusion Protein (150ng, 75ng, 37.5ng)
- Strip 2: HeLa cell extract (30µg, 15µg, 7.5µg)
- Strip 3: Phosphorylated AKT cell extract (15µg, 7.5µg, 3.75µg)
- Strip 4: Elk-1 fusion protein (150ng, 75ng, 37.5ng)
- Strip 5: HeLa cell extract (30µg, 15µg, 7.5µg)
- Strip 6: HeLa cell extract (30µg, 15µg, 7.5µg)

Probing and antibody conditions using the iBind Flex FD Solution were as follows:

Strip #	1° Ab	[1° Ab] Manual	[1° Ab] iBind Flex	2° Ab	[2° Ab] Manual	[2° Ab] iBind Flex
1	Phospho-AKT (red) Phospho-Elk-1 (green)	1:2,000 1:1,000	1:400 1:200	IRDye 680LT IRDye 800CW	1:20,000 1:15,000	1:4,000 1:3,000
2	SRC (red) beta-Catenin (green)	1:250 1:1,000	1:50 1:200	IRDye 680LT IRDye 800CW	1:20,000 1:15,000	1:4,000 1:3,000
3	Phospho-AKT	1:2,000	1:400	IRDye 680LT	1:20,000	1:4,000
4	Phospho-Elk-1	1:1,000	1:200	IRDye 800CW	1:15,000	1:3,000
5	SRC	1:250	1:50	IRDye 680LT	1:20,000	1:4,000
6	beta-Catenin	1:1,000	1:200	IRDye 800CW	1:15,000	1:3,000

After completion of Western processing, blots were imaged on the Odyssey CLx instrument.

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

iBind Western System

The Invitrogen™ iBind™ Western System for mini blots is an automated Western blot processing platform that requires less primary antibody and enables sensitive, reproducible Western results. All blocking, antibody incubation and washing steps are hands-free, allowing you to load your solutions and walk away. No electricity or battery is required. You can also use your existing chemiluminescent, chromogenic or fluorescent Western detection protocols, including primary or secondary antibody conjugates of horseradish peroxidase (HRP), alkaline phosphatase (AP) or fluorophores. Automated processing enables improved blot-to-blot consistency with coefficients of variation (CVs) typically less than 5% (compared to manual processing that can have CVs of 13%).

Features:

- **Cost savings** — use up to 80% less primary antibody than with traditional tray-based incubation steps for Western blotting
- **Reproducibility** — automated immunodetection enables improved blot-to-blot consistency
- **Simplicity** — load solutions and walk away using SLF technology; no batteries or electricity required

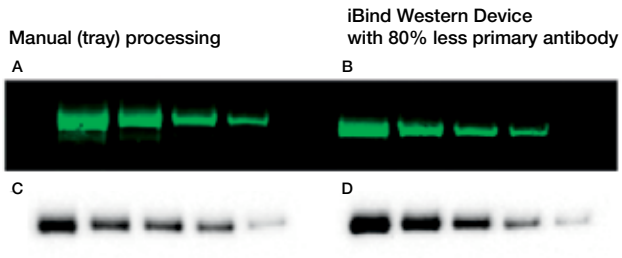


Figure 7. Using chemiluminescent or fluorescence-based detection methods, you can use up to 80% less primary antibody than manual methods. A 2-fold dilution series of EGF receptor control cell lysate (30µg, 15µg, 7.5µg, 3.75µg and 1.875µg) was used. Proteins were separated using the Bolt Bis-Tris Plus mini gels and transferred to PVDF membranes using the iBlot Dry Blotting System. The blots were probed with a mouse anti–phospho-EGF receptor [Tyr1068] (1H12) antibody (1:1,000 dilution, equated to 2µL antibody for the iBind device method and 10µL antibody for the manual method) followed by a goat anti–mouse IgG (H+L) peroxidase-conjugated antibody (1:360 for iBind device processing (5.55µL); 1:1,800 for manual method (5.55µL)). The standard iBind Solution Kit was used for the chemiluminescence blot (panel C, manual processing and panel D, iBind system processing); the iBind FD Solution Kit was used for fluorescence detection (panel A, manual processing and panel B, iBind processing). These results demonstrate that Western blots processed on the iBind device show comparable results to those obtained when Western blots are processed manually, with lower overall primary antibody requirements for the iBind device.

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

For ordering information refer to page 78.

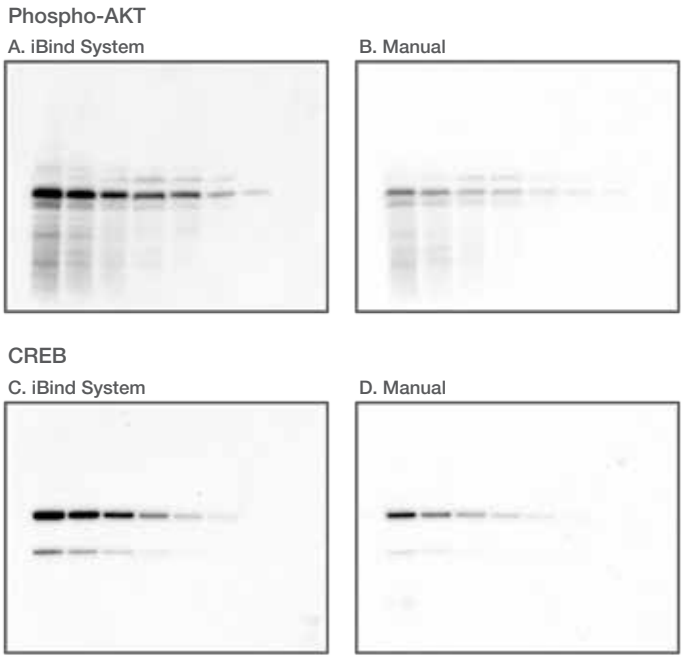


Figure 8. Western blots processed on the iBind device show superior sensitivity compared to Western blots processed manually. (Panel A and B) Western blots with phosphorylated AKT (left to right: 30µg–500ng cell lysate load) were processed either on the iBind device or using standard manual Western processing protocols as specified by the antibody manufacturer (monoclonal anti-phospho-AKT [pT308] (C31E5E) primary antibody; HRP-conjugated anti-rabbit secondary antibody). The blot processed with the iBind device detected phospho-AKT in 500ng of cell lysate, while the target was detected in 4µg on the manually processed blot. (Panel C and D) Western blots with cell lysate expressing CREB (left to right: 30µg–1µg cell lysate load) were processed either on the iBind device or using standard manual Western processing protocols as specified by the antibody manufacturer (polyclonal anti-CREB primary antibody; HRP-conjugated anti-rabbit secondary antibody). The blot processed with the iBind system detected CREB in 6µg of cell lysate, while 10µg of lysate was needed to detect CREB on the manually processed blot. For all blots, proteins were separated using the Bolt Bis-Tris Plus mini gels and transferred to PVDF membranes using the iBlot Dry Blotting System.

iBind Solutions Kits

The Invitrogen™ iBind™ Solution Kit and the iBind Fluorescence Detection (FD) Solution Kit are intended for use with the iBind and iBind Flex Western Devices for automated blot processing, from blocking to washes to antibody incubations. The iBind solutions act as the blocking, washing and antibody dilution solutions and have been optimized for compatibility with most antibodies and detection methods, including colorimetric, chemiluminescence and fluorescence. Each solution is released from the respective iBind device wells to the Invitrogen™ iBind™ or iBind™ Flex Card through SLF; the solutions are then wicked towards the stack region of the card. The glass fiber matrix of the card allows for homogenous and consistent flow of the solutions to the membrane, increasing the antigen-antibody interaction. Each iBind solutions kit contains sufficient solution for 10 mini blot reactions, while each iBind Flex Solution Kit and iBind Flex FD Solution Kit contains sufficient solution for 10 midi blot or 20 mini blot reactions.

Features:

- **SLF optimized** — solutions with ideal viscosity for blocking, washing and antibody dilution
- **Long shelf life** — store iBind solutions for 12 months at 4°C
- **Better antigen-antibody interaction** — solutions flow between the iBind Card and membrane for maximum interaction between protein and antibody

Manual probing methods

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 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-antigen 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.

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.

No single blocking agent is ideal for every occasion because each antibody-antigen pair has unique characteristics. For this reason, we offer a variety of buffers to suit your Western blot conditions.

For ordering information refer to page 78.

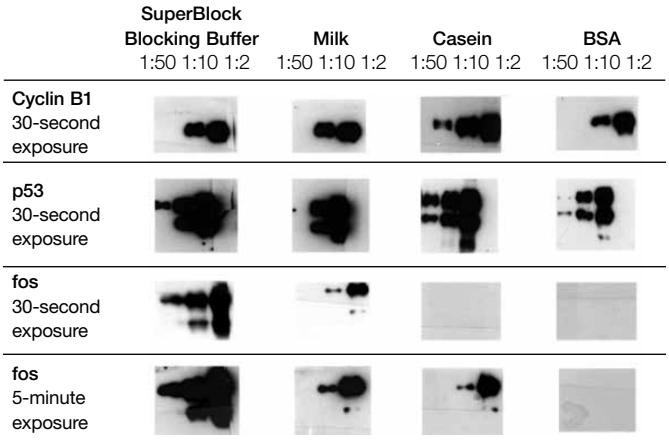


Figure 9. Blocking buffer optimization. Recombinant human cyclin B1, wild-type p53 and mouse fos baculovirus lysates were diluted in Lane Marker Reducing Sample Buffer (1:50, 1:10 or 1:2) and separated electrophoretically on a 12% SDS-polyacrylamide gel. The proteins were transferred to nitrocellulose membrane and cut into strips. The membrane strips were blocked for one hour at room temperature with shaking in Blocker Casein in TBS, 1% BSA in TBS, SuperBlock Blocking Buffer in TBS or 5% nonfat milk in TBS. Tween-20 (0.05%) was added to all blocking buffers. The membranes were then incubated with the appropriate primary antibody at 0.5µg/mL prepared in the different blocking solutions for one hour at room temperature with shaking. Each membrane strip was washed with TBS followed by a one-hour incubation in HRP-conjugated goat anti-mouse antibody prepared in the different blocking buffers at 25ng/mL. The membranes were washed with TBS. A working solution of SuperSignal West Pico Chemiluminescent Substrate was prepared and added to each membrane for five minutes. The membranes were placed in sheet protectors and exposed to film for 30 seconds and five minutes as indicated. The film was developed per the manufacturer's instructions.

Did you know?

BLOTTO is an acronym for Bovine Lacto Transfer Technique Optimizer.

Table 3. Western blot blocking buffers selection guide.

	Product	Description
Start with	StartingBlock Blocking Buffer	Single purified protein; fast blocking; broad applicability; excellent for reblocking stripped blots available in PBS and TBS with and without T20. Compatible with most antibodies as well as biotin-binding reagents
	SEA BLOCK Blocking Buffer	Steelhead salmon serum. Recommended for fluorescence. For reduced cross-reactivity and high signal-to-noise ratios
	SuperBlock Blocking Buffer	Free of biotin and albumin allowing for reduced undesirable binding and high signal-to-noise ratios. Single purified glycoprotein; fast blocking; broad applicability; stabilizes plate-coated antibodies for drying; available in PBS and TBS with and without Tween-20
Next	BSA Blocking Buffer	Purified bovine serum albumin in PBS or TBS
	Casein Blocking Buffer	Purified casein in PBS or TBS
Specialty	Pierce Fast Blocking Buffer	Developed especially for rapid, 5-minute blocking of Western blots as part of the Fast Western Blot system; proprietary proteins formulation in TBS
	Pierce Protein-Free Blocking Buffers	Proprietary non-protein blocking compound; available in PBS and TBS with and without Tween-20. Eliminate potential cross-reactivity associated with protein-based blockers and provide high signal-to-noise ratios
Generic	BLOTTO Blocking Buffer	Non-fat dry milk proteins in TBS
	Clear Milk Blocking Buffer	Milk proteins, clarified and stabilized in proprietary solution

StartingBlock Blocking Buffers

Although no blocking buffer is ideal for every system, you can improve the odds dramatically with Thermo Scientific™ StartingBlock™ Blocking Buffer because it is compatible with the widest variety of antibodies. For example, StartingBlock Blocking Buffers are compatible with biotin-containing systems, while milk-based protein blockers interfere. StartingBlock buffers rarely cross-react with rabbit antibodies, while many other blockers do. StartingBlock Blocking Buffers are also free of potentially interfering serum proteins.

StartingBlock Blocking Buffers offer a high level of performance — regardless of the system you choose for your Western blotting (Figure 10). They may be the only blockers you ever use.

Features:

- **Compatible with a wide range of detection systems** — serum-free, biotin-free and rarely cross-reacts with rabbit antibodies
- **Shorter blocking times** — 1–15 minutes for Western blots
- **Strip and reprobe** — no reblocking necessary

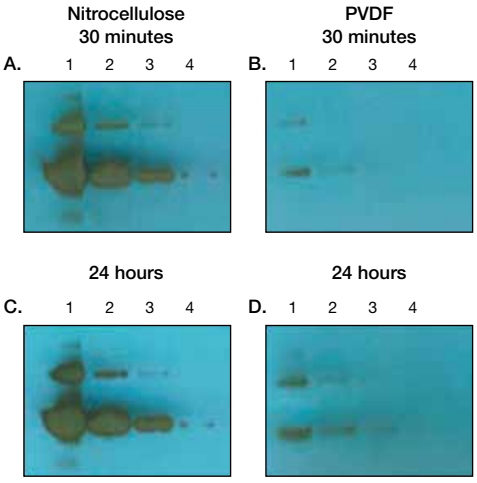


Figure 10. StartingBlock Blocking Buffer performance after stripping and reprobing. Nitrocellulose and PVDF membranes that had been blocked with StartingBlock Blocking Buffer and used to detect a different primary target were then stripped with Restore Western Blot Stripping Buffer (Cat. No. 21059) and probed for the transferrin receptor (CD71). Detection was with SuperSignal West Dura Extended Duration Substrate (Cat. No. 34075) and exposed to film for 30 minutes and then again for 24 hours. Very little background occurs with either membrane or exposure time, indicating exceptional blocking performance.

For ordering information refer to page 78.

SEA BLOCK Blocking Buffer

Thermo Scientific™ SEA BLOCK™ Blocking Buffer is steelhead salmon serum in PBS that is especially useful as a blocking agent in immunohistochemistry (IHC) and other detection methods involving mammalian samples.

Fish serum has several advantages over typical blocking buffers. Because salmon is phylogenetically distant from mammals, which are the source of antibodies and samples used in most experiments, its serum proteins are less likely to have specific binding interactions with proteins used in typical cell biology experiments. When normal serum causes background in immunohistochemistry (IHC), try this product. SEA BLOCK Blocking Buffer is particularly effective in applications involving fluorescence imaging. The blocker has been used to decrease background and increase signal-to-noise ratios with near-IR fluorescent probes and is validated for use with the LI-COR Odyssey Infrared Imaging System.

Features:

- **Non-mammalian** — fish proteins are less likely to have specific binding interactions with antibodies and other mammalian proteins present in typical methods
- **Convenient** — filtered and stabilized in PBS for compatibility with most assay systems
- **Easy to use** — can be used as supplied or diluted up to 10-fold as needed
- **Flexible** — may be used for many different applications, including as a diluent for antibodies
- **Fluorescence optimized** — recommended blocker for fluorescent Western detection

SuperBlock Blocking Buffers

Our most popular biotin-free blocking buffer, Thermo Scientific™ SuperBlock™ Blocking Buffer, now comes in both dry and liquid formats. Many researchers have discovered that SuperBlock Blocking Buffer is the only blocking buffer needed for all of their applications. Each packet of Thermo Scientific™ SuperBlock™ Dry Blend (TBS) Blocking Buffer produces 200mL of buffer.

Features:

- **Fast** — blocks Western blot membranes in five to 10 minutes
- **High sensitivity** — non-serum protein solution yields a high signal-to-noise ratio
- **Flexible formats** — liquid formulations available in PBS or TBS; powder formulation in TBS
- **Compatibility** — biotin-free

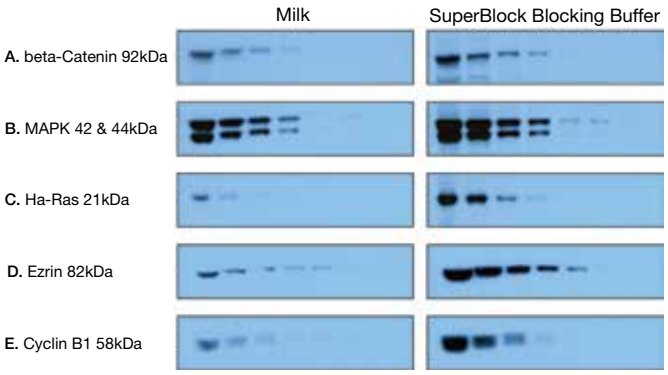


Figure 11. SuperBlock Blocking Buffer in PBS is better than milk for sensitive detection of target proteins. HeLa cell lysate (20, 10, 5, 2.5, 1.25, 0.625 and 0.3125µg) was separated by SDS-PAGE, and transferred to nitrocellulose (Panels A-C, Cat. No. 88014) or PVDF (Panels D-E, Cat. No. 88585) membrane. Membranes were blocked for one hour using 5% milk in Tris-buffered saline with 0.05% Tween-20 Detergent or SuperBlock Blocking Buffer in phosphate-buffered saline with 0.05% Tween-20 Detergent. Membranes were probed for the indicated targets. Blots were incubated in SuperSignal West Pico Chemiluminescent Substrate (Cat. No. 34080) for five minutes and exposed to Thermo Scientific™ CL-XPosure™ Film (Cat. No. 34091).

Blocker BSA

Thermo Scientific™ Blocker BSA in PBS or TBS is a ready-to-use, 10X solution of bovine serum albumin protein for blocking steps in Western blot detection methods. Blocker BSA is usually more effective than nonfat milk blocking buffers for biotin-avidin systems because it contains a single purified protein that is devoid of endogenous biotin.

Features:

- **Purified protein** — 10% solutions of high-quality bovine serum albumin
- **Convenient** — concentrated formulation saves storage space
- **Easy to use** — no powder to dissolve; ready-to-dilute liquid concentrate
- **Flexible** — available in PBS or TBS formulations

Blocker Casein

Thermo Scientific™ Blocker Casein is a ready-to-use, in either TBS or PBS. The blocking buffer contains casein protein that is purified from milk by the Hammarsten method. Blocker Casein Buffers are 1% (w/v) casein, which corresponds to the optimal concentration for most applications. Casein is not recommended for use when probing for phosphoproteins.

Features:

- **Purified casein** — single-protein blocking buffer provides fewer chances of cross-reaction with assay components than serum or milk solutions
- **Easy to use** — 1% casein solutions are ready to use; can be diluted further as needed
- **Flexible** — available in PBS and TBS formulations to suit a variety of applications
- **Safe** — stable, thimerosal-free formulations

Pierce Fast Blocking Buffer

Thermo Scientific™ Pierce™ Fast Blocking Buffer effectively and reliably blocks Western blots in just five minutes to provide low-background results and speed up traditional blotting protocols. The buffer is compatible with antibodies and biotin-avidin systems, as well as both nitrocellulose and PVDF membranes.

Features:

- **Fast** — shorten the typical Western blot development by up to one hour
- **Simple** — optimized protocol makes Western blot analysis easier than ever
- **Low background** — provides results comparable to classic Western blotting buffers

Protein-Free Blocking Buffers

Traditional blocking buffers contain proteins that can cross-react with a system, resulting in high background and reduced signal (Figure 12). Thermo Scientific™ Pierce™ Protein-Free Blocking Buffers eliminate or minimize cross-reactivity associated with protein-based blocking buffers in ELISA, Western blotting, arrays and other immunodetection applications. This buffer is especially useful when other protein-based blocking buffers have resulted in higher background noise.

Features:

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

For ordering information refer to page 78.

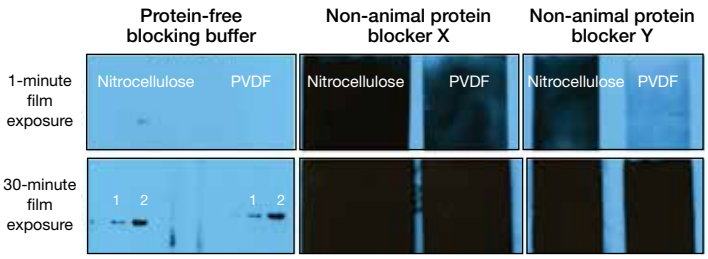


Figure 12. Pierce Protein-Free Blocking Buffer efficiently blocks Western blotting membranes. Jurkat apoptotic lysate (Lane 1: 0.25µg; lane 2: 0.50µg) was separated in 4–20% Tris-glycine gels and transferred to nitrocellulose or PVDF membranes. The membranes were blocked for one hour at room temperature with the indicated blocking buffer, probed with mouse anti-PARP (0.25µg/mL) followed by goat anti-mouse HRP (4ng/mL) and detected by SuperSignal West Dura substrate.

Blocker BLOTTO

Thermo Scientific™ Blocker BLOTTO Blocking Buffer is a ready-to-use 5% solution of nonfat powdered milk in Tris-buffered saline for Western blot and other detection methods.

Features:

- **Convenient** — supplied as a ready-to-use 1X TBS solution; can be diluted as needed
- **Easy to use** — formulated with antifoaming agent and thimerosal-free preservative
- **Flexible** — may be used for multiple applications, including as a diluent for antibody
- **Popular** — nonfat milk has been used for many years in a variety of protein methods, although it is not recommended for avidin-based techniques because it contains some endogenous biotin

Pierce Clear Milk Blocking Buffer

Thermo Scientific™ Pierce™ Clear Milk Blocking Buffer (10X) is a proprietary preformulated milk solution for blocking excess nonspecific binding sites, reducing background in Western blotting applications and diluting antibodies when used with nitrocellulose and PVDF membranes. It is similar to Blocker BLOTTO Buffer, but offers a space-saving feature as 10X concentrate. Pierce Clear Milk Blocking Buffer (10X) provides lower background, enhanced sensitivity, extended shelf life and reproducible results compared to homemade buffers based on dissolved nonfat dry milk.

Features:

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

Did you know?

Milk, the most popular blocking buffer additive, should not be used with avidin-biotin detection because the biotin in milk will result in high background.

Antibody incubations

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 particular antigens enable those targets to be "found" and detected in a complex sample. However, antibodies are themselves proteins, and they are not specifically detectable in an assay system unless they are tagged for visualization or secondarily probed with another molecule that is tagged.

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. Enzymatic tags such as horseradish peroxidase (HRP) or alkaline phosphatase (AP) are most commonly used for 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 antigen to be detected and what antibodies are available to that antigen. A large number of primary antibodies are available commercially. Alternatively, a primary antibody may be custom-made to recognize the antigen of interest.

Both polyclonal and monoclonal antibodies work well for Western blotting. Polyclonal antibodies are less expensive and less time-consuming to produce, and they often have a high affinity for the antigen. Monoclonal antibodies are valued for their specificity, purity and consistency that result in lower background. Crude antibody preparations such as serum or ascites fluid are sometimes used for Western blotting, but the impurities present may increase background. To obtain antibodies with the greatest specificity, you can affinity-purify the antibody using the immobilized antigen.

Primary antibodies

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

Custom antibody services

The Thermo Scientific™ Custom Antibody Development Service leverages our experience in making more than 18,500 antibodies to peptides and recombinant proteins. Our proprietary antigen design tools, including the Thermo Scientific™ Antigen Profiler Software, and targeted antigen display produces more robust antibodies that perform better in your targeted assays. When you initiate a custom antibody project with us we provide you access to our online project management tool. This secure account gives you easy access to project information and allows you to provide specific instructions for your projects.

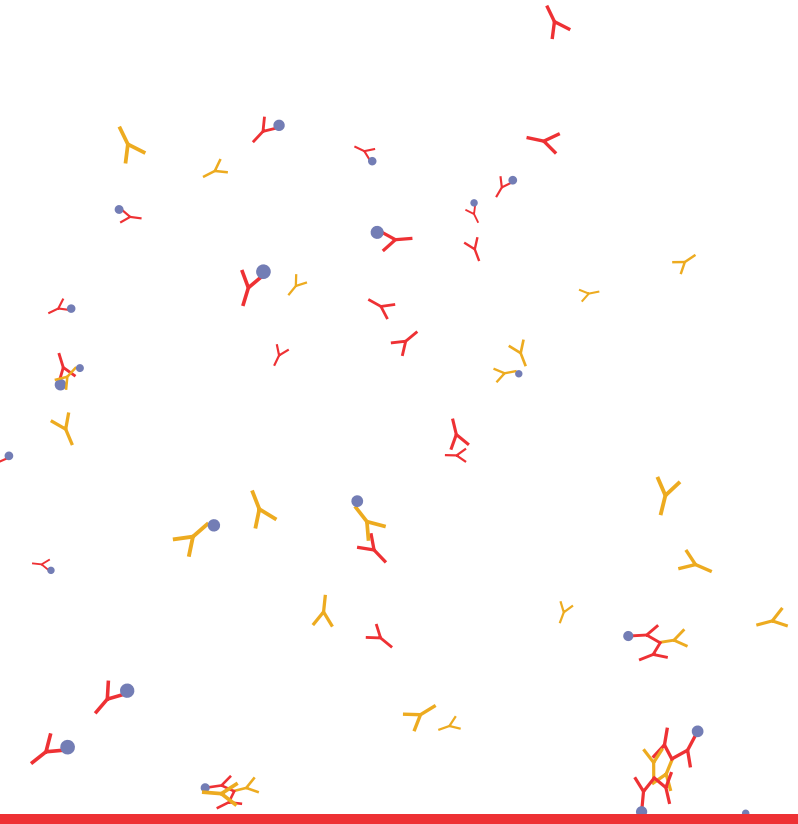
Learn more at **thermofisher.com/antibodies**

For ordering information refer to page 79.

Secondary antibody incubation

A secondary antibody aids in the detection of target antigens by binding to a primary antibody, which directly binds to the target antigen. 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 and economical for manufacturers to produce and supply ready-to-use, labeled secondary antibodies for most applications and detection systems. From a relatively small number of secondary antibodies, many features are available with regard to purity level, specificity and label type for a given application.

Antibody solutions for Western blotting are typically diluted from 1:100 to 1:500,000 beginning from a 1mg/mL stock solution. The optimal dilution of a given antibody with a particular detection system must be determined experimentally. More sensitive detection systems require less antibody, which can result in substantial savings on antibody costs and allow a limited supply of antibody to be used for many experiments. It also produces a side benefit of reduced background because the limited amount of antibody is specific for the target with the highest affinity. We offer a wide variety of labeled secondary antibodies for use in Western blotting. The labels include biotin, fluorescein, rhodamine, Invitrogen™ Alexa Fluor™ dyes, horseradish peroxidase and alkaline phosphatase.



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 share the same kappa light chains as IgG). In contrast, immunizing a goat with only mouse IgG_1 antibodies will only generate antibodies specific for mouse IgG_1 antibodies and molecules sharing the same conserved domains.

Because of the high degree of conservation in the structure of many immunoglobulin domains, class-specific secondary antibodies must be affinity purified and cross-absorbed to achieve minimal cross-reaction with other immunoglobulins. Using the example described above, immobilized mouse IgG_1 antibodies would be used to affinity purify all goat antibodies that bind to mouse IgG_1. These anti-mouse IgG_1 antibodies would then be further purified by passage through a chromatography column(s) containing mouse IgG_{2a}, IgG_{2b}, IgG_3, IgM, etc., to remove any antibodies that cross-react with non-IgG_1 isotypes. Additionally, secondary antibodies can be further purified by passage through columns containing the immobilized serum proteins from species other than those used to immunize the host. This method of cross-absorption (typically referred to as “Cross-Absorbed” 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 4).

Table 4. 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, light-emitting or fluorescent product. While reporter enzymes have been tried, alkaline phosphatase (AP) and horseradish peroxidase (HRP) are the two enzymes used most extensively as labels for protein detection. An array of chromogenic, fluorogenic and chemiluminescent substrates is available for use with either enzyme (see pages 28–54).

Alkaline phosphatase (AP) catalyzes the hydrolysis of phosphate groups from a substrate molecule resulting in a colored or fluorescent product or the release of light as a byproduct 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.

Horseradish peroxidase (HRP) catalyzes the oxidation of substrates by hydrogen peroxide, resulting in a colored or fluorescent product or the release of light as a by-product of the reaction. HRP functions optimally at a near-neutral pH and can be inhibited by cyanides, sulfides and azides. Antibody-HRP conjugates are superior to antibody-AP conjugates with respect to the specific activities of both the enzyme and antibody. In addition, its high turnover rate, good stability, low cost, and wide availability of substrates 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 for some researchers.

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), fluorescence-activated cell sorting (FACS) and immunohistochemistry using fluorescence microscopy, but are now expanding into Western blotting applications. The use of fluorophore-conjugated probes 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 special equipment and the sensitivity is lower than what can be obtained with enzymatic chemiluminescent systems. Although not as sensitive as enzymatic detection, fluorescent detection methods reduce chemical waste and have the added advantage of multiplex compatibility (using more than one fluorophore in the same experiment).

The growing demand for multiplex assays has driven the development of many new fluorescent dyes. These new fluorophores are brighter and more photostable than the traditional fluorescein and rhodamine molecules and comprise a broader range of non-overlapping spectra. Together with the advances in the digital imaging equipment, particularly infrared and near-infrared imaging, these new fluorophores enable extremely powerful analyses to be performed in all types of protein detection techniques.

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.

For ordering information refer to page 79.

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 in the graphic below are useful in the process of choosing a secondary antibody.

1 Determine origin of the primary antibody.

- Mouse monoclonal
- Rat monoclonal
- Rabbit polyclonal/monoclonal
- Others

2 Select an appropriate host species for the secondary antibody:

- Goat anti–mouse IgG
- Anti–rabbit IgG
- Others

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

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

4 Detection or purification method.

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

5 Consider requirements of the supplied secondary.

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

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

Superclonal Secondary Antibodies

Thermo Scientific™ Superclonal™ Secondary Antibodies represent a breakthrough in recombinant antibody technology designed to provide precise and accurate detection of mouse, rabbit and goat primary antibodies in a variety of applications. The proprietary screening and production process yields specific mixtures of recombinant goat or rabbit secondary antibodies that bind with the epitope-precision of monoclonal antibodies, while also achieving the multi-epitope coverage (e.g., H+L) and sensitivity of polyclonal antibodies. Each Superclonal Secondary Antibody is formulated and optimized to help achieve excellent results in ELISA, Western blot and cell imaging.

Features:

- Designed to eliminate cross-reactivity in detection of primary antibodies
- Developed as recombinant monoclonal antibodies to enable precise and accurate detection
- Formulated to recognize both heavy- and light-chain epitopes (H+L) of target IgG molecules
- Selected and optimized for use with cell imaging, ELISA and Western blotting applications
- Offered in four types: goat anti-mouse (GAM), goat anti-rabbit (GAR), rabbit anti-mouse (RAM), rabbit anti-goat (RAG)
- Available unconjugated and conjugated with biotin, horseradish peroxidase (HRP), and selected Alexa Fluor dyes

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

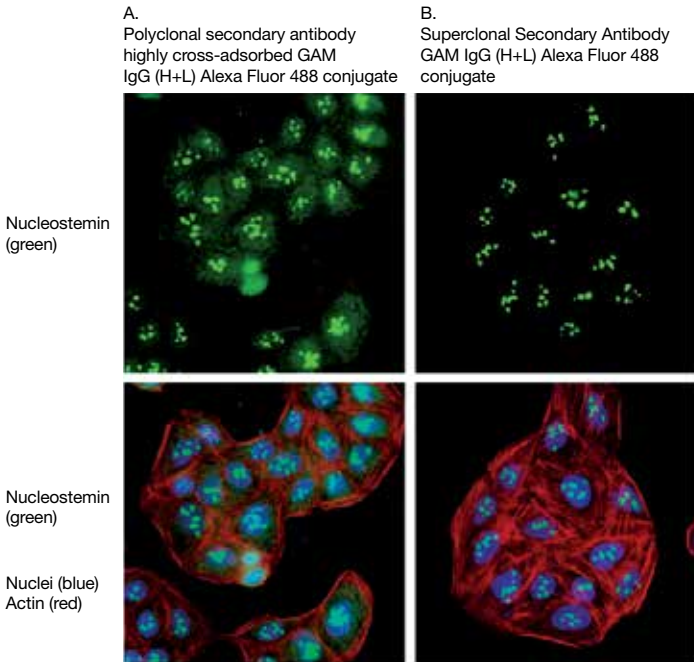


Figure 13. Superclonal Secondary Antibodies eliminate cross-reactivity for lower background. The nucleoli of HeLa cells were labeled with anti-nucleostemin primary antibody, which was then detected with respective types of Invitrogen™ Alexa Fluor™ 488 conjugated secondary antibodies (green): (A) highly cross-adsorbed goat anti-mouse IgG (H+L) secondary antibody, or (B) Goat Anti-Mouse IgG (H+L) SuperClonal Secondary Antibody. Cell nuclei were stained with DAPI (blue) and actin filaments were stained with Invitrogen™ Alexa Fluor™ 594 Phalloidin (red). Detection with the Superclonal Secondary Antibody exhibits significantly less cytoplasmic staining, indicating enhanced specificity.

Did you know?

Did you know that using too much secondary antibody can cause your chemiluminescent signal to fade, become undetectable or cause ghost bands?

For ordering information refer to page 79.

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 1mg/mL stock solution. These 1mg/mL stock 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. Anti-microbial 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; alkaline phosphatase 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.

Pierce Ethylene Glycol Solution

Thermo Scientific™ Pierce™ Ethylene Glycol Solution provides exceptional anti-freeze 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:

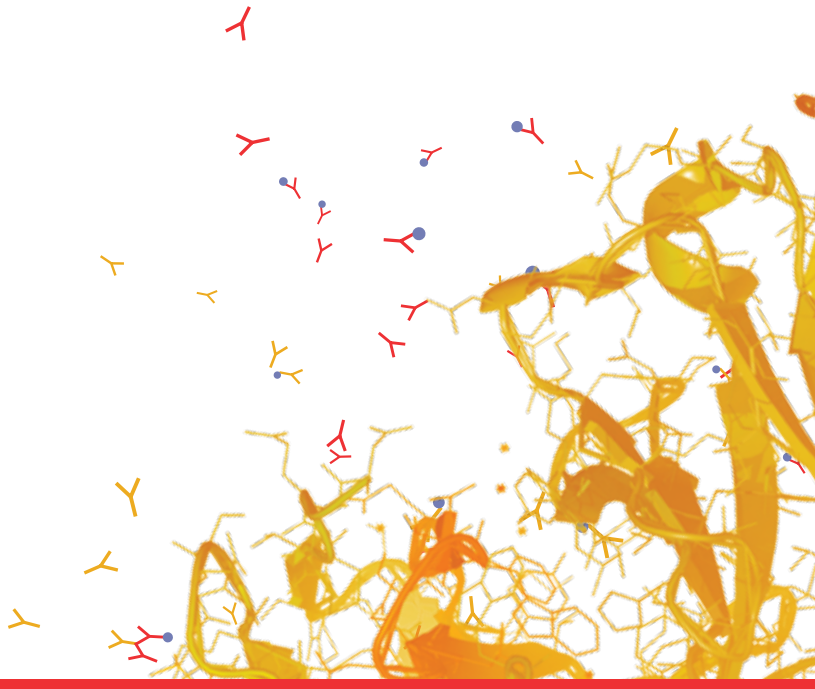
- Specially purified to remove impurities such as aldehydes, peroxides, iron and UV-absorbing hydrocarbons
- Suitable for enzyme storage without the worry of losing enzymatic activity
- Stable for months

Pierce Peroxidase Conjugate Stabilizer

Thermo Scientific™ Pierce™ Peroxidase Conjugate Stabilizer is an anti-freeze solution for storing aliquots and stocks of antibodies and other HRP-conjugates in liquid form so they can be pipetted for use without thawing. Mixing the Stabilizer in 2:1 or 1:1 ratio with HRP conjugate solutions (usually at least 1mg/mL) produces a stabilized conjugate stock solution that remains a liquid at –20°C, allowing pipetting without thawing.

Features:

- Provides a buffered anti-freeze environment for enzyme-conjugated antibodies and proteins
- Contains high-purity ethylene glycol, which maintains a liquid, low-viscosity solution at –20°C
- Contains preservatives to prevent anti-microbial growth during long-term storage
- No aliquoting of conjugate is necessary and sampling is convenient



Guardian Peroxidase Conjugate Stabilizer/Diluent

Thermo Scientific™ Guardian™ Peroxidase Conjugate Stabilizer/Diluent preserves the functional integrity and activity of horseradish peroxidase conjugated antibodies and other proteins at very dilute concentrations for long-term storage. With Guardian Peroxidase Conjugate Stabilizer/Diluent, typical 1mg/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 six 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 six-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
- **Saves money** — less expensive than competitor formulations or ordering new HRP conjugates

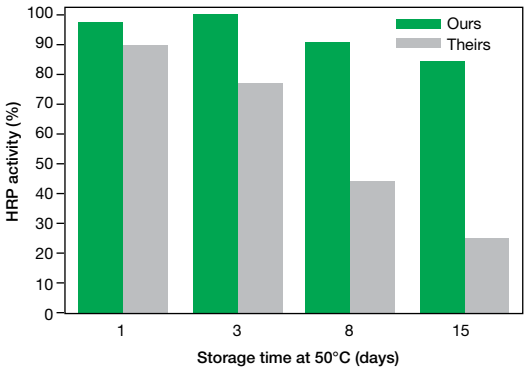
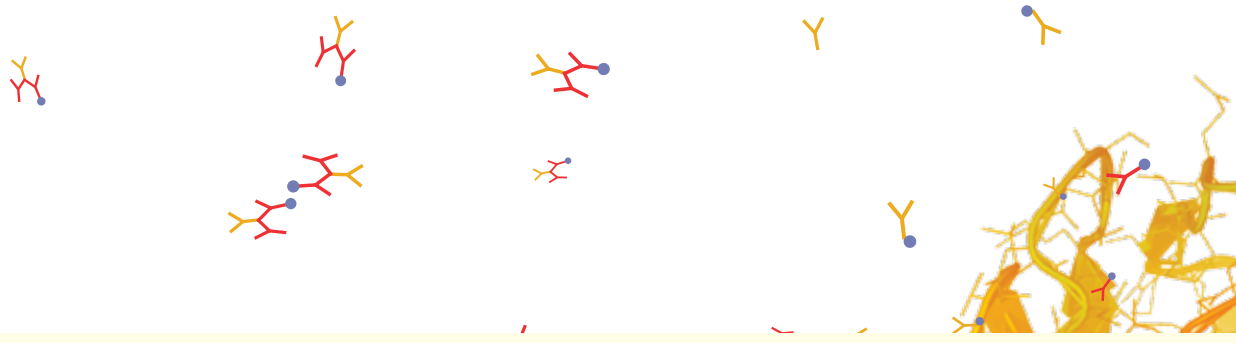


Figure 14. Better HRP-conjugate stability with Guardian Stabilizer-Diluent. Streptavidin-HRP (1mg/mL) was diluted 1:1,000 and stored at 50°C in Guardian Stabilizer-Diluent (Ours) or another supplier's HRP stabilizer (Theirs). At each time point, the HRP conjugate was diluted to 1:5,000,000 with PBS/SuperBlock Blocking Buffer and incubated for one hour in a white plate that had been coated with biotinylated BSA. The plate was then washed three times with 200µL PBS-T. Thermo Scientific™ SuperSignal™ ELISA Femto Substrate (100µL) was added to the plate and incubated for one 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 two weeks is equivalent to 12 months at 4°C.

Did you know?

Detergents such as Tween 20 can help minimize background when added to a blocking buffer, but did you know that too much detergent can prevent sufficient blocking? A final concentration of 0.05% often works well, but may need to be optimized specific to one's application.



For ordering information refer to page 79.

Protein Stabilizing Cocktail

The Thermo Scientific™ Protein Stabilizing Cocktail is a versatile stabilizing solution that increases the shelf life of purified or partially purified proteins during routine storage. The proprietary formulation of low-molecular weight, naturally occurring molecules helps protect proteins from environmental stresses that can otherwise lead to enzyme inactivation, aggregation and freeze-thaw damage. The Protein Stabilizing Cocktail is provided as an easily pipettable, buffered 4X concentrate. After addition of the cocktail, proteins may be refrigerated or frozen for storage without losing their activity or function. Although the degree of stabilization is protein-specific, the cocktail significantly stabilizes most proteins compared to conventional buffer alone. The Protein Stabilizing Cocktail is nontoxic and does not destabilize biomolecules; however, all cocktail components can be removed by dialysis or desalting before use in downstream assays, if desired.

Features:

- Stabilizes most enzymes and other proteins significantly better than ordinary buffers
- Protect proteins from environmental stresses
- Does not destabilize biomolecules in downstream assays
- Components are low molecular weight and fully dialyzable
- Low-viscosity reagent is easier to pipette than 50% glycerol
- Protein classes tested include kinases, phosphatases, peroxidases, restriction enzymes, luciferases, cytokines and antibodies

Did you know?

Did you know that too much HRP in a localized area produces an excess of free radicals in the presence of substrate? These free radicals can inactivate HRP and damage antibodies, target and the membrane, prohibiting effective re-probing.

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 and/or antigen from the blot. As with other steps in performing a Western blot, a variety of buffers may be used. Occasionally, washing is performed in a physiological buffer such as Tris-buffered saline (TBS) or phosphate-buffered saline (PBS) without any additives. More commonly, a detergent such as 0.05% Tween-20 Detergent (Cat. No. 28320) is added to the buffer to help remove nonspecifically bound material. Another common technique is to use a dilute solution of the blocking buffer along with some added detergent to help minimize background.

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

BupH Tris Buffered Saline (TBS)

Each pack of Thermo Scientific™ BupH™ Tris Buffered Saline yields 500mL of 25mM Tris, 0.15M sodium chloride, pH 7.2 when dissolved in 500mL deionized water (10 packs make 5L total; 40 packs make 20L 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 non-leaching high-density polyethylene (HDPE) bottles, ensuring its stability and eliminating the accumulation of peroxides and degradation products.

For ordering information refer to page 79.

Table 5. Wash buffer selection guide.

	TBS	PBS	TBST	PBST
Dry blend	**28376** — 40 packs **28379** — 10 packs	**28372** — 40 packs		
Liquid concentrate	**28358** — 500mL	**28348** — 500mL	**28360** — 500mL	**28352** — 500mL
Formulation	Both dry and liquid: • 25mM Tris • 0.15M NaCl • pH 7.2	Dry: • 0.1M sodium phosphate • 0.15M NaCl • pH 7.2 Liquid: • 10mM sodium phosphate • 0.15M NaCl • pH 7.5	• 25mM Tris • 0.15M NaCl • 0.05% Tween-20 • pH 7.5	• 10mM sodium phosphate • 0.15M NaCl • 0.05% Tween-20 • pH 7.5
Application	• ELISA, Western blotting and other immunoassays • Diluent or blocking buffer for plate-based assays	• ELISA, Western blotting and other immunoassays • Crosslinking, biotinylation and fluorescent labeling reactions requiring an amine-free buffer	• ELISA, Western blotting and other immunoassays • Diluent or blocking buffer for plate-based assays	• ELISA, Western blotting and other immunoassays • Diluent or blocking buffer for plate-based assays

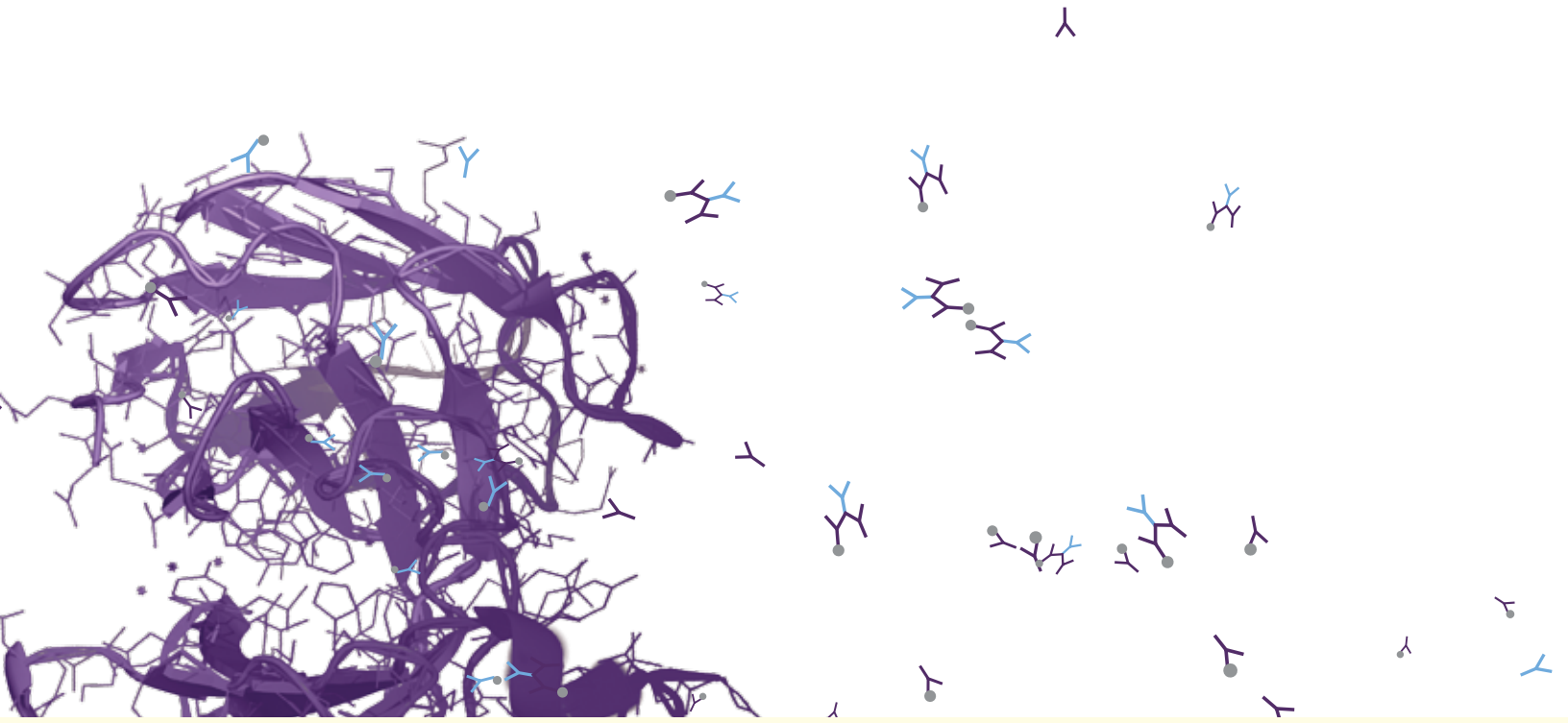
Detection

Introduction

Only 30 years ago, the main means to detect proteins on a Western blot relied on radioactive probes, typically ^{125}I. Over time non-radioactive methods have become predominant in Western blotting applications and center on chromogenic, chemiluminescence and fluorescence detection. Table 6 shows a comparison of these detection techniques.

Table 6. Comparison of Western blot detection methods.

Detection type	Chromogenic	Chemiluminescence	Fluorescence	Long-wavelength fluorescence
Sensitivity	++	+++++	++++	+++
Signal duration	Months	Hours	Months/Years	Years
Multiplexing	No	No	Yes	Yes
Means of visualization	Visual; no instrument required	X-ray film, CCD imagers	Fluorescent digital imager, NIR imaging system (dependent on the fluor used)	Fluorescent digital imager, UV transilluminator, UV gel documentation systems



For ordering information refer to page 80.

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. The resulting colored band or spot requires no special equipment for processing or visualizing. Chromogenic blotting substrates are available in a variety specifications and formats. The appropriate substrate choice depends on the enzyme label, desired sensitivity and form of signal or method of detection needed (Table 7).

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 substrate reaction is a colored precipitate, the signal is stable, therefore chromogenic substrates do not typically have issues with false negative results (ghost bands) that can occur with chemiluminescent substrates. Since chromogenic substrates are typically used to detect abundant proteins and reaction development can be monitored visually, this gives them greater flexibility for optimization compared to chemiluminescent or fluorescent blotting systems. However, unlike 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.

Table 7. Chromogenic substrates selection guide. Alkaline phosphatase (AP) and horseradish peroxidase (HRP) substrates for use with nitrocellulose or PVDF membranes. (Listed in order of increasing sensitivity).

Enzyme conjugate	Product	Cat. No.	Total assays[†]	Color	Detection limit[‡]	1° / 2° Ab dilution[‡] (from 1mg/mL stock)
HRP	1-Step Chloronaphthol	34012	2,500cm^2	Blue-purple	5ng	1° 1:500 / 2° 1:2,000–1:20,000
HRP	4-Chloro-1-Naphthol, 25g	34010	92,500cm^2	Blue-purple	5ng	1° 1:500 / 2° 1:2,000–1:20,000
HRP	DAB Substrate Kit	34002	1,000cm^2	Brown	1ng	1° 1:500 / 2° 1:2,000–1:20,000
HRP	DAB, 10g	34001	80,000cm^2	Brown	1ng	1° 1:500 / 2° 1:2,000–1:20,000
HRP	1-Step TMB-Blotting	34018	2,500cm^2	Dark blue	1ng	1° 1:500 / 2° 1:2,000–1:20,000
HRP	CN/DAB Substrate Kit	34000	1,000cm^2	Black	500pg	1° 1:500 / 2° 1:2,000–1:20,000
AP	1-Step NBT/BCIP plus Suppressor	34070	1,000cm^2	Black-purple	30pg	1° 1:500 / 2° 1:5,000–1:50,000
AP	1-Step NBT/BCIP	34042	2,500cm^2	Black-purple	30pg	1° 1:500 / 2° 1:5,000–1:50,000
HRP	1-Step Ultra TMB Blotting	37574	2,500cm^2	Dark blue	20pg	1° 1:1,000 / 2° 1:5,000–1:10,000
HRP	Metal Enhanced DAB Substrate Kit	34065	2,500cm^2	Brown-black	17pg	1° 1:1,000 / 2° 1:5,000–1:50,000

[†] Value is the area of membrane that can be processed. For additional information about required materials and assay considerations that determine the number of assays that may be performed, please see the product instructions.

[‡] Detection limits and recommended antibody dilutions have been generalized as a means to begin optimization. Individual assays may require conditions outside the ranges suggested here.

Chromogenic HRP substrates

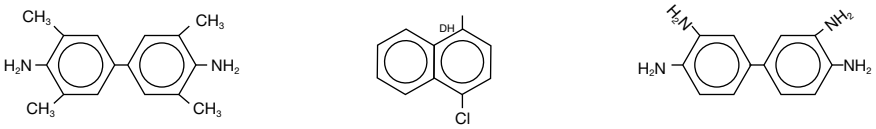
There are numerous options for chromogenic HRP substrates, but all require the presence or addition of peroxide for colorimetric detection. Because of its extremely short shelf life at the desired concentration, hydrogen peroxide traditionally was added to a buffer, along with the substrate, immediately 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™ Pierce™ 1-Step™ TMB-Blotting is a single-component peroxidase substrate for Western blotting and immunohistochemistry. Precipitating the product results in dark blue bands where the enzyme is located. 1-Step TMB-Blotting is well suited to applications that require a large 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.

4-CN has and 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, 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
M.W. 240.4

4-CN
M.W. 178.6

DAB
M.W. 214.1

Figure 15. Chromogenic substrates for Western blotting with HRP.

Table 8. Which chromogenic detection kit is right for you?

	1-Step Chloronaphthol	Metal Enhanced DAB Substrate Kit	CN/DAB Substrate Kit	1-Step Ultra TMB Blotting Solution
Format	Ready-to-use liquid	Two-component reagent kit	Two-component reagent kit	Ready-to-use liquid
Stability	Stable at 4°C for 1 year	Working solution is stable for over six hours at RT	Stable at 4°C for 1 year	Stable at 4°C for 1 year
Sensitivity	5ng	17pg	500pg	20pg
Color	Blue-purple	Brown-black	Black	Dark blue

For ordering information refer to page 80.

Pierce 1-Step Ultra TMB-Blotting Solution

Thermo Scientific™ Pierce™ 1-Step Ultra TMB-Blotting Solution is an enhanced single-component HRP substrate for Western blotting and immunohistochemistry. This precipitating, colorimetric Western blot substrate for HRP provides high sensitivity, increased signal-to-noise ratio and low background compared to many other chromogenic substrates. The blotting solution contains soluble TMB (3,3',5,5'-tetramethylbenzidine), which reacts very quickly with horseradish peroxidase 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

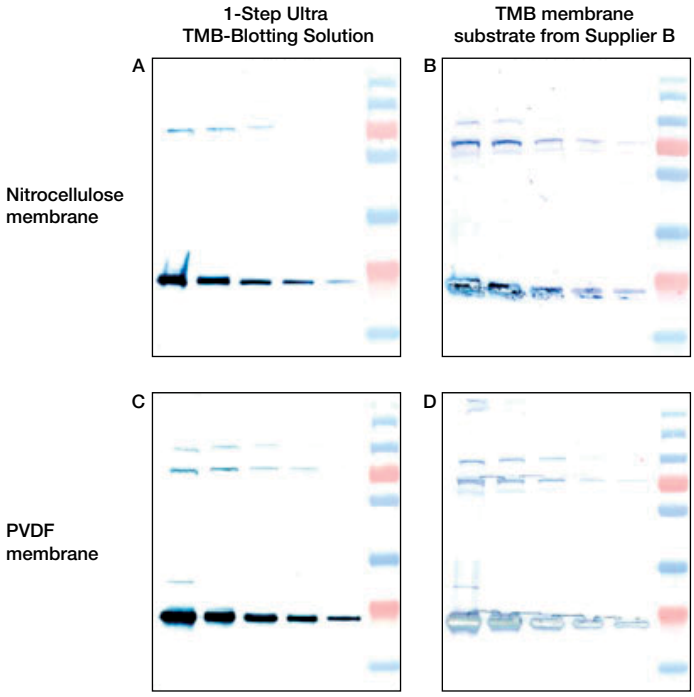


Figure 16. Detection with 1-Step Ultra TMB-Blotting Solution and another commercial TMB substrate. Serial dilutions of HepG2 cell lysates (15, 7.5, 3.45, 1.88, 0.94µg) were prepared and separated by electrophoresis. The proteins were transferred to nitrocellulose (top) membranes (Cat. No. 88013) and PVDF (bottom) membranes (Cat. No. 88518). The membranes were blocked with Clear Milk Blocking Buffer 1X (Cat. No. 37587). After blocking, the membranes were incubated with Thermo Scientific™ Pierce™ Mouse Anti-PLK-1 Monoclonal Antibody (Cat. No. MA1-848) and Rabbit Anti-Cyclophilin B Polyclonal Antibody (Cat. No. PA1-027A). The membranes were washed and then incubated with 0.2µg/mL of Thermo Scientific™ Pierce™ Goat Anti–Mouse IgG (H+L) Secondary Antibody, HRP Conjugate (Cat. No. 31430) and Thermo Scientific™ Pierce™ Goat Anti–Rabbit IgG (H+L) Secondary Antibody, HRP Conjugate (Cat. No. 31460) and then washed again. The membranes were placed in 10mL of 1-Step Ultra TMB-Blotting Solution (Cat. No. 37574) (A), TMB substrate from Supplier B. The color development was stopped at three minutes (bottom) and five minutes (top) by rinsing the membranes with ultrapure water.

Did you know?

Did you know you can test the activity of your HRP-conjugated antibody by spiking in undiluted HRP-conjugated antibody into a tube of substrate in the dark (darkroom test)?

1-Step TMB-Blotting Solution

Thermo Scientific Pierce 1-Step TMB-Blotting Solution is a ready-to-use solution of the blue TMB peroxidase (HRP) substrate that is specially formulated for chromogenic detection in Western blot and IHC experiments. 1-Step TMB-Blotting Solution is formulated to precipitate and localize the blue reaction product onto membrane surfaces or tissue samples at sites where the peroxidase-substrate reaction occurs. The 1-Step TMB-Blotting substrate produces a very large signal-to-noise ratio, although its high sensitivity requires careful washing and blocking to avoid background staining.

Features:

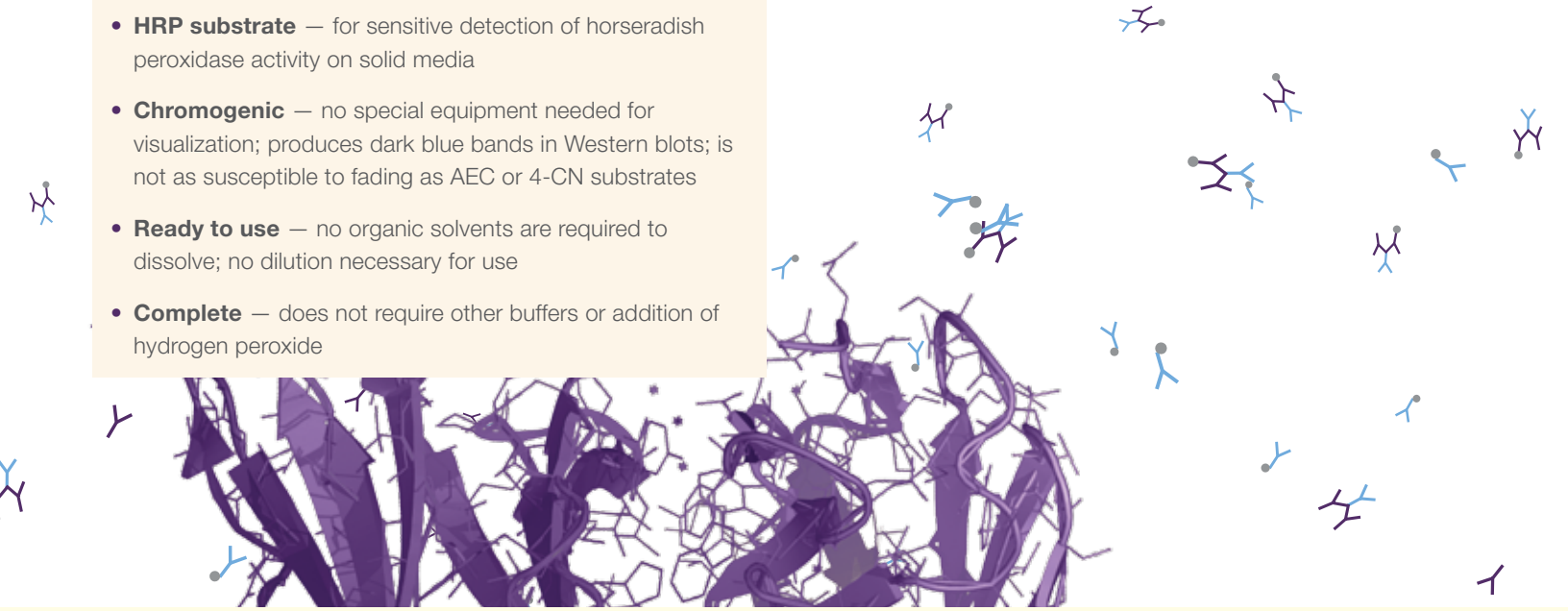
- **HRP substrate** — for sensitive detection of horseradish peroxidase activity on solid media
- **Chromogenic** — no special equipment needed for visualization; produces dark blue bands in Western blots; is not as susceptible to fading as AEC or 4-CN substrates
- **Ready to use** — no organic solvents are required to dissolve; no dilution necessary for use
- **Complete** — does not require other buffers or addition of hydrogen peroxide

Pierce Chloronaphthol (4-CN) substrate powder

Thermo Scientific™ Pierce™ Chloronaphthol (4-CN) substrate powder is for the chromogenic detection of HRP activity in Western blot and tissue staining methods. Chloronaphthol (4-CN) 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:

- **Cost effective** — no special equipment needed for visualization
- **Easy** — easy to photograph in color or black-and-white



For ordering information refer to page 80.

1-Step Chloronaphthol Solution

Thermo Scientific™ 1-Step Chloronaphthol Solution is a solution of 4-chloro-1-naphthol for the chromogenic detection of HRP activity in Western blot and tissue staining methods. Chloronaphthol (4-CN) 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 Metal Enhanced DAB Substrate

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:

- **Incredible sensitivity** — fifty times more sensitive than the traditional DAB method and thirty 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. And, 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
- **Six-hour stability** — the innovative working solution is stable for more than six hours at room temperature, while other DAB substrates must be used immediately

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 (4-chloro-1-naphthol) and DAB (3,3'-diaminobenzidine tetrahydrochloride)
- **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

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 alkaline phosphatase 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. 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.

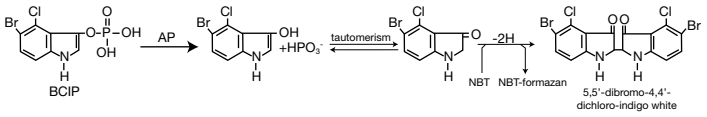


Figure 17. Chemical reaction of NBT and BCIP substrates with alkaline phosphatase. BCIP is hydrolyzed by alkaline phosphatase to form an intermediate that undergoes dimerization to produce an indigo dye. The NBT is reduced to the NBT-formazan by the two reducing equivalents generated by the dimerization. This reaction proceeds at a steady rate, allowing accurate control of the relative sensitivity and control of the development of the reaction.

For ordering information refer to page 80.

WesternBreeze 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 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

Pierce NBT and BCIP Substrates

Thermo Scientific™ Pierce™ NBT and BCIP Substrates are powders and ready-made solutions of nitro-blue tetrazolium and 5-bromo-4-chloro-3'-indolyphosphate for chromogenic blot and IHC staining with alkaline phosphatase probe, yielding an intense, insoluble black-purple precipitate. Pierce NBT and BCIP powders are available individually and in two convenient, ready-to-use Thermo Scientific™ Pierce™ 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 pre-formulated, ready-to-use Pierce 1-Step NBT/BCIP substrate solutions, with or without levamisole suppressor

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:

- Allow for multiple exposures to be made in order to obtain the best image
- Allow for blots to be reprobed to optimize detection, or to visualize a second protein
- Detect and quantitate 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. This is in contrast to chromogenic substrates (see pages 30–34) 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.

As with chromogenic substrates, there are a variety of chemiluminescent substrates available with different formats and sensitivities (Table 9).

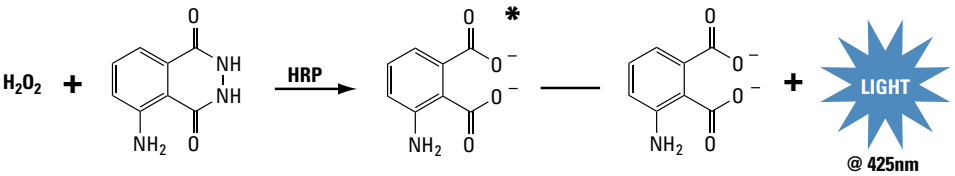


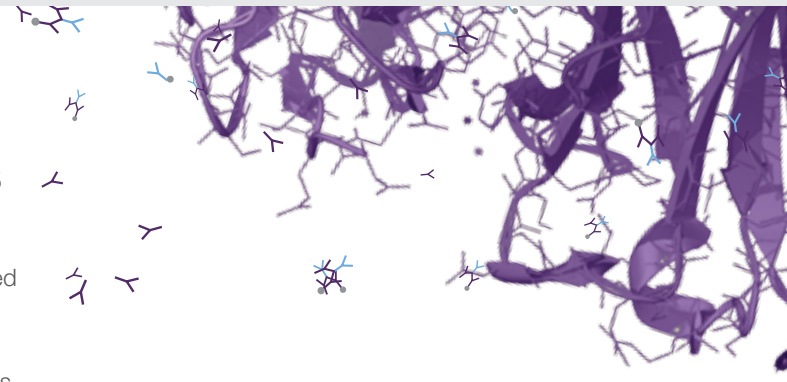
Figure 18. Chemiluminescent reaction of luminol. Chemiluminescence is a property of chemical reactions which emit light as a byproduct. Luminol is one of the most widely used chemiluminescent reagents. The oxidation of luminol by peroxide results in creation of an excited state product called 3-aminophthalate. This product decays to a lower energy state by releasing photons of light.

Table 9. Chemiluminescent Western blotting substrates. Alkaline phosphatase (AP) and horseradish peroxidase (HRP) substrates for use with film and CCD imaging systems, listed in order of increasing sensitivity. Pierce ECL Plus substrate can also be detected with certain phosphor imagers.

Enzyme conjugate	Product	Cat. No.	Total assays†	E_{max} color‡	Detection limit††	1° / 2° Ab dilution†† (from 1mg/mL stock)
HRP	Pierce ECL substrate	32106	4,000cm²	425nm blue/green	20pg	1° 1:1,000 2° 1:5,000–1:10,000
AP	Novex AP Chemiluminescent substrate	WP20002	1,000cm²	461–466nm	0.5pg	1° 1:1,000 2° 1:5,000–1:10,000
HRP	SuperSignal West Pico substrate	34080	5,000cm²	425nm blue/green	1pg	1° 1:1,000 2° 1:20,000–1:100,000
HRP	Pierce ECL Plus substrate	32132	1,000cm²	425nm blue/green	0.5pg	1° 1:1,000 2° 1:25,000–1:200,000
HRP	SuperSignal West Dura substrate	34076	2,000cm²	425nm blue/green	250fg	1° 1:5,000 2° 1:50,000–1:250,000
HRP	SuperSignal West Femto substrate	34096	2,000cm²	425nm blue/green	60fg	1° 1:5,000 2° 1:100,000–1:500,000

† Value is the area of membrane that can be processed. For additional information about required materials and assay considerations that determine the number of assays that may be performed, please see the product instructions.
‡ The peak emission wavelength is given for reference. However, for best sensitivity, capture total light output using film or without the use of filters.
†† Detection limits and recommended antibody dilutions have been generalized as a means to begin optimization. Individual assays may require conditions outside the ranges suggested here.

For ordering information refer to page 80.



Chemiluminescent HRP substrates

Chemiluminescent substrates for HRP are two-component systems consisting of a stable peroxide solution and an enhanced luminol solution. In most cases, to make a working solution, the 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 425nm which can be captured with X-ray film and CCD camera imaging devices that detect chemiluminescence. Although X-ray film provides qualitative and semi-quantitative data and is useful to confirm the presence of target proteins, cooled CCD cameras offer the advantages of quantitative analysis, instant image manipulation, higher sensitivity, greater resolution and a larger dynamic range than film. Plus, you don't have to spend quality time in the darkroom; imaging can be performed right at your lab bench.

Use Table 10 to select the most appropriate HRP chemiluminescent substrate based on abundance of your target protein of interest, abundance of sample containing the target protein, and the level of sensitivity and type of instrumentation available for detection.

Did you know?

Sodium azide is an inhibitor of the HRP enzyme and should be avoided during Western blot processing involving HRP-based detection. Sulfide, cyanide, fluoride and superoxide ions also inhibit HRP to some extent.

Table 10. Which HRP chemiluminescent substrate is right for you?

	Pierce ECL substrate	SuperSignal West Pico substrate	Pierce ECL Plus substrate	SuperSignal West Dura substrate	SuperSignal West Femto substrate
Detection level	~20pg	~1pg	~0.5pg	~250fg	~60fg
Recommended antibody dilution	1°: 0.2–10µg/mL 2°: 0.07–1µg/mL	1°: 0.2–1µg/mL 2°: 10–50ng/mL	1°: 0.05–1µg/mL 2°: 5–40ng/mL	1°: 0.02–1µg/mL 2°: 4–20ng/mL	1°: 10–200ng/mL 2°: 2–10ng/mL
Signal duration	1–2 hours	6–8 hours	5 hours	24 hours	6–8 hours
Detection methods	X-ray film, CCD imager	X-ray film, CCD imager	X-ray film, CCD imager, fluorescence imager	X-ray film, CCD imager	X-ray film, CCD imager
Select when…	Target and sample is abundant	Target is less abundant, sample is limited and improved sensitivity is required	Target is less abundant, sample is limited and using chemifluorescent detection	Target is less abundant, sample is limited and using CCD imaging Recommended for CCD imaging	Target is least abundant, sample is precious and maximum sensitivity is required

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. 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
- **A name you can rely on** — count on the strong quality assurance, technical support and reputation behind Thermo Scientific™ Protein Research Products

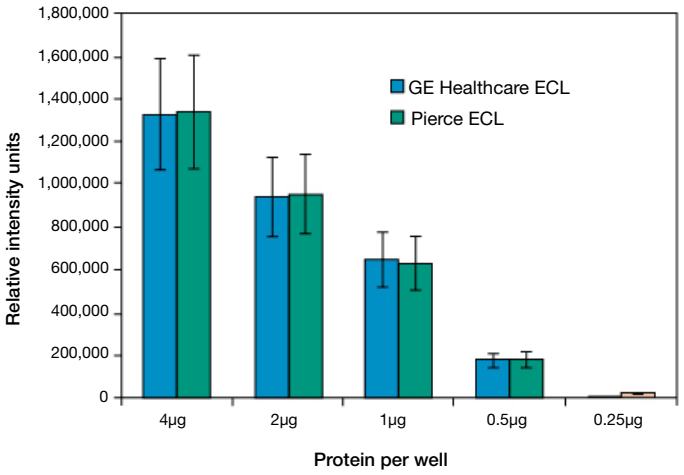


Figure 19. Signal intensity of Pierce ECL Western Blotting Substrate is comparable to GE Healthcare ECL Western Blotting Detection Reagent. HeLa cell lysate was separated by SDS-PAGE and transferred to nitrocellulose membrane to detect β-actin. The signal was detected and analyzed using Kodak™ 1D Image Analysis Software. Error bars represent ±20% difference in relative intensity units.

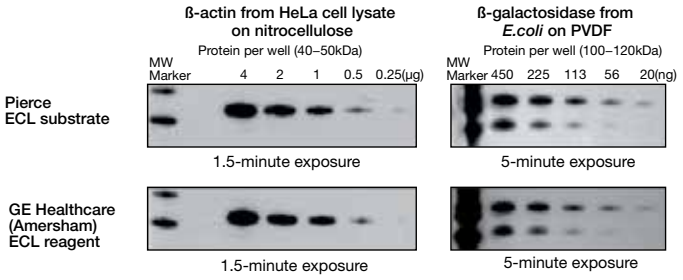


Figure 20. ECL Western blotting substrate for detection of proteins on nitrocellulose or PVDF. β-actin and β-galactosidase protein in HeLa cell and *E. coli* lysates, respectively, were detected by Western blotting. The membranes were blocked with 5% nonfat milk and probed with primary antibody at 1µg/mL. The membranes were washed, then incubated with 0.2µg/mL of HRP-conjugated goat anti-mouse IgG (Cat. No. 31430) and washed again. Working solutions of the Pierce ECL Western Blotting Substrate and GE Healthcare Amersham ECL Western Blotting Detection Reagent (Cat. No. RPN2134) were prepared according to the manufacturers' instructions and added to replicate membranes for one minute. The membranes were removed from the substrates and placed in plastic sheet protectors and exposed to CL-XPosure Film (Cat. No. 34090) and developed.

For ordering information refer to page 80.

Pierce ECL Plus Substrate

Thermo Scientific™ Pierce™ ECL Plus Substrate is an acridan-based chemiluminescent and chemifluorescent HRP substrate for Western blot detection using X-ray film or CCD or laser-based imagers. This product is sold as Pierce ECL 2 Substrate (Cat. No. PI80196) through Fisher Scientific and other channels. Pierce ECL Plus Substrate enables the detection of low-picogram amounts of target protein on nitrocellulose or PVDF membrane when probed with appropriate primary and secondary antibody concentrations. Its broad dynamic range, high sensitivity and long-lasting signal make Pierce ECL Plus Substrate an excellent choice for Western blot analysis.

Features:

- **Easy to use** — can be substituted for the discontinued GE ECL Plus Substrate without any re-optimization
- **Higher sensitivity** — detect targets down to the low-picogram level
- **Longer signal duration** — sustained light output for as long as five hours
- **More imaging options** — X-ray, CCD or laser-based imagers
- **More affordable** — high quality and performance at a lower price than other suppliers' similar substrates

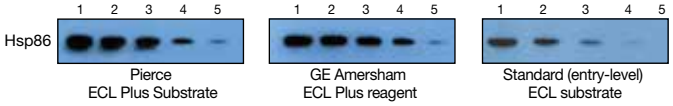


Figure 21. Pierce ECL Plus Substrate delivers the same performance you've come to expect. HeLa cell lysate was diluted in electrophoresis sample buffer and heated to 95°C for five minutes. Lane 1 contained 10µg of total protein. Four 1:1 dilutions were prepared and applied to lanes 2–5 at 10µL/well. After electrophoresis, the proteins were transferred to nitrocellulose membranes (Cat. No. 88013). 5% milk in TBST was used as a blocking buffer. Rabbit Anti-Hsp86 Antibody (Cat. No. PA3-013) at 1:1,000 dilution and Goat Anti-Rabbit HRP (Cat. No. 31460) at 6.6ng/mL (1:150,000 dilution of 1mg/mL stock solution) were used for target detection. Blots were prepared using Pierce ECL Plus Substrate (Cat. No. 32132) or other indicated substrates, and then exposed to CL-XPosure Film (Cat. No. 34090).

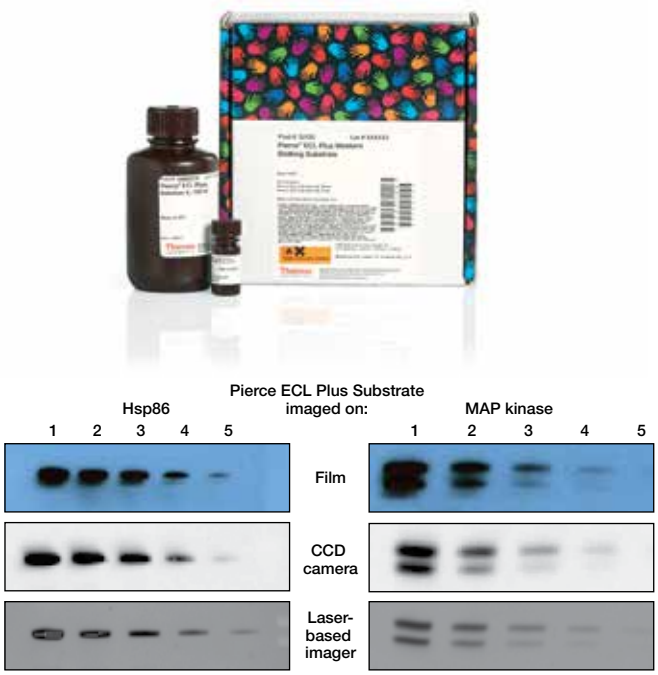


Figure 22. One substrate detected with three methods: X-ray film, CCD imager and laser-based imager. HeLa cell lysate was diluted in electrophoresis sample buffer and heated to 95°C for five minutes. Lane 1 contained 10µg of total protein. Four 1:1 dilutions were prepared and applied to lanes 2–5 at 10µL/well. After electrophoresis, proteins were transferred to nitrocellulose membranes (Cat. No. 88013). 5% milk in TBST was used as a blocking buffer. The membranes were incubated with primary antibody (Rabbit Anti-MAP Kinase, Millipore or Rabbit Anti-Hsp86, Cat. No. PA3-013) at 1:1,000 dilution and then with Goat Anti-Rabbit HRP (Cat. No. 31460) at 6.6ng/mL (1:150,000 dilution of 1mg/mL stock solution). Pierce ECL Plus Substrate (Cat. No. 32132) was used for detection. The membranes were exposed to CL-XPosure Film (Cat. No. 34090) for five seconds and scanned using the Typhoon™ 9410 Variable Mode Imager (excitation at 457nm, emission at 510nm) and Syngene™ G:BOX iChemi XT Imager (one-minute exposure).

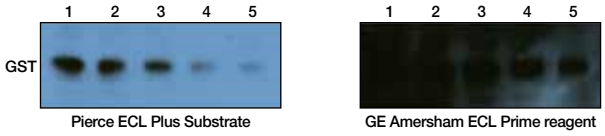


Figure 23. Better sensitivity does not always translate to better results. Purified Thermo Scientific™ Pierce™ GST (Cat. No. 20237) was diluted in Thermo Scientific™ Pierce™ LDS Sample Buffer (Cat. No. 84788) and heated to 95°C for five minutes. Lane 1 contained 50ng of GST at 10µL/well. Four 1:1 serial dilutions were prepared and applied to lanes 2–5. After electrophoresis proteins were transferred to nitrocellulose membranes (Cat. No. 88024). StartingBlock (TBS) Blocking Buffer (Cat. No. 37542) was used for blocking. Biotinylated Rabbit Anti-GST Antibody (Santa Cruz, Cat. No. sc-459B) at 1:2,000 dilution and Thermo Scientific™ Pierce™ High Sensitivity Streptavidin-HRP (Cat. No. 21130) at 6.66ng/mL were used for target detection. Blots were prepared using Pierce ECL Plus Substrate (Cat. No. 32132) or GE Healthcare Amersham ECL Prime Western Blotting Detection Reagent (Cat. No. RPN2232) and then exposed to CL-XPosure Film (Cat. No. 34090).

SuperSignal West Pico Chemiluminescent Substrate and Kits

Thermo Scientific™ SuperSignal™ West Pico Chemiluminescent Substrate is an enhanced chemiluminescent HRP substrate for low-picogram-level detection by Western blot analysis. It is compatible with different membranes, blocking reagents and a wide range of antibody dilutions. SuperSignal West Pico substrate provides excellent performance, versatility and economy for routine Western blotting needs.

SuperSignal West Pico kits contain substrate and a secondary antibody HRP conjugate (either anti-mouse or anti-rabbit). Complete kits also contain wash and blocking buffer. The kits are ideal for busy researchers or new beginners to Western blot analysis.

Features:

- **Picogram sensitivity** — detect low-picogram amounts of protein in bands on nitrocellulose or PVDF membrane
- **Good signal duration** — incubated blots provide six to eight hours of usable light output when conditions are optimized
- **Stable reagent** — 24-hour working solution stability; one-year kit stability at room temperature
- **Economical** — optimized for dilute antibody concentrations
- **Standard or complete kit options also available** — choose a kit with substrate and antibody only, or a complete kit that also includes TBS wash buffer and high-performance SuperBlock Blocking Buffer

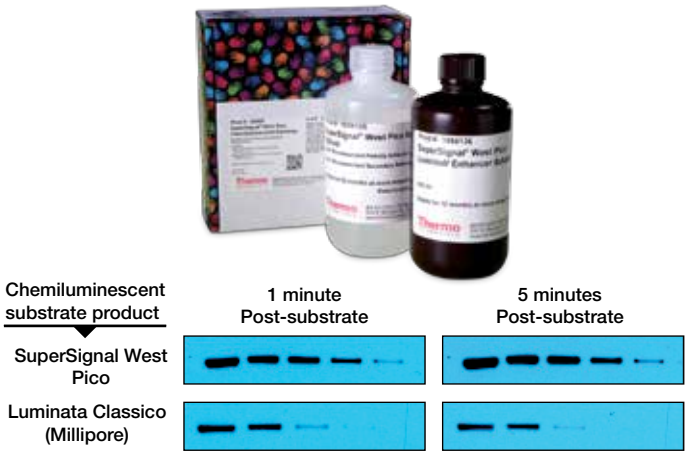


Figure 24. Product comparison. Blots detected using SuperSignal West Pico Chemiluminescent Substrate (top) or Luminata™ Classico Western HRP Substrate (Millipore) (bottom) at one and five minutes post substrate incubation. Bands are Hsp86 in HeLa cell lysate samples (10µg total protein in first lane; 2-fold dilutions thereafter), probed with rabbit anti-Hsp86 antibody (Cat. No. PA3-013) at 1:1,000 dilution, followed by goat anti-rabbit HRP antibody (Cat. No. 31460) at 10ng/mL and exposure to CL-XPosure Film (Cat. No. 34091).

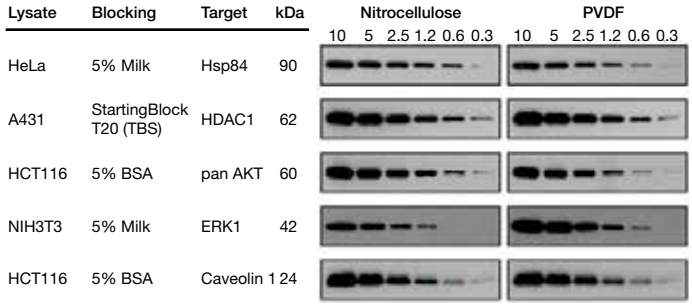


Figure 25. SuperSignal West Pico Chemiluminescent Substrate performs well with different lysates, targets, blocking agents and membranes. Whole cell lysate was serial diluted in reducing sample buffer at 10, 5, 2.5, 1.2, 0.6 and 0.3µg/well with a 15µL/well load and separated by SDS-PAGE. After electrophoresis, proteins were transferred to nitrocellulose membrane (Cat. No. 88018) using the Thermo Scientific™ Pierce™ Power Blotter (Cat. No. 22834) and Thermo Scientific™ Pierce™ 1-Step Transfer Buffer (Cat. No. 84731). The membrane was blocked with StartingBlock T20 (TBS) Blocking Buffer (Cat. No. 37543), 5% non-fat dry milk, or 5% BSA as indicated above. Then the membrane was incubated with Rabbit Anti-Hsp84 Antibody (Cat. No. PA3-012) at 0.3µg/mL, Rabbit Anti-HDAC1 Antibody (Cat. No. PA1-860) at 0.5µg/mL, Rabbit (Monoclonal) Anti-pan AKT Antibody (Cat. No. MA5-14916) at 1µg/mL, Mouse Anti-ERK1 Antibody (Cat. No. MA1-13041) at 1µg/mL, or Rabbit Anti-Caveolin 1 (Cat. No. PA1-064) at 0.5µg/mL followed by incubation with Goat anti-Mouse IgG (H+L) Secondary Antibody, HRP conjugate (Cat. No. 31430) or Goat anti-Rabbit IgG (H+L) Secondary Antibody, HRP conjugate (Cat. No. 31460) at 20ng/mL. SuperSignal West Pico substrate (Cat. No. 34078) was used for detection. Exposures were acquired on CL-XPosure Film (Cat. No. 34091).

For ordering information refer to page 80.

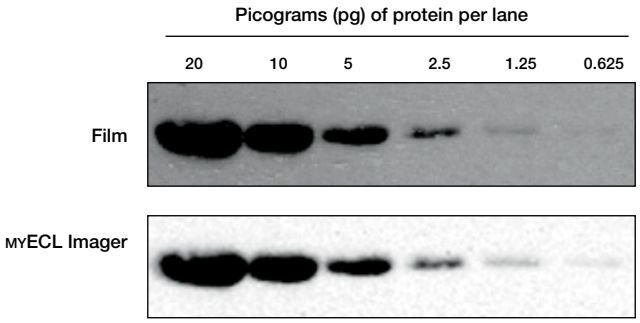


Figure 26. Low-picogram detection with SuperSignal West Pico Chemiluminescent Substrate. Turbo GFP-His-HA-FLAG was diluted in electrophoresis reducing sample buffer. Lane 1 contained 20pg of Turbo GFP. Five 1:1 serial dilutions were then prepared and applied at 10µL/well. After electrophoresis, proteins were transferred to nitrocellulose membrane (Cat. No. 88018) using the Pierce Power Blotter (Cat. No. 22834) and Pierce 1-Step Transfer Buffer (Cat. No. 84731). The membrane was blocked with SuperBlock (TBST) Blocking Buffer (Cat. No. 37536). Then the membrane was incubated with Mouse Anti-6x-His Epitope Tag Antibody (HIS.H8) (Cat. No. MA1-21315) at 1µg/mL, followed by incubation with Goat anti-Mouse IgG (H+L) Secondary Antibody, HRP conjugate (Cat. No. 31430) at 50ng/mL. SuperSignal West Pico substrate (Cat. No. 34078) was used for detection. One-minute exposures were acquired on CL-XPosure Film (Cat. No. 34091) and the MYECL Imager (Cat. No. 62236). Exposures from the MYECL Imager were inverted and contrasted (black=64,000, white=65,535, gamma=1.0).

Did you know?

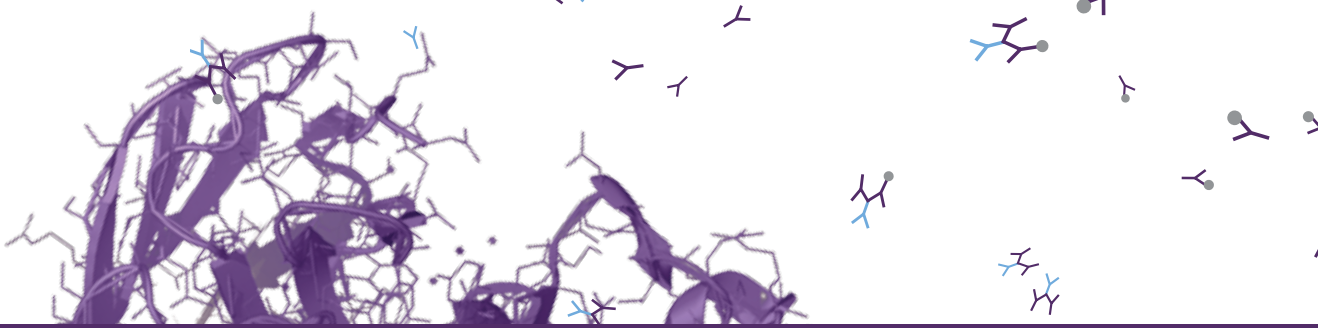
Use plastic tweezers to handle the membrane for HRP-based detection because metallic tools may have rust, which can cause high background.

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 with CCD-based imagers.

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
- **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



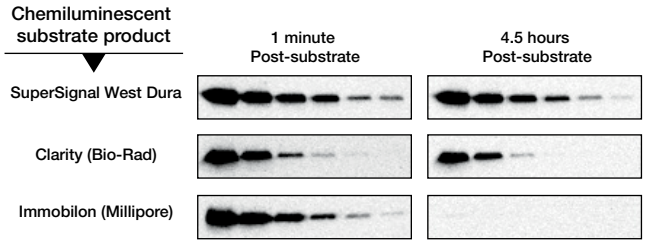


Figure 27. Better sensitivity and signal duration with SuperSignal West Dura Extended Duration Substrate. A431 cell lysate was diluted in electrophoresis reducing sample buffer. Lane 1 contained 5µg of A431 lysate. Five 1:1 serial dilutions were then prepared and applied at 10µL/well. After electrophoresis, proteins were transferred to nitrocellulose membrane (Cat. No. 88018) using the Pierce Power Blotter (Cat. No. 22834) and Pierce 1-Step Transfer Buffer (Cat. No. 84731). Membranes were blocked with 5% milk in TBST Buffer. The membranes were incubated with Mouse Anti-beta-Catenin Antibody (Cat. No. MA1-300) at 0.3µg/mL and then with Goat Anti-Mouse IgG HRP Conjugate (Cat. No. 32430) at 20ng/mL. Identical blots were incubated in either SuperSignal West Dura substrate (Cat. No. 34076), 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 (Cat. No. 62236) with the following settings: black = 65,296, white = 65,535, gamma = 1.0).

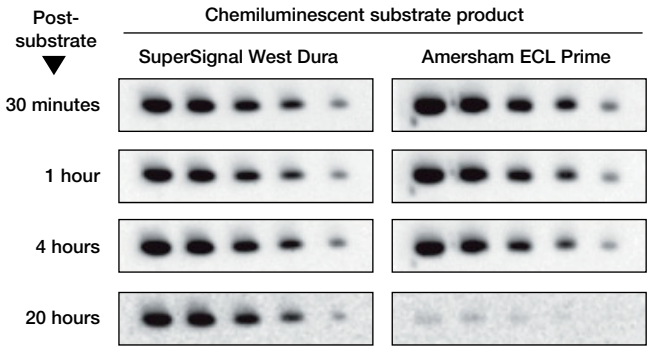


Figure 28. Longer signal duration with SuperSignal West Dura Extended Duration Substrate. Detection of Hsp86 in HeLa cell lysate on nitrocellulose membranes. HeLa cell lysate was diluted in sample buffer and heated to 95°C for five minutes. Lane 1 contained 10µg total protein (total 10µL/well). Four 1:1 serial dilutions were then prepared and loaded at 10µL/well (lanes 2–5). After transfer to nitrocellulose membranes (Cat. No. 88024), blots were blocked with StartingBlock (TBS) Blocking Buffer (Cat. No. 37542), probed with Rabbit Anti-Hsp86 Antibody (Cat. No. PA3-013) at 1:2,000 dilution, followed by goat anti-rabbit IgG HRP secondary antibody (Cat. No. 31460) at 6.6ng/mL. Finally, respective blots were incubated with SuperSignal West Dura substrate (Cat. No. 34076) or GE Healthcare Amersham™ ECL Prime Western Blotting Detection Reagent (Cat. No. RPN2232) 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.)

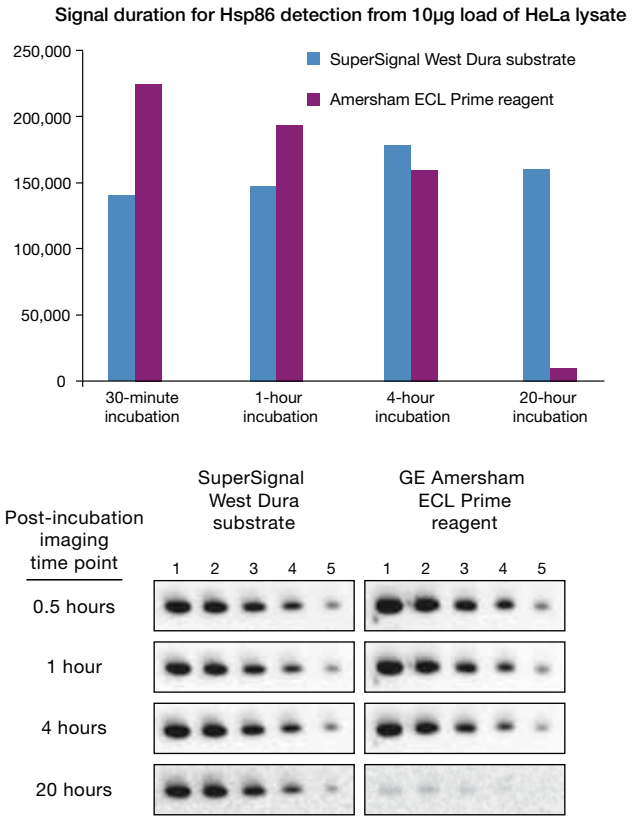
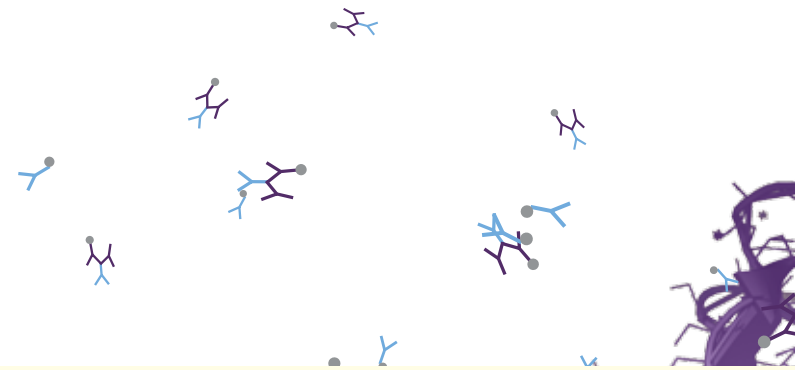


Figure 29. SuperSignal West Dura Extended Duration Substrate has better signal duration than GE Healthcare Amersham ECL Prime Western Blotting Detection Reagent. HeLa cell lysate was diluted in sample buffer and heated to 95°C for five minutes. Lane 1 contained 10µg total protein (total 10µL/well). Four 1:1 serial dilutions were then prepared and loaded at 10µL/well (Lanes 2-5). After electrophoresis proteins were transferred to nitrocellulose membranes (Cat. No. 88024). StartingBlock (TBS) Blocking Buffer (Cat. No. 37542) was used for blocking. Rabbit Anti-Hsp86 Antibody (Cat. No. PA3-013) was used as primary antibody at 1:2,000 dilution and Goat Anti-rabbit HRP (Cat. No. 31460) was used as secondary antibody at 6.6ng/mL. SuperSignal West Dura substrate (Cat. No. 34076) and GE Healthcare Amersham ECL Prime Western Blotting Detection Reagent (Cat. No. RPN2232) was used for detection. The blots were imaged using Syngene G:BOX iChemi XT Imager at the indicated times. Quantitation of signal intensity was performed using GeneSnap image acquisition software.



SuperSignal West Femto Maximum Sensitivity Substrate

Thermo Scientific™ SuperSignal™ West Femto Maximum Sensitivity Substrate is an ultrasensitive enhanced chemiluminescent HRP substrate for low-femtogram-level detection by Western blot analysis. When combined with optimized antibody concentrations and blocking buffers, SuperSignal West Femto Substrate enables detection of target proteins in amounts that are too low to be seen with typical ECL substrates.

Features:

- **Sensitive** — detect low-femtogram (mid-zeptomole) amounts of protein in bands on nitrocellulose or PVDF membranes when probed with appropriate primary and secondary antibodies
- **Quantitative** — produces quantitative signal that is measurable over two orders of magnitude
- **Intense signal** — easy to capture an image by exposure to film or imaging system
- **Excellent signal duration** — eight hours of usable light output when conditions are optimized
- **Stable reagent** — kit components are stable for one year at 4°C or six months at room temperature
- **Economical** — optimized for extremely dilute antibody concentrations

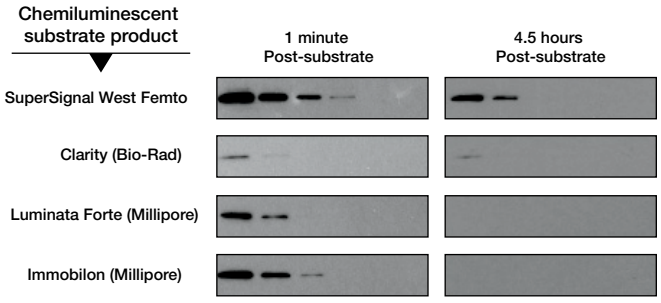


Figure 30. Obtain maximum sensitivity and signal duration with SuperSignal West Femto Maximum Sensitivity Substrate. NIH3T3 lysate was diluted in electrophoresis reducing sample buffer. Lane 1 contained 5µg of NIH3T3 lysate. Five 1:1 serial dilutions were then prepared and applied at 10µL/well. After electrophoresis, proteins were transferred to nitrocellulose membrane (Cat. No. 88018) using the Pierce Power Blotter (Cat. No. 22834) and Pierce 1-Step Transfer Buffer (Cat. No. 84731). Membranes were blocked with 5% milk in TBST Buffer. The membranes were incubated with Mouse Anti-ERK1 Antibody (Cat. No. MA1-13041) at 0.2µg/mL and then with Goat Anti-Mouse Horseradish Peroxidase Conjugate (Cat. No. 32430) at 5ng/mL. Identical blots were incubated in either SuperSignal West Femto substrate (Cat. No. 34096), Clarity Western ECL Substrate, Luminata™ Forte Western HRP Substrate or Immobilon Western Chemiluminescent HRP Substrate according to each respective manufacturer's instructions. Two-minute exposures of the resulting blots were simultaneously acquired on a single CL-XPosure Film (Cat. No. 34091).

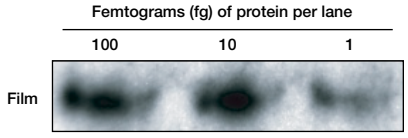
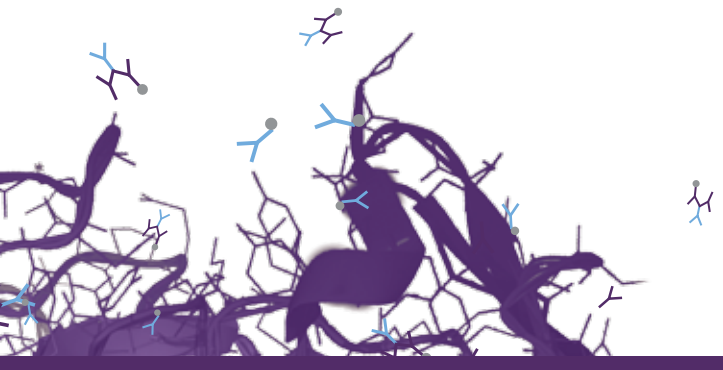


Figure 31. True femtogram levels of detection with SuperSignal West Femto Maximum Sensitivity Substrate. Purified IκB was serially diluted from 100 to 1fg and then electrophoresed on a 4–20% mini gel. The protein was transferred to PVDF membrane and blocked with StartingBlock Blocking Buffer for one hour at room temperature. The blot was incubated in Rabbit Anti-IκBα (1mg/mL) at 1:1,000 dilution overnight at 4°C, followed by incubation in HRP-conjugated goat anti-rabbit IgG (1mg/mL) at 1:200,000 dilution for one hour at room temperature. The membrane was exposed to CL-XPosure Film for one minute.



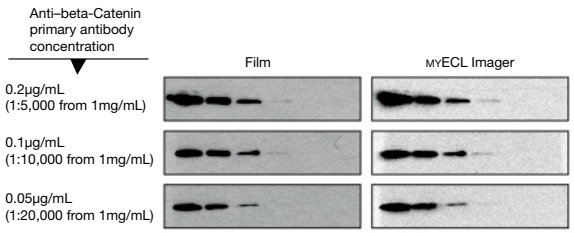


Figure 32. Use less and see more with SuperSignal West Femto Maximum Sensitivity Substrate. A431cell lysate was diluted in electrophoresis reducing sample buffer at 5, 2.5, 1.25, 0.625, 0.3125 and 0.15625µg/well with a 10µL/well load. After electrophoresis, proteins were transferred to nitrocellulose membrane (Cat. No. 88018) using the Pierce Power Blotter (Cat. No. 22834) and Pierce 1-Step Transfer Buffer (Cat. No. 84731). The membrane was blocked with 5% milk in TBST and cut into three strips to optimize antibody dilutions. Membranes were then incubated with Mouse Anti–beta-Catenin Antibody (Cat. No. MA1-300) as indicated, followed by incubation with the appropriate dilution of Goat anti-Mouse Horseradish Peroxidase Conjugate (Cat. No. 31430). SuperSignal West Femto substrate (Cat. No. 34095) was used for detection. Ten-second exposures were acquired on CL-XPosure Film (Cat. No. 34091) and the MYECL Imager (Cat. No. 62236). Exposures from the MYECL Imager were inverted and contrasted (black = 62,000, white = 65,535, gamma = 1.0).

Chemiluminescent AP substrates

A variety of AP Western blot detection substrates exist which can be used as an alternative to the more popular HRP-based systems. With AP-based detection, reaction rates remain linear, sensitivity can be improved by allowing the reaction to proceed for longer periods of time. The activity of calf intestinal alkaline phosphatase 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 inhibited by cysteine, cyanides, arsenate, inorganic phosphate and divalent cation chelators, such as EDTA.

For AP-based Western blot detection, we offer our Invitrogen™ CDP-Star™ Substrate that delivers picogram-level sensitivity and is compatible with both traditional X-ray film and CCD-based imaging. Choose from a standalone substrate, Novex AP Chemiluminescent Substrate or a complete kit, such as the WesternBreeze Chemiluminescent kits containing all solutions necessary for your application.

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.

For ordering information refer to page 81.

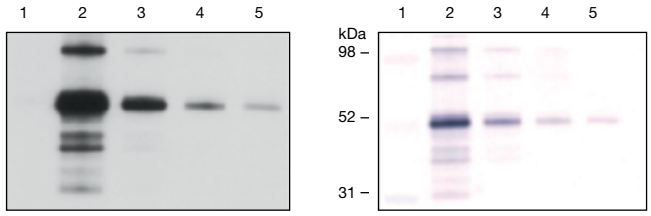
WesternBreeze Chemiluminescent kits

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 a CDP-Star-compatible imaging system.

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** — high specificity, clean background
- **High sensitivity** — femtogram levels detectable
- **Long signal duration** — up to five days
- **Fast** — results in less than three hours



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

Blot B - Detected with the WesternBreeze Chromogenic Kit.

Figure 33. Human IgG detected with the WesternBreeze Kits. Human IgG (h-IgG) was separated on a 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 indicated WesternBreeze anti-rabbit kit. Lane 1: 3µL Invitrogen™ MultiMark™ Multi-Colored Standard (no longer available); lane 2: 10ng of h-IgG; lane 3: 1ng of h-IgG; lane 4: 100pg of h-IgG; lane 5: 10pg of h-IgG.

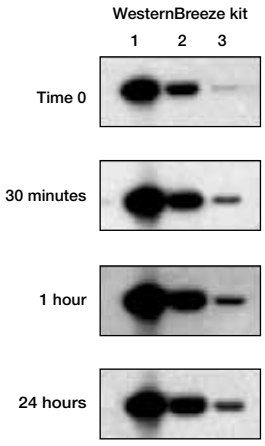


Figure 34. Signals achieved with the WesternBreeze Chemiluminescent kits. A 53kDa protein containing a V5 epitope (Invitrogen™ Positope™ Control Protein) was separated on a NuPAGE 4–12% Bis-Tris gel (with MES buffer) then transferred to a Novex PVDF membrane. The blot was probed with a 1:5,000 dilution of mouse anti-V5 primary antibody. Detection was performed using anti-mouse versions of WesternBreeze detection system with film exposures taken over time. Exposures were two minutes in duration with all three blots exposed on the same piece of film. 'Time 0' refers to an exposure taken less than five minutes after excess substrate removal. Subsequent time points are number of minutes/hours after Time 0. Lane 1: 20ng Positope Control Protein; lane 2: 2ng Positope Control Protein; lane 3: 200pg Positope Control Protein.

Did you know?

Did you know that speckled background on film can be caused by aggregate formation in the HRP conjugate? (Filtering the conjugate through a 0.2µm filter may help, or use a new HRP conjugate.)

Novex AP Chemiluminescent Substrate

Invitrogen™ Novex™ AP Chemiluminescent Substrate is a non-radioactive 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:

- **High specificity, clean background**
- **Ultra-sensitivity** — low picogram to femtogram levels detectable
- **Long-lasting signals** — up to five days
- **Results in less than three 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 Chemiluminescent Substrate

Invitrogen™ CSPD™ Substrate is a chemiluminescent AP substrate, ready to use for protein or nucleic acid blotting on nitrocellulose membranes. This substrate can also be used in solution-based assays. 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 alkaline phosphatase labels with the highest possible sensitivity and signal-to-noise

For ordering information refer to page 81.

Data imaging for chemiluminescence detection

With chemiluminescent detection methods, the chemical reaction (HRP- or AP-driven) produces light which can be captured with X-ray film, CCD camera imaging devices that detect chemiluminescence. Although X-ray film provides qualitative and semi-quantitative data and is useful to confirm the presence of target proteins, cooled CCD cameras offer the advantages of qualitative analysis, instant image manipulation, higher sensitivity, greater resolution and a larger dynamic range than film. Plus, you don't have to spend quality time in the darkroom.

Although electronic data capture with digital cameras and imagers is growing in popularity as the technologies improve and equipment prices decline, most of the data obtained from Western blotting with chemiluminescence is still captured on film. Often, it is necessary to expose several films for different time periods to obtain the proper balance between signal and background. The goal is to time the exposure of the membranes to the film so that the desired signal is clearly visible while the background remains low. This is difficult to accomplish 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 lost in the background or separate bands may become blurred together.

MYECL Imager

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

Features:

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

For ordering information refer to page 82.

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

Analyze images by adjusting display settings (saturation, invert and contrast) onscreen with the imager to ensure that the captured data are of suitable quality for the intended use. Then, once the original image is transferred to another computer that is running the Thermo Scientific™ MyImageAnalysis™ Software, it can be fully analyzed to identify lanes and bands, overlay molecular weight markers, calculate molecular weights, measure densitometry, among other features and tool options. The MyECL Imager is easier to set up and use than most instruments of its type, allowing researchers to adapt and use the new technology quickly without the need for an engineering visit. The imager eliminates the need for a darkroom, replacing it with a machine that has a small footprint. Unlike film, the MyECL Imager does not require the use of silver-containing chemicals that require hazardous materials handling and disposal.

▶▶ Learn more at **thermofisher.com/myeclimager**

Did you know?

Did you know that Western blot X-ray film can be developed entirely by hand?

CL-XPosure Film

Thermo Scientific™ CL-XPosure™ Film is an economically priced clear-blue X-ray film especially for detection and documentation of ECL-based Western blots and other chemiluminescent protein or nucleic acid assay methods. CL-XPosure Film is an excellent photographic film for use with ECL-type substrates for HRP or AP. Convenient sizes are offered for protein Western blotting, standard film cassettes and film developing equipment. The autoradiography film also works well for chemiluminescent or isotopic DNA and RNA methods, including Southern and Northern blotting and gel-shift assays (EMSA). The quality and price of this film make it the smart choice for life science research applications.

Features:

- **High performance** — just as sensitive as other commercially available autoradiography films
- **Affordable** — costs up to 75% less than other brands of comparable quality X-ray film
- **Convenient** — available in five sheet sizes in packages containing 50 or 100 non-interleaved sheets
- **Perfect match** — validated for use with SuperSignal Chemiluminescent Substrates and Pierce ECL Chemiluminescent Substrate

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

Figure 35. CL-XPosure Film vs. Kodak Film. Three types of X-ray film were tested using identical Western blotting conditions (2 blue, 1 grey). The results showed no appreciable difference between any of these films. The only significant difference is the cost per sheet of film.

Fluorescent Western blot detection

Fluorescent blotting applications differ from other detection systems in that the signal 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 molecule returns back to its normal state. This is in contrast to enzyme-substrate systems that produce a colored product or light emission as a result of a chemical reaction. Optimized fluorescent applications can be more quantitative than enzyme systems.

Reagents for fluorescent detection have excitation and emission wavelengths that are selected to avoid auto fluorescence from common membranes. Special low-fluorescence membranes are also available for fluorescent Western blots.

Fluorescent reagents are growing in popularity for Western blotting because they offer increased time savings over chemiluminescent detection and reduced chemical waste compared to both chemiluminescent or 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.

WesternDot Antibodies

Fluorescence detection enables quantitative, multiplex analysis of Western blots right at your bench — without the need for ECL optimization, film, or a darkroom. Invitrogen™ WesternDot™ Antibodies are detected on membranes with high sensitivity and minimal background signal. Detect both strong and weak signals at the same time with a greater than 4,000-fold linear dynamic range. With an appropriate reader you can multiplex up to three probes on the same blot, providing an extra level of precision and biological context for your measurements.

Simply replace your HRP- or AP-conjugated secondary antibody with an appropriate Invitrogen™ WesternDot™ conjugate. After a 15-minute incubation, wash the membrane and you're ready to view your results.

With multiple fluorescence detection channels using WesternDot conjugates, you can save both time and precious sample by detecting multiple proteins in the same assay. No need to strip and reprobe, or run a second blot. You can also normalize your intensity values to a control protein to correct for any inaccuracies in loading.

Features:

- Simple, quantitative Western blots
- Uses existing benchtop equipment
- Wide linear dynamic range
- Multiplexing capability

▶▶ Learn more at **thermofisher.com/westerndot**

For ordering information refer to page 82.

Invitrogen™ WesternDot™ reporters are based on quantum dots, which are fluorescent materials that are approximately 10–100 times brighter than traditional fluorescent dyes due to their high molar absorptivity (approximately 10^6–$10^7 M^{-1}cm^{-1}$ compared to approximately 10^4–$10^5 M^{-1}cm^{-1}$ for dyes). The optical properties of these materials make quantum dots good probes for protein detection on Western blots. The narrow emission spectra ensure very little spillover between different emission channels, making detection of multiple protein targets on a single blot with minimal filter bleed-through. In addition, the stability of quantum dots makes it possible to collect multiple images and store dried blots for months while retaining detectable fluorescent signal.

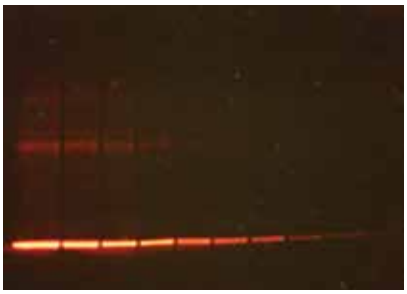


Figure 36. Detection with transilluminator. The standard benchtop transilluminator you used for ethidium bromide or Invitrogen™ SYBR™ Green detection is an ideal imaging system for simple fluorescent Western blots. Invitrogen™ WesternDot™ 625 conjugates exhibit a robust fluorescent signal with most transilluminator wavelengths. A standard gel imager with an ethidium bromide filter is an ideal starting point for simple Invitrogen™ WesternDot™ probe detection. Place your blot face down on the stage for the best signal, and for rapid documentation you can even capture the result on a smartphone.

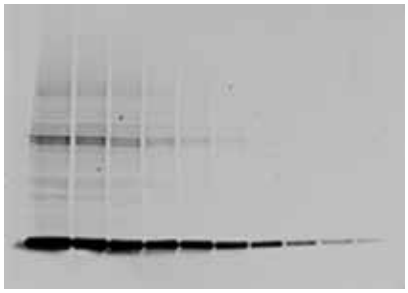


Figure 37. Detection with a gel imager. You can obtain precise, quantitative data from your Invitrogen™ WesternDot™ gel blots using either a camera-based or scanner-based gel imaging system. Select the right wavelength for your imager and WesternDot conjugate so that you maximize signal and minimize background. With an appropriate detector your blots will provide up to four orders of magnitude of linear dynamic range. That's about 16 times more than chemiluminescent detection, with lower cost and a vastly simpler workflow. Use any of the WesternDot conjugates for robust quantitative results.

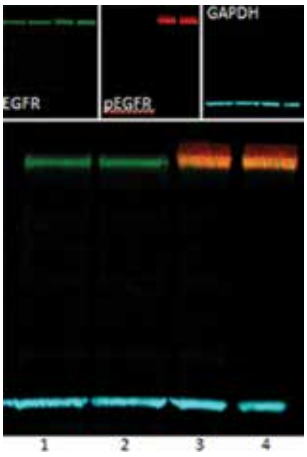


Figure 38. Detection with multiplex imager. With a multi-wavelength imaging system you can take advantage of the full power of WesternDot conjugate detection. Use the multiplexing capability to monitor housekeeping proteins, correlate different protein concentrations, or simply run reference standards. No need for duplicate blots or reprobing — you can directly compare band intensities for multiple proteins on a single blot. Combine Invitrogen™ WesternDot™ 585 conjugates with Invitrogen™ WesternDot™ 655 and 800 conjugates for the best spectral separation and multicolor detection. The wide range of available conjugates offers you the flexibility you need.

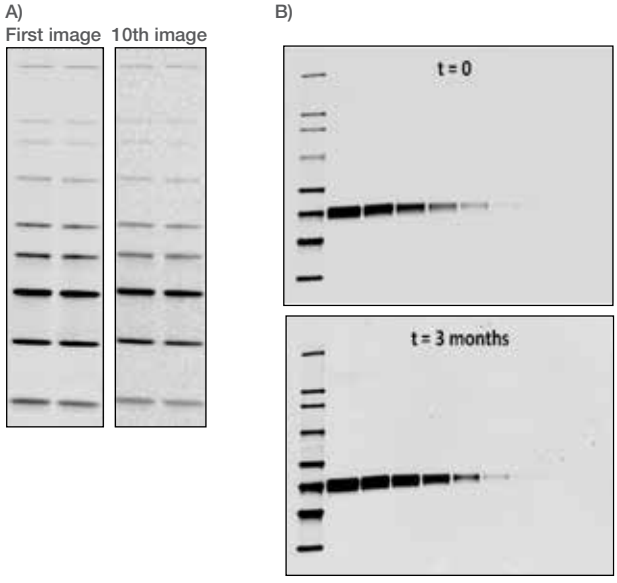


Figure 39. Multiple images and long-term storage of blots. (A) Photostable Invitrogen™ WesternDot™ reagents enable taking multiple images without loss of signal intensity. Invitrogen™ MagicMark™ XP Western Protein Standard (Cat. No. LC5602) labeled with WesternDot 655 goat anti-mouse secondary antibody conjugate. Image was taken on E-Gel Imager with a UV lamp as an excitation source. (B) The stability of WesternDot reagents also enables long-term storage of dried blots. Shown is a β-tubulin blot stained with WesternDot 655 goat anti-mouse reagent. The signal intensity did not reduce after storage for three month under ambient conditions. Images were acquired on the Fujifilm LAS 4000 gel imager.

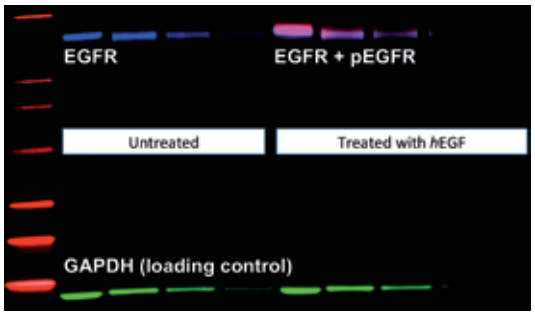


Figure 40. Simultaneous detection of three proteins on a single blot using WesternDot antibody conjugates. A Western blot containing serial dilutions (20–3µg protein) of lysates from unstimulated (lanes 2–5) and hEGF-stimulated (lanes 6–9) A431 cell lysate was probed with mouse anti-EGFR, rabbit anti–phospho-EGFR and chicken anti-GAPDH antibodies, followed by WesternDot 800 goat anti-mouse (pseudocolored blue), WesternDot 585 goat anti-rabbit (pseudocolored red) and WesternDot 655 goat anti-chicken (pseudocolored green) conjugates. The merged image shows overlaid red and blue bands as purple. The blot contains MagicMark XP Western Protein Standard (lane 1, Cat. No. LC5603) and was imaged using the Fujifilm LAS 4000 gel imager.

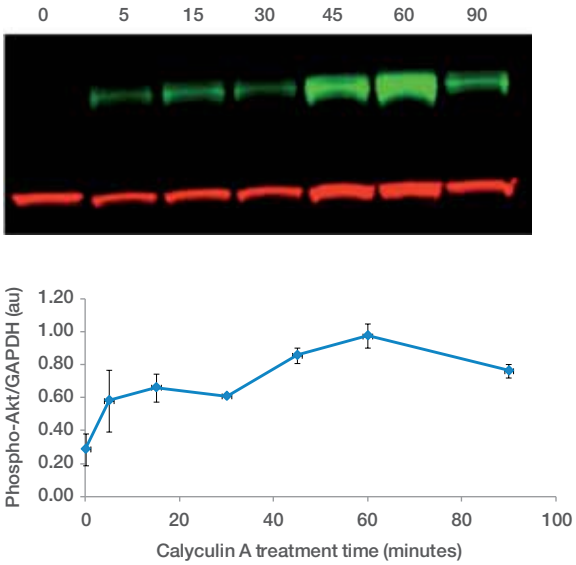


Figure 41. Quantitative analysis of AKT phosphorylation level changes following stimulation of Jurkat cells with calyculin A. Jurkat cells were treated with 60nM calyculin A for 0, 5, 15, 30, 45, 60 and 90 minutes at 37°C. 30µg cell lysate per well was loaded into a 10-well Bolt 4–12% Bis-Tris Plus Protein Gels, then transferred to PVDF membrane. Phospho-AKT and the loading control GAPDH were probed with rabbit anti-phospho-AKT (Cell Signaling Technology, Cat. No. 4060p) and mouse anti-GAPDH (Cat. No. 398600), respectively, followed by detection with WesternDot 800 goat anti-rabbit and WesternDot 655 goat anti-mouse. Images were acquired on the Fujifilm LAS 4000 gel imager. Fluorescence signals were quantified with Image J.

iBind Fluorescent Detection (FD) Solution Kit

The Invitrogen™ iBind™ Fluorescent Detection (FD) Solution Kit is intended for use with the iBind Western Device and is optimized for western blotting with fluorescent-conjugated antibodies, including those commonly used for infrared detection and dual wavelength semi-quantitative analysis. For more information on automated blot processing methods, please see page 7.

iBind Flex Fluorescent Detection (FD) Solution is a proprietary formulation compatible with downstream fluorescent detection (e.g., infrared dual wavelength) and serves as a combined blocking, washing and antibody diluent solution to process up to one midi blot, two mini blots or six vertically cut strip blots when used with the iBind Flex Western Device and iBind Flex Cards. Each kit contains reagents sufficient for 10 midi blots or 20 mini blots.

Features:

- **Optimized** — solutions with ideal viscosity for blocking, washing and antibody dilution
- **Long shelf life** — store iBind Fluorescent Detection (FD) Solution Kit for 12 months at 4°C
- **Better antigen** — antibody interaction solutions flow between the iBind Card and membrane for maximum interaction between protein and antibody

For ordering information refer to page 82.

Data imaging for fluorescence detection

Recording and documenting the results of fluorescence-based Western blotting requires special instrumentation, namely a fluorescent imaging system. Several manufacturers offer fluorescence imagers, most of which use either filter-based or laser-based technologies to deliver the appropriate excitation wavelength and then record the emission light output. Captured images are saved digitally.

The number of commercially available fluorescent dyes with different excitation and emission spectra continues to increase. Fluors with non-overlapping spectra enable multiplex analysis, whereby two or three different targets can be detected and independently distinguished in the same lane and blot. However, the imager used must be equipped with the appropriate filters or lasers for the fluors used.

Certain instruments are specialized for detection of infrared and near-infrared fluors, while others provide for analysis of only one or two particular fluors in the visible range. Increasingly, new instruments are being offered with capabilities for detection of nearly any combination of excitation and emission wavelengths.

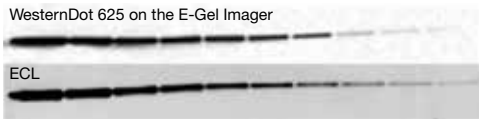


Figure 42. A Western blot of a dilution series of Jurkat lysate (10µg to 4ng) was probed with an anti-GAPDH primary antibody followed by a WesternDot 625 Donkey Anti-Rabbit Secondary Antibody. The blot was imaged on the E-Gel Imager. When compared to a duplicate Western blot detected using ECL followed by imaging on the Fujifilm FLA-9000 image scanner, the sensitivity was comparable.

Did you know?

Did you know that some fluorophores, like those found on WesternDot antibodies, can be excited with UV light of the appropriate wavelength?

E-Gel Imager

The Invitrogen™ E-Gel™ Imager is a compact, affordable, easy-to-use imaging system for stained gels and WesternDot 625 probe-stained Western blots. Equipped with a high-resolution, scientific-grade digital camera, the E-Gel Imager can be used to image gels incorporating fluorescent stains (e.g., ethidium bromide and SYBR stains) or colorimetric stains (Coomassie and silver stains). Additionally, you can image Western blots detected with the WesternDot 625 detection kit reagents, avoiding the need for film processing. Setup is simple, and the system features intuitive software for band analysis. The E-Gel Imager can also be used for the documentation and densitometric analysis of stained nucleic acids and proteins in agarose and polyacrylamide gels.

Features:

- **Faster time-to-results** — eliminate cumbersome HRP and ECL optimization
- **Cost savings** — image the blot directly; no more films to process
- **Blot signal is stable for months and does not suffer from photo-bleaching** — obtain multiple exposures without significant reduction in signal

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

Specialized Western blotting systems

In addition to our traditional SuperSignal Western blotting substrates and kits, we offer specialized kits for the detection of histidine-tagged proteins, phosphoproteins and O-Glc-NAc posttranslational modifications.

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.

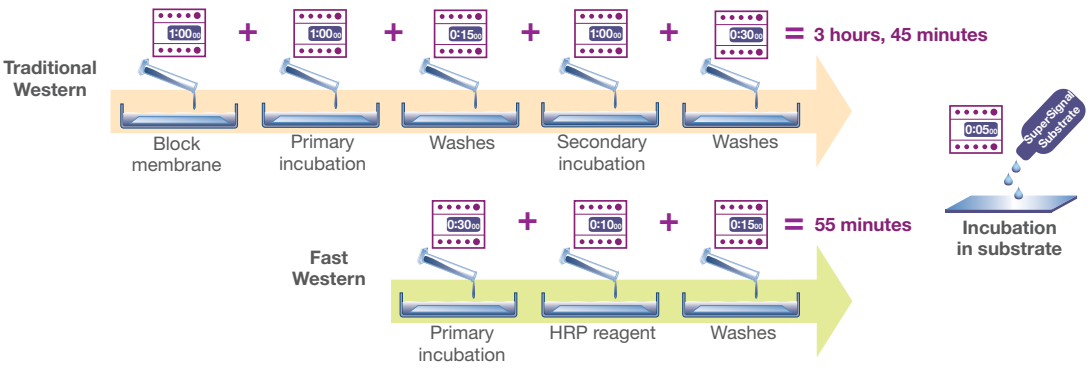
Pierce Fast Western Blot Kits

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

After transferring proteins to a membrane, traditional Western blotting procedures can take more than four hours to complete. Our kits provide all the reagents (except your primary antibody) necessary to complete a Western blot in about 55 minutes. The protocol is easy and requires little or no special optimization. Simply use a mouse or rabbit primary antibody at the same concentration used for typical detection with the Pierce ECL or SuperSignal substrate. Because it is a reagent-based system, there is no investment in equipment or costly consumables to purchase. Our specially formulated blocking buffers and titered detection antibodies accelerate Western blotting by minimizing incubation times without sacrificing sensitivity.

Features:

- **Fast** — save up to four hours compared to ordinary chemiluminescent Western blots
- **Convenient** — no expensive hardware or vacuum required
- **Simple** — optimized protocol simplifies Western blot analysis
- **Economical** — no expensive consumables or extra equipment required
- **Stable** — kits are stable for one year stored at 4°C
- **Easy** — complete kits contain all components needed to block, probe and develop a blot with your mouse or rabbit primary antibody



For ordering information refer to page 83.

Table 11. Which fast Western product is right for you?

	Fast Western Blot Kit, Pierce ECL	Fast Western Blot Kit, SuperSignal West Pico	Fast Western Blot Kit, SuperSignal West Dura	Fast Western Blot Kit, SuperSignal West Femto
Detection substrate	Pierce ECL Substrate	SuperSignal West Pico Chemiluminescent Substrate	SuperSignal West Dura Extended Duration Substrate	SuperSignal West Femto Maximum Sensitivity Substrate
Sensitivity	Picogram	Low picogram	Femtogram	Low femtogram
Species	Combined rabbit and mouse antibodies	Separate mouse and rabbit kits	Separate mouse and rabbit kits	Separate mouse and rabbit kits

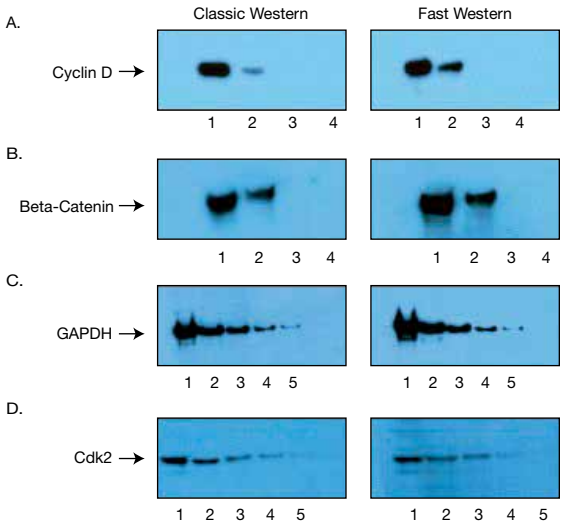


Figure 43. Comparable results obtained with classic Western blotting protocol and Pierce Fast Western Blot Kit, ECL Substrate. The detection sensitivity for various target proteins was compared using the Fast Western Blot Kit and the classical Western blot protocol.

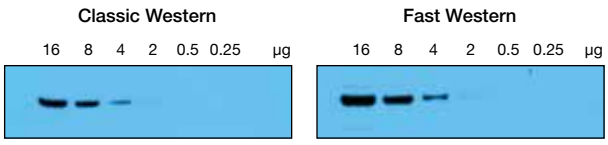


Figure 44. Classic vs. fast Western protocol using SuperSignal West Pico Chemiluminescent Substrate. 293T cell lysate was diluted in reducing sample buffer and loaded to a gel at 16, 8, 4, 2, 0.5 and 0.25µg/well. After electrophoresis, proteins were transferred to nitrocellulose membranes using the Pierce Fast Blotter for 10 minutes at 25V. One membrane was developed using the classic Western protocol, and the other was developed using the fast Western protocol. The blots were probed with mouse anti-XIAP antibody (1µg/mL, BD Pharmingen). Each blot was incubated in SuperSignal West Pico Chemiluminescent Substrate Working Solution for five minutes and exposed to film for one minute.

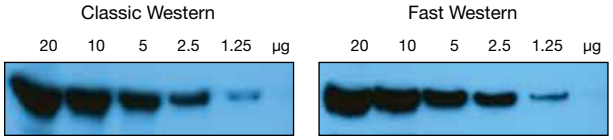


Figure 45. Classic vs. fast Western protocol using SuperSignal West Dura Extended Duration Substrate. HeLa cell lysate was diluted in reducing sample buffer and loaded onto a gel at 20, 10, 5, 2.5 and 1.25µg/well. After electrophoresis, the proteins were transferred to PVDF membranes using the Pierce Fast Blotter for 10 minutes at 25V. The membranes were probed with rabbit anti-GRB14 antibody (0.5µg/mL, Millipore). Membranes were developed using the classic Western protocol or the fast Western protocol. Each blot was incubated in SuperSignal West Dura Extended Duration Substrate Working Solution for five minutes and exposed to film for one minute.

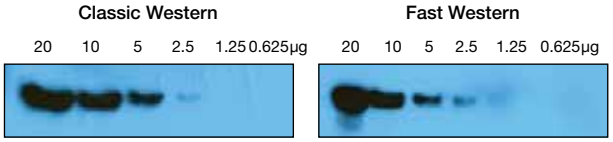


Figure 46. Classic vs. fast Western protocol using SuperSignal West Femto Maximum Sensitivity Substrate. HeLa cell lysate was diluted by 50% and loaded onto a gel at 20, 10, 5, 2.5, 1.25 and 0.625µg. After electrophoresis, the proteins were transferred to PVDF membranes using the Pierce Fast Blotter for 10 minutes at 25V. Membranes were developed using either the classic Western blotting protocol or the fast Western protocol. The membranes were probed with mouse anti-ezrin antibody (1µg/mL, Lab Vision). Each blot was incubated in SuperSignal West Femto Maximum Sensitivity Substrate Working Solution for five minutes and exposed to film for one minute.

Pierce O-GlcNAc Western Blot Detection Kit

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

Features:

- Highly specific monoclonal antibody against O-GlcNAc modification
- Speed and sensitivity of chemiluminescent detection
- Complete kit contains cell lysis and detection reagents

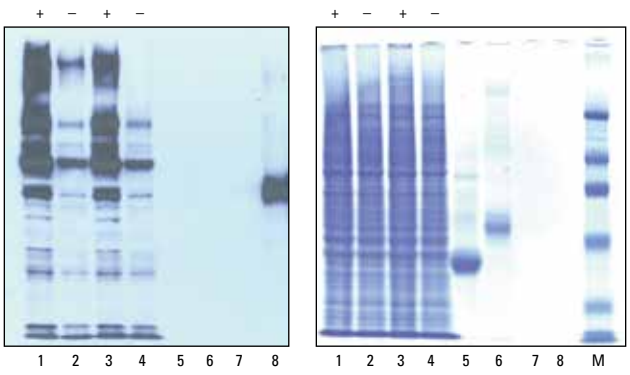


Figure 47. Western blot detection of O-GlcNAc-modified proteins after SDS-PAGE. Lanes 1–4 are proteins from Jurkat cell extract. Lanes 5, 6 and 7 are the negative controls: ovalbumin (5µg), fetuin (5µg) and O-β-GalNAc-modified BSA (10ng). Lane 8 is O-β-GlcNAc-modified BSA (5ng, positive control). The (+) and (–) refer to plus and minus treatment with PUGNAc and glucosamine, and M represents the molecular weight marker.

SuperSignal West Pico HisProbe Kit

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

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 anti-6XHis-tag antibodies
- **High specificity** — HisProbe-HRP can recognize His-tags independent of adjacent tags
- **Less steric hindrance** — HisProbe-HRP is smaller than antibodies and detects his tagged fusion proteins that are often undetectable when using some anti-His-tag antibodies

For ordering information refer to page 83.

Pierce Far-Western Blot kit

The Thermo Scientific™ Pierce™ Far-Western Blot kit for biotinylated proteins enables in-gel or on-membrane Western blot detection of target proteins via interaction with any biotin-labeled test protein used as the primary probe. The kit is successful for analysis of protein-protein interactions in which the target (prey) proteins can be bound after they have been separated by gel electrophoresis. The kit includes reagents and procedures for probing and chemiluminescent detection with blots or directly from mini gels.

Features:

- **On-membrane or in-gel detection options** — on-membrane detection offers greater sensitivity; in-gel detection is faster and prevents problems associated with incomplete or inefficient transfer
- **Nonradioactive alternative for far-Western analysis** — reliable and sensitive biotin/streptavidin-HRP chemistry combined with chemiluminescent detection offers a practical and safe alternative to radiolabeling the bait protein
- **Useful interaction range** — effective for interaction pairs that have moderate to strong binding associations between the prey and the biotinylated bait protein
- **Primary antibody-free detection** — uses a biotinylated protein as the probe, eliminating the need for primary antibodies or the need to have prior knowledge of target-protein identities
- **Compatible with both SDS-PAGE and native gels** — provides option to probe for prey proteins in a more native environment because reduced or denaturing systems may not always allow for the intended interaction
- **Reduced nonspecific binding** — biotin/streptavidin-HRP systems demonstrate less nonspecific binding compared to antibodies directed against the bait protein
- **Compatible with protein staining** — gels can be used for total protein staining after the in-gel chemiluminescent detection step, eliminating the need to run two gels

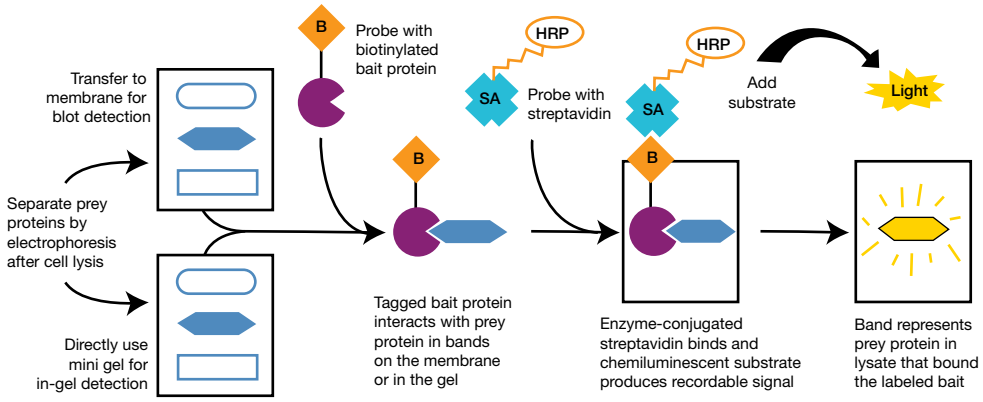


Figure 48. Protocol scheme for the Far-Western Blot kit for biotinylated proteins. The method allows identification and characterization of protein interactions, whether native or artifactual.

Clean-Blot IP Detection Reagent

Thermo Scientific™ Clean-Blot™ IP Detection Reagent is a horseradish peroxidase 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 50kDa) and light-chain (25kDa) IgG-fragments of antibodies used for the initial immunoprecipitation assay.

Features:

- **Universal** — bind and detect most species IgG subclasses and isotypes of primary antibodies that are commonly used for Western blotting
- **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
- **Unobstructed detection** — clear Western blot results for immunoprecipitation assays without significant interference from denatured IgG bands

Table 12. Clean-Blot IP Detection Reagent recognizes the various polyclonal antibodies and the specific monoclonal antibodies listed. To determine specific antibody compatibility, perform a dot-blot analysis.

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

For ordering information refer to page 83.

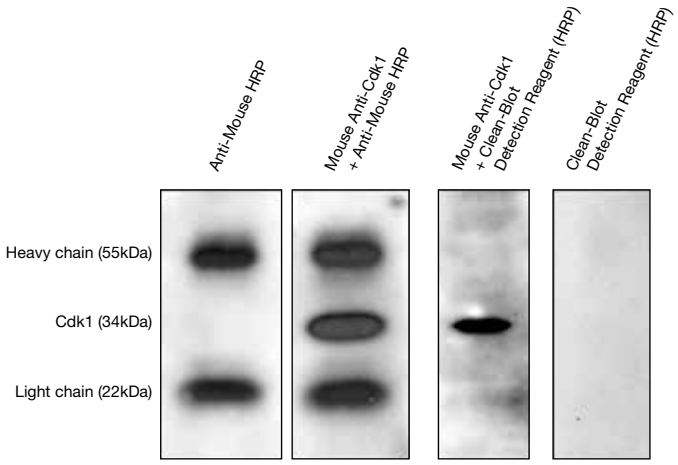


Figure 49. Easily distinguish your target protein on a Western blot with Clean-Blot Detection Reagent (HRP). Mouse liver extract (50µg) total protein was separated on a Bio-Rad Criterion™ Gel, transferred to PVDF membrane and blocked with 5% milk in TBST. The membrane was probed with mouse monoclonal anti-Cdk1 (LabVision, 0.2µg/mL) and goat anti-mouse HRP (0.16µg/mL) or Clean-Blot Detection Reagent (HRP) (0.2µg/mL). SuperSignal West Pico substrate (Cat. No. 34080) was used for detection of Cdk1 protein.

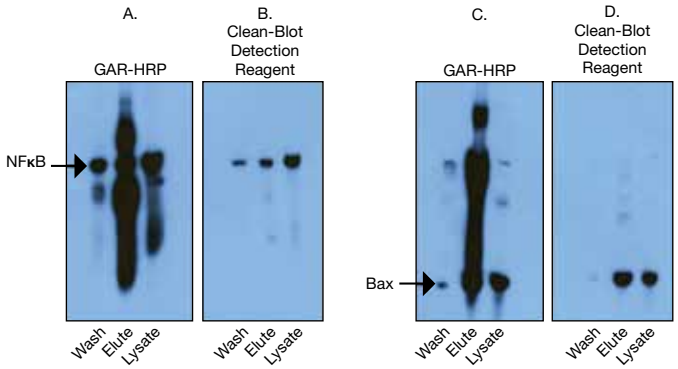
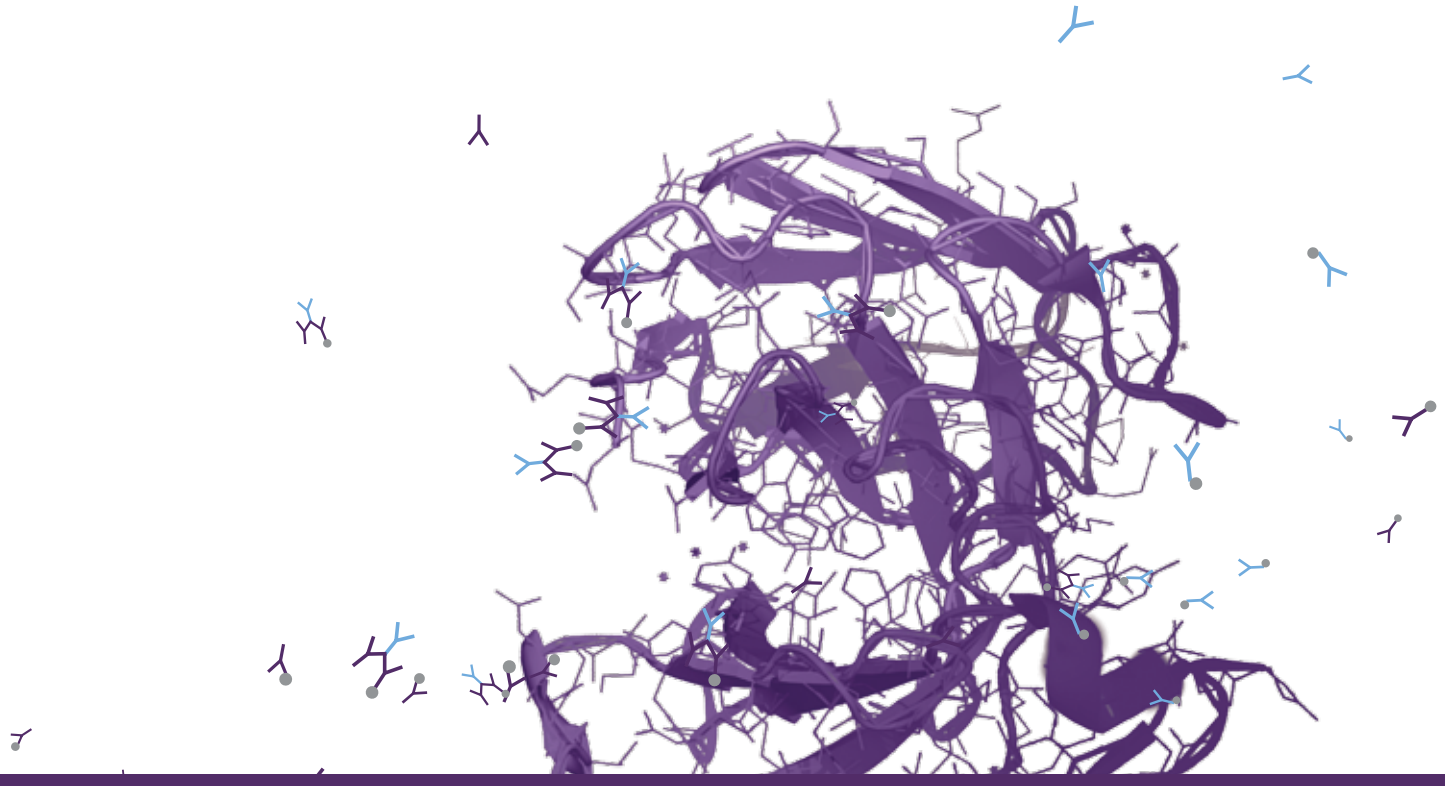


Figure 50. Reveal your target protein with Clean-Blot Detection Reagent (HRP). To demonstrate unmasking of the target protein, we performed IP and Western blot experiments. NF-κB and Bax were immunoprecipitated from A549 lysate using Protein A/G Agarose Resin and rabbit anti-NF-κB (panels A and B) and rabbit anti-Bax (panels C and D). Panels A and C were detected with goat anti-rabbit HRP, which masked the target. Panels B and D were detected with the Clean-Blot Detection Reagent (HRP), revealing the target protein.



Post-detection methodologies

Stripping and reprobing Western blots

One of the major advantages offered by chemiluminescent and fluorescent detection methods is the ability to strip reagents from a blot and then reprobe the same blot. 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 13). With chemiluminescence and fluorescence, all of the reagents can be removed from the membrane because the product detected is light rather than a colored precipitate absorbed on the membrane.

Table 13. 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 analyzed with the same or different antibodies.
Saves time	It is time-consuming to run an SDS-polyacrylamide gel and then transfer the proteins to a membrane. By using the same blot for several different detections, you save time.
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.
Correct mistakes	Immunoblotting requires many steps, providing ample opportunity for mistakes to occur. By stripping the membrane, the blot can be reused.

For ordering information refer to page 83.

Stripping Western blot membranes

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

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 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.

Table 14. Which stripping buffer is right for me?

	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 minutes at room temperature	5–15 minutes at room temperature	15 minutes 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 • Works on nitrocellulose and PVDF • 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. The Restore Western blot stripping procedure only takes 15 to 30 minutes, depending on the affinity of the primary antibody.

Features:

- **Saves time** — no need to re-run 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 antigen during stripping allowing efficient reprobing
- **Odor-free** — no mercaptans means no acrid odors
- **Economical** — less expensive than other commercial stripping buffers

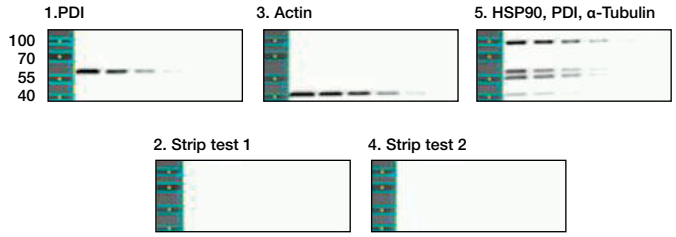


Figure 51. Stripping and reprobing blots for similar molecular weight targets with Restore Western Blot Stripping Buffer. Stripping and reprobing blots for similar molecular weight targets with Restore Western Blot Stripping Buffer. A431 cell lysate was diluted to 125µg/mL in electrophoresis reducing sample buffer and 1:1 serial dilutions were made. 10µL of each dilution (1,250ng to 39ng of total protein) were separated by SDS-PAGE and the protein transferred to 0.45µm nitrocellulose membranes (Cat. No. 88018). The membrane was blocked with 5% non-fat dry milk in 1X PBS Tween-20 Buffer (Cat. No. 28352) and analyzed by Western blot using SuperSignal West Dura Extended Duration Substrate (Cat. No. 34076) and the MYECL Imager (3 × 3 binning) (Cat. No. 62236). The first target (panel 1) was detected by probing with Anti-PDI Monoclonal Antibody (Cat. No. MA3-019) at 0.33µg/mL followed by Goat anti-Mouse Horseradish Peroxidase Conjugate (Cat. No. 31430) at 6.7ng/mL and imaged. Next, the blot was stripped in Restore Western Blot Stripping Buffer (Cat. No. 21059) for 15 minutes at 37°C, washed in 1X PBS Tween-20, incubated with substrate and imaged to check for stripping efficiency (panel 2). The second target (panel 3) was detected by reblocking the membrane and probing with Anti-Actin Monoclonal Antibody (Cat. No. MA1-744) at 0.5µg/mL followed by the anti-mouse HRP conjugate at 6.7ng/mL and imaged. The blot was stripped again (panel 4) and then probed for multiple targets (alpha-Tubulin, Cat. No. 62204 at 0.2µg/mL; PDI as above; and Hsp90, Cat. No. PA3-013 at 0.14µg/mL), and imaged as described above.

For ordering information refer to page 83.

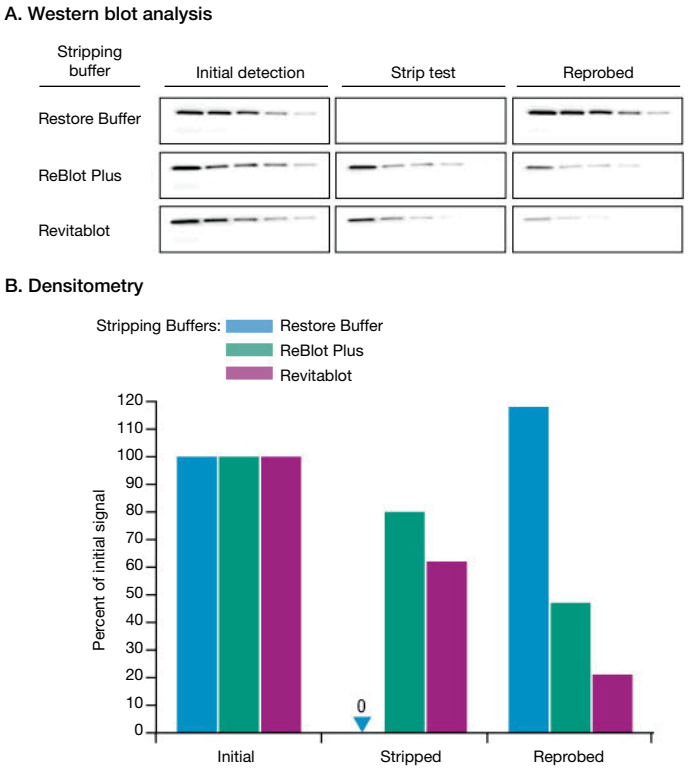


Figure 52. Western blot stripping and reprobing performance of three stripping buffers on nitrocellulose. HeLa cell lysate was diluted to 1mg/mL in electrophoresis reducing sample buffer and 1:1 serial dilutions were made. Three sets of 10µL/dilution (10µg to 0.31µg of total protein) were separated by SDS-PAGE and the protein transferred to 0.45µm nitrocellulose membrane (Cat. No. 88018). A. The membrane was blocked with 5% non-fat dry milk in 1X TBS Tween-20 Buffer (Cat. No. 28360) and analyzed by Western blot using SuperSignal West Dura Extended Duration Substrate (Cat. No. 34076) and the MYECL Imager (3 x 3 binning) (Cat. No. 62236). The membrane was probed with Anti-Hsp90 Polyclonal Antibody (Cat. No. PA3-013) at 0.5µg/mL followed by Goat anti-Rabbit Horseradish Peroxidase Conjugate (Cat. No. 31460) at 5.7ng/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 (Cat. No. 21059) (15 minutes at 37°C), Reblot Plus Stripping Solution (Millipore, Cat. No. 2502) (15 minutes at room temperature) or Revitablot™ Western Blot Stripping Buffer (Rockland Immunochemicals Inc., Cat. No. MB-085-0050) (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 repeated as described above. B. Densitometry analysis shows that the Restore Stripping Buffer permitted both complete signal removal and maintained nearly identical levels of detection between the initial and reprobed Western blot analysis.

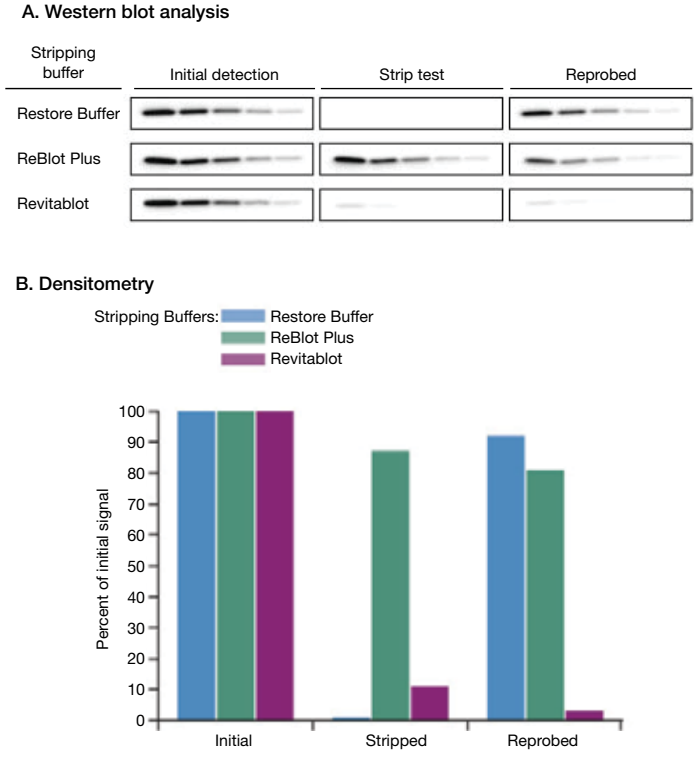


Figure 53. Western blot stripping and reprobing performance of three stripping buffers on PVDF. HeLa cell lysate was diluted to 1mg/mL in electrophoresis reducing sample buffer and 1:1 serial dilutions were made. Three sets of 10µL/dilution (10µg to 0.31µg of total protein) were separated by SDS-PAGE and the protein transferred to 0.45µm PVDF membrane (Cat. No. 88518). A. The membrane were as blocked with 5% non-fat dry milk in 1X TBS Tween-20 Buffer (Cat. No. 28360) and analyzed by Western blot using SuperSignal West Dura Extended Duration Substrate (Cat. No. 34076) and the MYECL Imager (3 x 3 binning) (Cat. No. 62236). The membrane was probed with Anti-Cyclophilin B Polyclonal Antibody (Cat. No. PA1-027A) at 0.2µg/mL followed by Goat anti-Rabbit Horseradish Peroxidase Conjugate (Cat. No. 31460) at 5.7ng/mL and imaged. Following the initial detection, the blot was cut into three strips to separate the serial dilution sets and east part of the blot was stripped in either Restore Western Blot Stripping Buffer (Cat. No. 21059) (15 minutes at 37°C), Reblot Plus Stripping Solution (Millipore, Cat. No. 2502) (15 minutes at room temperature) or Revitablot™ Western Blot Stripping Buffer (Rockland Immunochemicals Inc., Cat. No. MB-085-0050) (15 minutes at room temperature) according to manufacturer's instructions. 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 repeated as described above. B. Densitometry analysis shows that the Restore Stripping Buffer permitted both 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 viable; strip the same blot up to five times
- **Flexible** — strip and reprobe to optimize antibody concentrations or to detect a new antigen with different antibodies

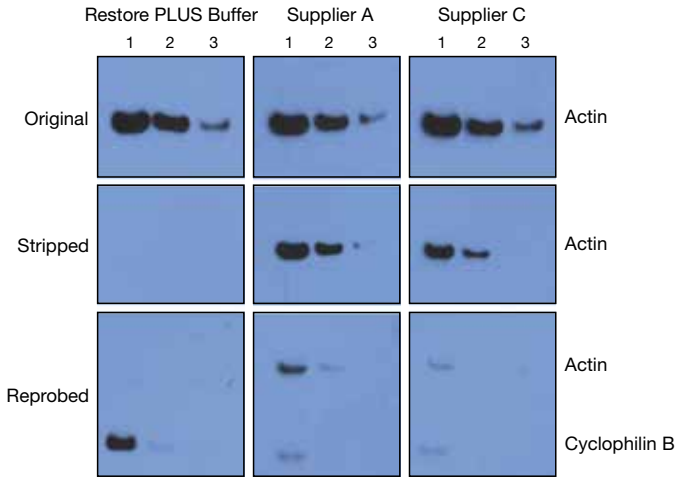
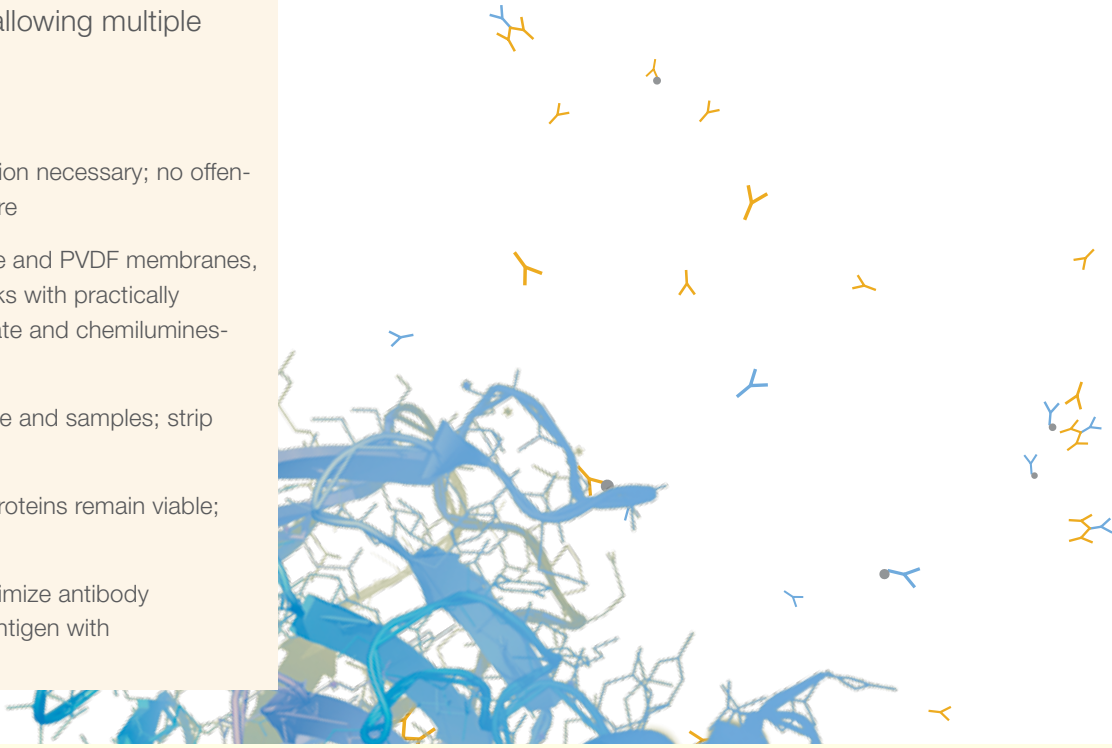


Figure 54. Restore PLUS Western Blot Stripping Buffer is most effective for reprobing with different antibodies. Reprobing with different antibodies. HeLa cell lysate was probed for actin and detected with Pierce ECL Substrate (original panel). Blots were then stripped with either Restore PLUS Stripping Buffer or other suppliers' stripping buffers (stripped panel). The blots were then re-blocked and reprobed for cyclophilin B and detected with Pierce ECL Substrate (reprobed panel).



For ordering information refer to page 83.

Restore Fluorescent Western Blot Stripping Buffer

The Thermo Scientific™ Restore™ Fluorescent Western Blot Stripping Buffer is a gentle and highly effective reagent for quickly removing primary and NIR dye-labeled secondary antibodies from Western blots. Restore Fluorescent Western Blot Stripping Buffer enables the reuse of PVDF membranes, simplifying the Western blot optimization process and allowing the same blot to be reprobed with different primary antibodies to detect alternative targets. Restore Fluorescent Western Blot Stripping Buffer is for use with low-fluorescence PVDF membrane only (Cat. No. 22860).

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

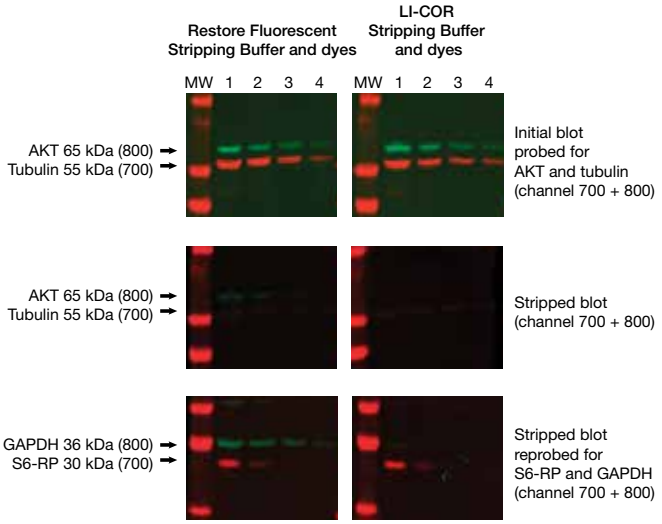


Figure 55. Effective stripping and reprobing of near-IR fluorescent Western blots on PVDF. A549 whole cell lysates (20, 10, 5 and 2.5µg of protein in lanes 1, 2, 3 and 4, respectively) were transferred to PVDF membranes. Top panels: The blots were probed with anti-AKT and anti-tubulin antibodies and detected using Thermo Scientific™ Pierce™ DyLight™ 680 Goat Anti-Mouse IgG (Cat. No. 35519) and DyLight™ 800 Goat Anti-Rabbit IgG (Cat. No. 35571), or IRDye™ 680 Goat Anti-Mouse and IRDye™ 800CW Goat Anti-Rabbit (LI-COR). The Odyssey Infrared Imaging System (channel 700 and 800) was used for imaging. Middle panels: Blots were stripped with Restore Fluorescent Stripping Buffer or NewBlot™ PVDF 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 and mouse anti-GAPDH antibodies. Targets were detected using DyLight 800 Goat Anti-Mouse IgG and DyLight 680 Goat Anti-Rabbit IgG (Cat. No. 35568), or IRDye 800CW Goat Anti-Mouse and IRDye 680 Goat Anti-Rabbit and imaged as described above.

Pierce Background Eliminator

Another method by which the signal-to-noise ratio can be improved is to “erase” the background on exposed film, leaving just the signal with little to no interference. Thermo Scientific™ Pierce™ Background Eliminator does just that without altering the integrity of the data. The Pierce Background Eliminator works on overexposed film, lightening the entire film evenly. This is done directly in the lab while viewing the film. No darkroom is required. The process can be halted when the signal is clearly visible and the background is at a minimum, thereby increasing the signal-to-noise ratio without altering the data’s integrity (Figure 57). Pierce Background Eliminator works on exposed X-ray film for Western, Northern or Southern blots.

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

Features:

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

For ordering information refer to page 83.

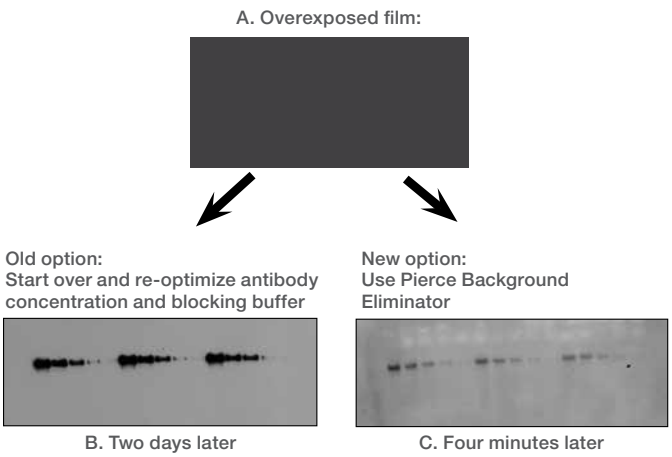


Figure 56. Pierce Background Eliminator lightens the entire film evenly in four minutes vs. the two days traditional methods require to start over and reoptimize experiment conditions. A431 cell lysate was electrophoresed on a 4–12% NuPage Gel and transferred overnight to nitrocellulose. The membrane was blocked with SuperBlock Blocking Buffer in PBS (Cat. No. 37515) for one hour and incubated with 1.25ng/mL of HRP-labeled mouse anti-phosphotyrosine (PY20) for one hour. After the membrane was washed for 30 minutes, SuperSignal West Dura substrate was added. The blot was exposed to film for 10 seconds and resulted in a completely black image caused by the antibody cross-reacting with the blocking buffer (A). Using the old option, another gel was prepared to optimize assay conditions. The proteins were transferred overnight and then the membrane was blocked with a 5% dry milk solution for one hour. The blot was detected with 2.5ng/mL of anti-phosphotyrosine (PY20)-HRP and SuperSignal West Dura substrate. The blot was exposed to film for 10 seconds. This optimization required a two-day procedure (B). Using the new option, the initial dark film (A) was treated with Pierce Background Eliminator to allow the band images to appear in four minutes (C).

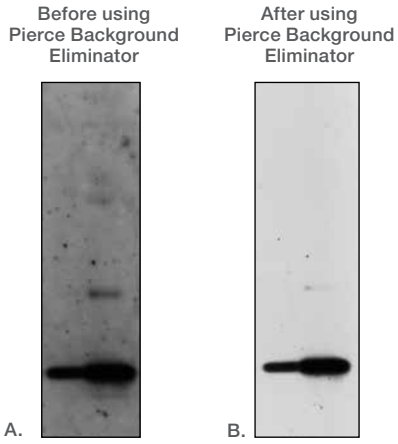
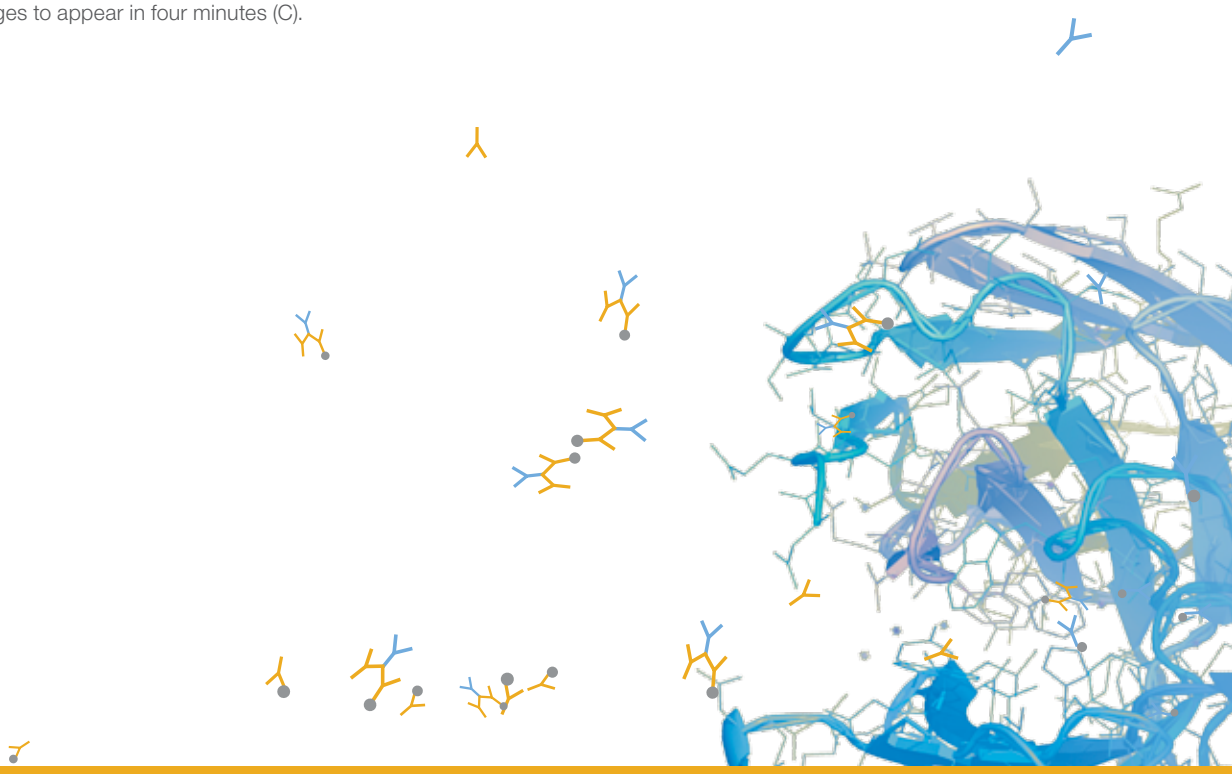


Figure 57. Pierce Background Eliminator erases speckling. Recombinant Human TNFα was electrophoresed on a 4–20% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane. The membrane was blocked and detected with Mouse anti-Human TNFα followed by Goat anti-Mouse-HRP (Cat. No. 31434) and SuperSignal West Dura substrate (Cat. No. 34075). The blot was exposed to film for 30 seconds, resulting in considerable background speckling (A). The film was then treated with Pierce Background Eliminator for two minutes to eliminate the background speckling (B).



Protocol quick reference

iBind Western System

Cat No. SLF1000

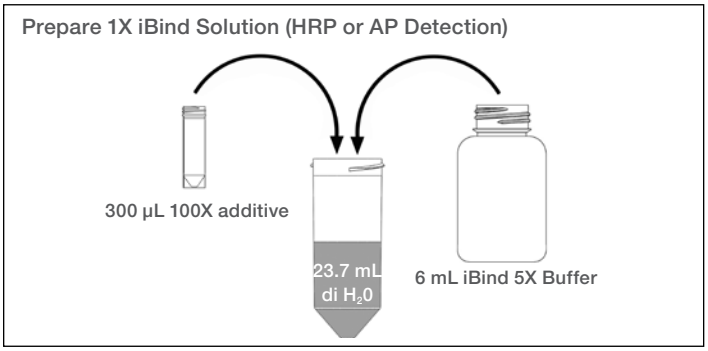
Publication Part No. 25-1075 **Publication No.** MAN0008932 **Rev.** C.0

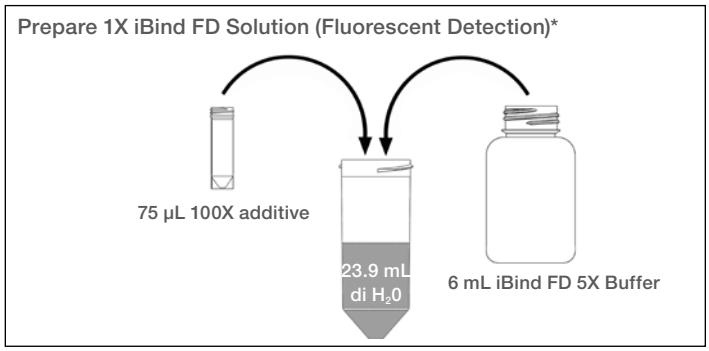
Instructions for using the Invitrogen™ iBind™ Western Device in a Western blot workflow are described below. For detailed instructions and guidance on optimizing results, refer to the manual available from http://www.thermofisher.com/us/en/home/technical-resources.html.

General guidelines

- Store membranes in iBind/iBind FD Solution, in distilled water, or dry.
- If you mark your membrane with ink, mark the membrane near the low molecular weight region.
- **Important!** Make sure that the wells are not positioned over the membrane when the lid of the iBind device is closed.
- Do not move the iBind device or open the lid until the incubation is complete (2.5 hours to overnight).
- Perform the Western detection protocol according to the following steps:
  - Prepare 1X iBind Solution (HRP or AP detection) or 1X iBind FD Solution (fluorescent detection).
  - Prepare membrane (membranes should only be blocked with iBind/iBind FD Solution).
  - Prepare diluted antibody solutions.
  - Perform Western blot procedure and detection.

Prepare solutions





* For the Optional 1X iBind FD Solution, add 300 µL 100X Additive, and 1.5 mL iBind FD 5X Buffer to 28.2 mL distilled water.

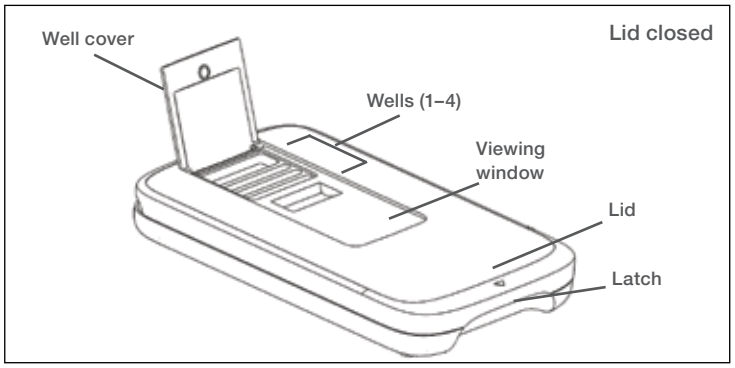
Table 1.1 Prepare primary antibody solutions

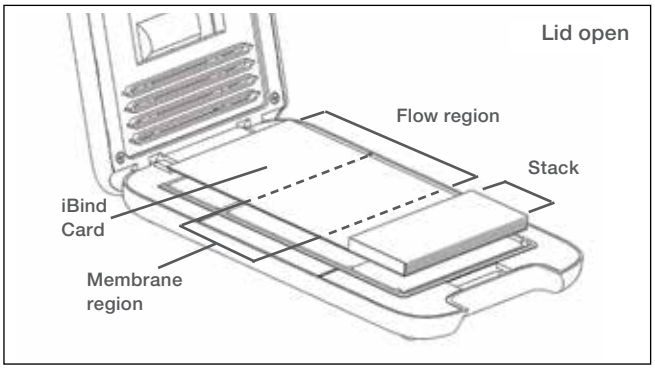
Component	HRP detection
1X iBind Solution	2mL
1° Antibody	Final antibody concentration equal to the manufacturer's recommended dilution.
Component	**AP Detection Kit only**
1X iBind Solution	2mL
1° Antibody	Final antibody concentration equal to the manufacturer's recommended dilution.
Component	**Fluorescent detection**
1X iBind FD Solution	2mL
1° Antibody	Final antibody concentration equal to the manufacturer's recommended dilution.

Table 1.2 Prepare secondary antibody solutions

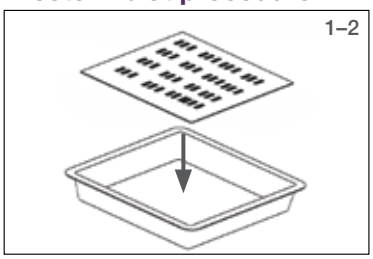
Component	HRP detection
1X iBind Solution	2mL
2° Antibody	Final antibody concentration at 5X the manufacturer's recommended dilution.
Component	**AP Detection Kit only**
1X iBind Solution	2mL
Anti–Mouse 2° Antibody **OR** Anti–Rabbit 2° Antibody	2µL (1:1,000 dilution) **OR** 1µL (1:2,000 dilution)
Component	**Fluorescent detection**
1X iBind FD Solution	2mL
iBind FD 10% SDS	10µL
Alexa Fluor 680 **OR** IRDye 680LT	1µL (1:2,000 dilution) 0.5µL (1:4,000 dilution)
Alexa Fluor 790 **OR** IRDye 800CW	1µL (1:2,000 dilution) 0.67µL (1:3,000 dilution)

Description of parts



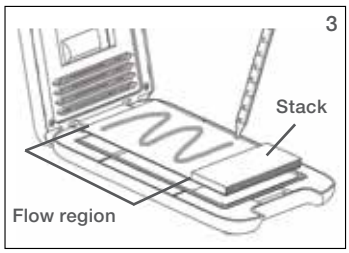


Western blot procedure

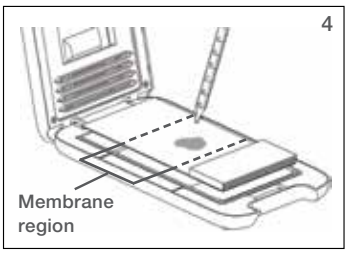


1. Immerse blotted membrane in 5 mL 1X iBind/iBind FD Solution.

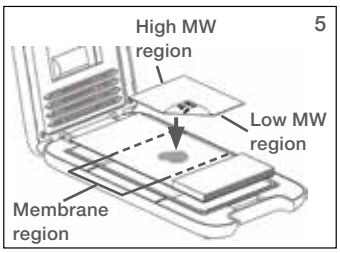
2. Dilute antibodies right after wetting the membrane (refer to Table 1).



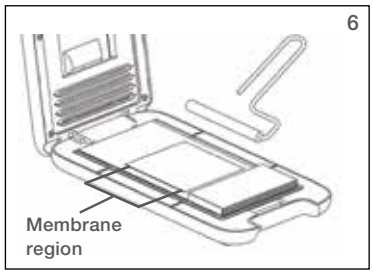
3. Place the iBind Card on the stage and pipette 5mL of 1X iBind/iBind FD Solution evenly across the flow region.
Note: Do not wet the Stack.



4. Add 1mL of 1X iBind/ iBind FD Solution so that it pools at the center of the membrane region on the iBind Card.



5. Place the membrane on top of the pooled solution with the **protein-side down**, and the low molecular weight region closest to the stack.



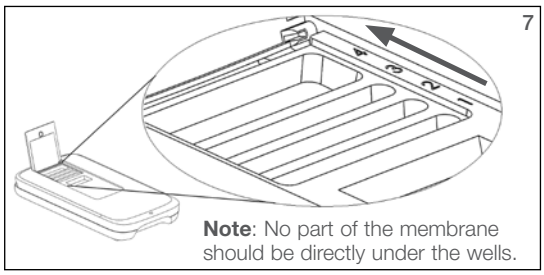


Table 2

⚠ Add solutions in the following order:
Well 1: 2mL diluted 1° antibody
Well 2: 2mL iBind/iBind FD Solution
Well 3: 2mL diluted 2° antibody
Well 4: 6mL iBind/iBind FD Solution

6. Use the Blotting Roller to remove any air bubbles.

7. Close the lid and add solutions sequentially to the iBind Wells starting with well 1 (see Table 2).

8. Place the window cover over the viewing window, and close the well cover. Incubate 2.5 h to overnight.

9. Rinse the membrane in water and proceed to immunodetection protocol.

Maintenance

Rinse the iBind Western Device under running water after each use and allow the device to dry before additional usage.
Store the iBind Western Device with the latch unlocked, and the lid open.

Protocol quick reference

iBind Flex Western System

Catalog No. SLF2000

Publication No. MAN0010926 **Rev.** A.0

Instructions for using the Invitrogen™ iBind™ Flex Western Device in a Western blot workflow are described below. For detailed instructions and guidance on optimizing results, refer to the manual available from http://www.thermofisher.com/us/en/home/technical-resources.html.

General guidelines

- Store membranes in iBind Flex/iBind Flex FD Solution, in distilled water, or dry.
- If you mark your membrane with ink, mark the membrane near the low molecular weight region.
- **Important!** Make sure that the wells are not positioned over the membrane when the lid of the iBind Flex device is closed.
- Do not move the iBind Flex device or open the lid until the incubation is complete (2.5 hours to overnight).
- Select a well insert based on the blot size being processed and place it into the iBind Flex device:
  - **Midi insert** – single midi-sized membrane.
  - **Mini insert** – 1 or 2 mini-sized membranes.
  - **Multi-strip insert** – membranes cut into vertical strips (**Not recommended** for membranes cut into **horizontal strips**).
- Perform the western detection protocol according to the following steps:
  - Prepare solutions (fluorescent detection protocol or HRP or AP detection protocol).
  - Perform western blot procedure and detection.

Prepare solutions

HRP or AP detection

1. Prepare 1X iBind Flex Solution:

Component	Volume
100X Additive	500µL
iBind Flex 5X Buffer	10mL
Distilled Water	39.5mL

2. Immerse blotted membrane in 10 mL 1X iBind Flex Solution.

3. Prepare primary antibody solutions:

Component	Midi blot	Mini blot	Vertical strip
1X iBind Flex Solution	4mL	2mL	0.7mL
1° Antibody	Use final antibody concentration equal to the manufacturer's recommended dilution.		

4. Prepare secondary antibody solutions:

Component	Midi blot	Mini blot	Vertical strip
1X iBind Flex Solution	4mL	2mL	0.7mL
2° Antibody	Use final antibody concentration at 5X the manufacturer's recommended dilution. (e.g. 1:1,000 dilution if 1:5,000 dilution recommended)		

Fluorescent detection

1. Prepare 1X iBind Flex FD Solution*:

Component	Volume
100X Additive	125µL
iBind Flex FD 5X Buffer	10mL
Distilled Water	39.9mL

* If using the Optional 1X iBind Flex FD Solution, add 500µL 100X Additive, and 2.5mL iBind Flex FD 5X Buffer to 47mL distilled water.

2. Immerse blotted membrane in 10 mL 1X iBind Flex FD Solution.

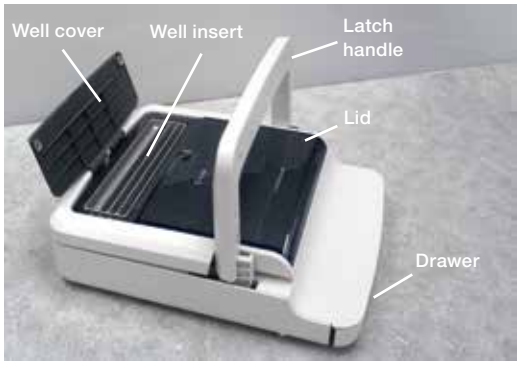
3. Prepare primary antibody solutions:

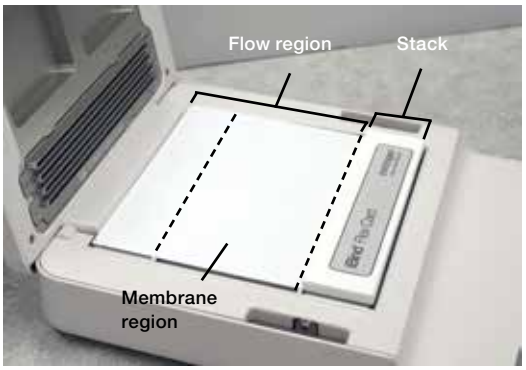
Component	Midi blot	Mini blot	Vertical strip
1X iBind Flex FD Solution	4mL	2mL	0.7mL
1° Antibody	Use final antibody concentration equal to the manufacturer's recommended dilution.		

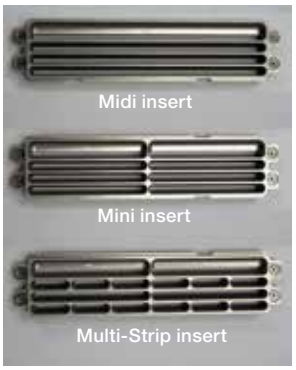
4. Prepare secondary antibody solutions:

Component	Midi blot	Mini blot	Vertical strip
1X iBind Flex FD Solution	4mL	2mL	0.7mL
iBind Flex FD 10% SDS	20µL	10µL	3.5µL
Alexa Fluor 680 **OR** IRDye 680LT	• 2µL (1:2,000 dilution) • 1µL (1:4,000 dilution)	• 1µL (1:2,000 dilution) • 0.5µL (1:4,000 dilution)	• 0.35µL (1:2,000 dilution) • 0.18µL (1:4,000 dilution)
Alexa Fluor 790 **OR** IRDye 800CW	• 2µL (1:2,000 dilution) • 1.3µL (1:3,000 dilution)	• 1µL (1:2,000 dilution) • 0.67µL (1:3,000 dilution)	• 0.35µL (1:2,000 dilution) • 0.23µL (1:3,000 dilution)

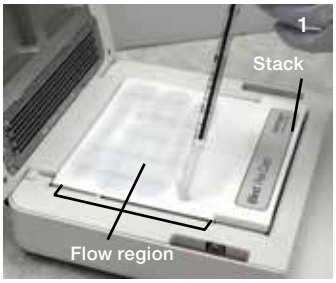
Description of parts



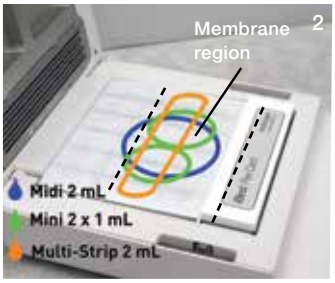




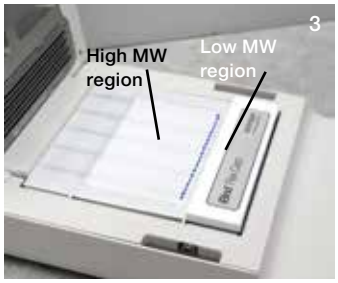
Western blot procedure



1. Place the iBind Flex Card on the stage and pipette 10 mL of 1X iBind Flex/iBind Flex FD Solution across the Flow Region. Lines appear to help align membranes with wells.
Note: Do not wet the Stack.



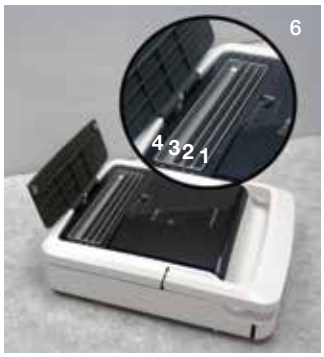
2. Add 1X iBind Flex/iBind Flex FD Solution based on the size of the membrane so that it pools in the indicated regions on the iBind Flex Card.



3. Place the membrane on top of the pooled solution with the **protein-side down**, and the low molecular weight region closest to the stack.

4. Use the Blotting Roller to remove any air bubbles.

5. Close the lid of the iBind Flex device and lower the latch handle to lock the lid.



Note: No part of the membrane should be directly under the wells.

Table 1

Add solutions in the following order:	Volume/well		
	Midi blot	Mini blot	Vertical strip
Row 1: diluted 1° antibody	4mL	2mL	0.7mL
Row 2: iBind Flex/iBind Flex FD Solution	4mL	2mL	2mL
Row 3: diluted 2° antibody	4mL	2mL	0.7mL
Row 4: iBind Flex/iBind Flex FD Solution	12mL	6mL	6mL

6. Add solutions sequentially to each well starting with Row 1 (see Table 1).

7. Close the well cover and record the time for the start of incubation.

8. Incubate 2.5 hr to overnight.

9. Rinse the membrane in water and proceed to immunodetection protocol.

Maintenance

Handle well inserts with care. Rinse the iBind™ Flex well inserts under running water after each use and allow to dry before additional usage. Store inserts in the drawer of the iBind Flex Western Device.
Store the iBind Flex Western Device with the latch unlocked, and the lid not fully closed.

Protocol quick reference

MYECL Imager

Start up

IMPORTANT Review and implement guidelines for proper set-up before installation. Locate the imager away from water, solvents, corrosive materials, strong magnetic fields and vibration sources.

1. Using the appropriate power cord supplied in the accessory pack, connect the imager to an outlet.
2. Switch on the hard power button located on the imager's rear panel (Figure 1).
3. Push the soft power button just below the touch screen.
4. The computer boots up and the touch screen displays the initial message "Welcome to Thermo Scientific MYECL Imager" (Figure 2).
5. The user is then prompted to enter the current date, time and time zone. Upon completion, the screen will automatically move to the default **Chemi** acquire screen (Figure 3).

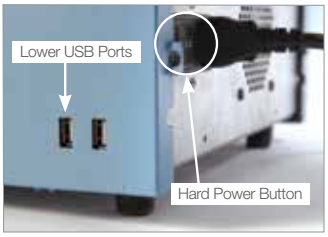


Figure 1. Locations for power center and USB ports.

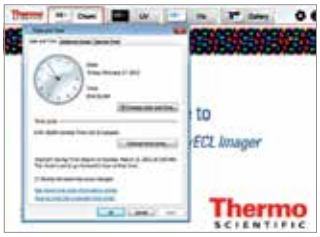


Figure 2. Initial "Welcome" screen with clock setting window open.

Soft Shut Down

The **Soft Shut Down** procedure uses only the front soft power switch.

1. Remove any USB flash drives.
2. Turn off the **soft power switch**.
3. The computer and touch screen shut down and turn off. The fans continue running as needed. The camera cooler remains powered and continues to maintain the camera at the optimal -25°C.
4. The transilluminator and epi-white lights turn off.

Hard Shut Down

The **Hard Shut Down** procedure uses the hard power switch on the rear of the imager. This turns off all power to the imager, including the computer and camera cooler.

1. Follow the **Soft Shut Down** procedure.
2. Turn off the **hard power switch** at the rear of the imager.

Image Information

Information for each captured image is stored within the individual .xml file.

1. With the captured image on the screen, select the **Image Info** icon at the bottom right of the touch screen.
2. A box appears with all of the relevant image information for that image.

Figure 7. Image information input screen.

Beneath the box is a keyboard for inputting personal data into the open fields. Only the fields for **File name**, **User**, **Project** and **Notes** can be added and modified by the user.

3. After the capture of each image, the imager assigns a default name to the image file. This image file name can be altered within the **Acquisition Information** screen.

File management

All image files and image information are saved and exported in individual folders. These folders contain both the .xml file with the image information and the .tif file containing the image.

Figure 8. File management buttons.

Export: The user can select and save highlighted images to a flash drive or external hard drive available through connections to the USB ports.

Note: Users may need to momentarily wait for the imager to detect the external drive once it is connected to the USB port.

Print: The Print button allows the highlighted image to be printed to a selected external printer. A print preview window will appear before printing begins.

Delete: Delete allows the user to delete the highlighted image.

Image gallery

1. Select the Gallery tab at the top of the touch screen.
2. Click on the Browse button to open the gallery.
3. Thumbnail will display previously taken images. Images are separated into sections of weeks, months and years. The user can use the right arrow buttons or the vertical scrollbar to move and view between the sections.
4. Choose an image or series of images by touching the image on the touch screen.

Figure 9. Image gallery with thumbnails.

Note: Before selecting an image, the user can choose to view only the Chemi image or both the Chemi and ChemiV images of the same sample by selecting the **Open ChemiV Image** button at the bottom right of the **Gallery** screen. This function is useful for establishing band locations in a Chemi image against known protein weight markers in a ChemiV image.

Note: Selecting one ChemiM image will open all of the images associated with that specific multiple exposure.

Chemiluminescence (Chemi) Mode

Chemiluminescence (Chemi) Mode is the default acquisition mode. **Chemi** mode is used for the capture of sample images using sample-transmitted light. ChemiV images are automatically captured in **Chemi** mode using reflected visible light.

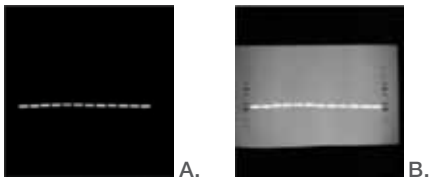
1. Select the **Chemi** tab on the touch screen (Figure 3).
2. (Optional) Select the **Live Camera** button if the live image is not displayed.
3. The epi-white lights and live image display are activated. Open the imager door and install the **Chemiluminescence** exposure screen (black) on the transilluminator glass.
4. Insert and center the sample on top of the chemiluminescence exposure screen (black) using the live image display for reference.
5. Close the imager door.

6. While viewing the live image, adjust the settings for **Focus**, **Digital Zoom** and **Resolution/Sensitivity** in the **Camera Settings** menu.
7. Select a **Preset**, **Custom** or **Multi** exposure time.
   Alternatively, use the **Interactive Chemi** tool to evaluate and optimize imaging exposure time. With Interactive Chemi, the imager acquires a 15-second exposure and displays a projected image based on the calculated optimal exposure time. This calculation takes into account maximum dynamic range and minimum pixel saturation (Figure 4).

Figure 3. Chemi acquire screen.

Figure 4. Interactive Chemi Screen.

8. **ChemiV Overlay:** After capturing an image in **Chemi** mode, superimpose that image onto the corresponding ChemiV image by selecting the **ChemiV Overlay** button. The ChemiV Overlay button is enabled only when user is viewing a **Chemi** image (Figure 5).



A. B.

Figure 5. Original Chemi image (A) and ChemiV Overlay (B).

Ultraviolet (UV) mode

Ultraviolet (UV) mode **is** used for the capture of images using transmitted UV light.

CAUTION UV mode uses the UV lights in the transilluminator. Long-term exposure to UV light can be harmful. Use the appropriate eye and skin PPE (personal protective equipment) if there is any chance of exposure to the UV light.

Note: If the UV light is needed after opening the imager door, the **white door peg** can be pulled out to turn on the UV bulbs. **Appropriate PPE must be worn if performing this operation.** The white door peg returns to a normal position once the door is fully closed.

1. Select the UV tab on the touch screen.
2. (Optional) Enable the UV button and select the **Live Camera** button if the live image is not displayed.
3. Open the imager door and confirm that the UV lights are off. The chamber LED lights are activated once the door is opened. Place the clear sample screen on top of the transilluminator.
4. Insert and center the sample on top of the clear sample screen using the live image display for reference. Once the sample is centered, close the imager door.
5. While viewing the live image, adjust the necessary settings for **Focus**, **Digital Zoom**, **Sensitivity/Resolution**, **Aperture** and **Filter** to produce the desired image.
   Note: Camera Settings are available only with the live image display.
6. Select a Preset or Custom exposure time.

Extended “UV on” position
White door peg in extended “UV on” position.

Retracted “UV off” position
White door peg in retracted “UV off” position (standard position).

Figure 6. UV white door peg in “on” and “off” positions.

Note: Images are captured and saved to the **Gallery**; however, they are not seen in the image tab if **Live Camera** is enabled.

Visible (Vis) mode

Visible (Vis) mode is used for the capture of gel images using reflected and/or transmitted light.

CAUTION Vis mode uses the UV lights in the transilluminator. Long-term exposure to UV light can be harmful. Use the appropriate eye and skin PPE if there is any chance of exposure to the UV light.

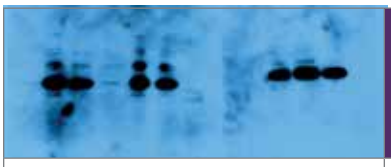
Vis mode uses the same functional camera settings as the Chemi mode, but adds the instrument setting functions for **Aperture**, **UV Light** and **Filter Wheel Position**.

1. Select the **Vis** tab on the touch screen. Changing to **Vis** mode automatically turns on the UV lights within the transilluminator.
2. (Optional) Select the **Live Camera** button and enable the UV button if the live image is not displayed.
3. Open the imager door, confirm that the UV lights are off and install the white light conversion screen on top of the transilluminator glass.
4. Insert and center the sample on top of the white light conversion screen using the live image display for reference.
5. Once the sample is centered, close the Imager door. The UV lights will turn on and show as visible light transmitting through the white sample screen.
6. While viewing the live image, adjust the necessary settings for **Focus**, **Digital Zoom**, **Sensitivity/Resolution**, **Aperture** and **Filter** to produce the desired image.

Troubleshooting

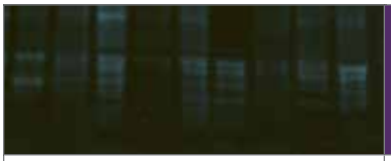
High background that is uniformly distributed

Possible causes	Precautions/Solutions
Antibody concentrations are too high	• High concentrations of primary and/or secondary antibody can cause high background. • Decrease antibody concentrations.
Incompatible blocking buffer was used	• Optimize blocking buffer. The best blocking buffer is system-dependent. • Increase the concentration of protein in the blocking buffer. • Optimize blocking time and/or temperature. Block for at least one hour at room temperature or overnight at 4°C. • Add 0.05% Tween-20 Detergent to the blocking buffer at a final concentration of 0.05%. This is not applicable to StartingBlock T20 Blocking Buffer in PBS or TBS or SuperBlock T20 Blocking Buffer in PBS or TBS. • Prepare antibody dilutions in blocking buffer that contains 0.05% Tween-20 Detergent.
Cross-reactivity of antibody with other proteins in blocking buffer	• Use blocking buffers that contain no proteins of plant or animal origin, for example Pierce Protein-Free Blocking buffers. • Do not use milk with avidin-biotin systems. Milk contains biotin. • Test for cross-reactivity. Block a clean piece of membrane, incubate with antibodies and then detect with SuperSignal Chemiluminescent Substrate. • Reduce the concentration of the HRP conjugate.
Insufficient washing	• Increase number of washes and the volume of buffer used. • Add Tween-20 Detergent to wash buffer at a final concentration of 0.05%. (Note: If the concentration of Tween-20 Detergent is too high, it can strip proteins off the membrane.) Skip this step if you use StartingBlock T20 Blocking Buffer in PBS or TBS or SuperBlock T20 Blocking Buffer in PBS or TBS.
Exposure time is too long	• Reduce the time the blot is exposed to film.
Membrane problems	• Wet membranes thoroughly according to the manufacturer's instructions. • Use new membranes. • Cover the membrane with liquid at all times to prevent it from drying. • Use agitation during all incubations. • Handle membranes carefully – damage to the membrane can cause nonspecific binding. • Do not handle membrane with bare hands. Always wear clean gloves or use forceps.
Contamination or growth in buffers	• Prepare new buffers.



High background that is blotchy or speckled

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

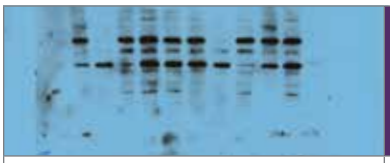


Black blots with white bands or signal that decreases quickly

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

Weak signal or no signal

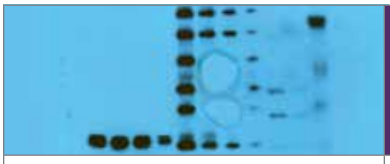
Possible causes	Precautions/Solutions
Proteins did not transfer properly to the membrane	• After transfer, stain the gel with a total protein stain to determine transfer efficiency. (Note: Total protein stains may not be able to detect low quantities of antigen.) • Use Thermo Scientific™ Pierce™ Reversible Membrane Stain to check membrane for transfer efficiency. • Ensure sufficient contact between the gel and membrane during transfer. • Make sure the transfer sandwich is assembled correctly. • Wet membrane according to manufacturer's instructions. • Make sure transfer unit does not overheat during electroblotting procedure. • Use positive control and/or molecular weight markers. • Optimize transfer time and current. • Use Thermo Scientific™ Pierce™ Lane Marker Sample Buffer. The tracking dye transfers to the membrane. • Make sure sample preparation conditions have not destroyed antigenicity of the sample. (Note: Some proteins cannot be run under reducing conditions.)
Insufficient binding to membrane	• Add 20% methanol to the transfer buffer to help binding. Low MW antigen may pass through the membrane. Use a membrane with a smaller pore size.
Insufficient amount of antibodies	• Increase antibody concentrations. Antibody may have poor affinity for the target protein. • Antibody may have lost activity. Perform a dot blot to determine activity.
Antibody concentrations are too high	• Using too much primary or secondary antibodies can cause the signal to fade quickly.
Insufficient amount of antigen present	• Load more protein onto the gel.
The antigen is masked by the blocking buffer	• Try different blocking buffers. • Optimize blocking buffer protein concentration.
Buffers contain sodium azide	• Do not use sodium azide, an inhibitor of HRP, as a preservative in buffers.
Exposure time is too short	• Lengthen the film exposure time.
Substrate incubation is too short	• SuperSignal substrates require a 5-minute substrate incubation.
Inactive substrate	• SuperSignal West Pico Chemiluminescent Substrate and SuperSignal West Dura Extended Duration Substrate are stable for up to 12 months at room temperature. SuperSignal West Femto Maximum Sensitivity Substrate is stable for at least six months at room temperature. • To evaluate the substrate activity, prepare a small amount of working solution. In a darkroom, add a small amount of HRP conjugate. A blue light should be observed. If no glow is observed, either the substrate or the HRP conjugate is inactive. • Ensure there is no cross-contamination between the two bottles of substrate, which can cause a decline in activity.
Membrane has been stripped and reprobed	• Optimize stripping procedure to prevent any loss of antigen or denaturation. • Reprobe only when necessary. • Avoid repeated reprobing of the same membrane.
Digestion of antigen on the membrane	• Blocking substance may have proteolytic activity (e.g., gelatin).
Protein degradation from blot storage	• Prepare a new blot.



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

Diffuse bands

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



Partly developed area or blank areas

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

Ordering information

Product	Quantity	Cat. No.
Block, wash, probe		
Automated		
iBind Flex Western Starter Kit	1 kit	SLF2000S
iBind Flex Western Device	1 device	SLF2000
iBind Flex Cards	10 cards	SLF2010
iBind Flex 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 FD Solution Kit	1 kit	SLF1019
iBind Solution Kit	1 kit	SLF1020
Blocking solutions		
Membrane Blocking Solution	1L	00-0105
WesternBreeze Blocker/Diluent (Part A and B)	80mL	WB7050
Blocker BLOTTO in TBS	1L	37530
Blocker BSA in PBS (10X)	200mL	37525
Blocker BSA in TBS (10X)	125mL	37520
Blocker Casein in PBS	1L	37528
Blocker Casein in PBS	100mL	37582
Blocker Casein in TBS	1L	37532
Pierce Clear Milk Blocking Buffer (10X)	100mL	37587
Pierce Fast Blocking Buffer	500mL	37575
Pierce Fast Blocking Buffer	100mL	37576
Blocker Casein in TBS	100mL	37583
Pierce Protein-Free (PBS) Blocking Buffer	1L	37572
Pierce Protein-Free (PBS) Blocking Buffer	100mL	37584

Product	Quantity	Cat. No.
Pierce Protein-Free (TBS) Blocking Buffer	1L	37570
Pierce Protein-Free (TBS) Blocking Buffer	100mL	37585
Pierce Protein-Free T20 (PBS) Blocking Buffer	1L	37573
Pierce Protein-Free T20 (TBS) Blocking Buffer	1L	37571
SEA BLOCK Blocking Buffer	500mL	37527
SEA BLOCK Blocking Buffer	3 x 500mL	37527X3
StartingBlock (PBS) Blocking Buffer	1L	37538
StartingBlock (PBS) Blocking Buffer	100mL	37578
StartingBlock (TBS) Blocking Buffer	1L	37542
StartingBlock (TBS) Blocking Buffer	100mL	37579
StartingBlock T20 (PBS) Blocking Buffer	1L	37539
StartingBlock T20 (TBS) Blocking Buffer	1L	37543
SuperBlock (PBS) Blocking Buffer	1L	37515
SuperBlock (PBS) Blocking Buffer	5L	37518
SuperBlock (PBS) Blocking Buffer	100mL	37580
SuperBlock (PBS) Blocking Buffer - Blotting	1L	37517
SuperBlock (TBS) Blocking Buffer	1L	37535
SuperBlock (TBS) Blocking Buffer	100mL	37581
SuperBlock (TBS) Blocking Buffer - Blotting	1L	37537
SuperBlock (TBS) Blocking Buffer Dry Blend	5 packs	37545
SuperBlock T20 (PBS) Blocking Buffer	1L	37516
SuperBlock T20 (TBS) Blocking Buffer	1L	37536
I-Block Protein-Based Blocking Reagent	30g	T2015

Ordering information

Product	Quantity	Cat. No.
Secondary Abs and solutions		
Stabilized Goat Anti-Mouse IgG (H+L), Peroxidase Conjugated (10µg/mL)	2mL	32430
Stabilized Goat Anti-Rabbit IgG (H+L), Peroxidase Conjugated (10µg/mL)	2mL	32460
Pierce High Sensitivity Streptavidin-HRP (1mg/mL)	0.5mL	21130
Pierce High Sensitivity Streptavidin-HRP (1mg/mL)	5mL	21132
Pierce High Sensitivity Streptavidin-HRP, pre-diluted (10µg/mL)	2mL	21134
Pierce High Sensitivity NeutrAvidin-HRP (1mg/mL)	0.5mL	31030
Pierce High Sensitivity NeutrAvidin-HRP	5mL	31032
Goat Anti-Mouse IgG (H+L), Peroxidase Conjugated	2.0mL	31430
Goat Anti-Rabbit IgG (H+L), Peroxidase Conjugated	2.0mL	31460
Pierce Goat Anti-Mouse Poly-HRP (0.5mg/mL)	0.5mL	32230
Pierce Goat Anti-Rabbit Poly-HRP (0.5mg/mL)	0.5mL	32260
Ethylene Glycol (50% solution)	200mL	29810
Pierce Peroxidase Conjugate Stabilizer	25mL	31503
Guardian Peroxidase Conjugate Stabilizer/Diluent	200mL	37548
Guardian Peroxidase Conjugate Stabilizer/Diluent	1L	37552
Protein Stabilizing Cocktail	10mL	89806
HRP-Conjugate Diluent/Stabilizer	1L	00-2018
Pierce Stable Peroxide Substrate Buffer (10X)	100mL	34062
Pierce Peroxidase Conjugate Stabilizer	25mL	31503
Poly-HRP Dilution Buffer	1L	N501
Poly-HRP Dilution Buffer	100mL	N500

Product	Quantity	Cat. No.
Wash buffers		
Pierce Fast Wash Buffer, 10X	1L	37577
Wash Buffer (25X)	100mL	WB01
Wash Buffer (25X)	1L	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
PBS (Phosphate-Buffered Saline) Powder	12 x 1L	00-3000
PBS (Phosphate-Buffered Saline) Tablets	100 tablets	00-3002
PBS (Phosphate-Buffered Saline) (10X) pH 7.4	500mL	AM9624
PBS (Phosphate-Buffered Saline) (10X) pH 7.4	1L	AM9625
Pierce 20X PBS Tween-20 Buffer	500mL	28352
Pierce 20X Phosphate Buffered Saline	500mL	28348
Pierce 20X TBS Buffer	500mL	28358
Pierce 20X TBS Tween-20 Buffer	500mL	28360
TBS Buffer	1 pack	R017R.0000
WesternBreeze Wash Solution (16X)	2 x 100mL	WB7003

Ordering information

Product	Quantity	Cat. No.
Chromogenic HRP substrates		
1-Step ABTS Substrate Solution	250mL	37615
1-Step Chloronaphthol Substrate Solution	250mL	34012
1-Step TMB-Blotting Substrate Solution	250mL	34018
1-Step Ultra TMB-Blotting Solution	250mL	37574
ABTS Substrate Tablets	50 tablets	34026
ABTS, powder	6 x 1mL	00-2001
ABTS, solution	500mL	00-2024
Chloronaphthol Substrate Powder (4-chloro-1-naphthol)	25g	34010
DAB Substrate Powder (3,3'-diaminobenzidine tetrahydrochloride)	10g	34001
DAB-Plus Reagent Set	12mL	00-2020
Novex HRP Chromogenic Substrate (TMB)	250mL	WP20004
Pierce CN/DAB Substrate Kit	275mL	34000
Pierce DAB Substrate Kit	275mL	34002
Stable DAB	250mL	750118
TMB	60mL	7588925
TMB	500mL	7588927
TMB Solution (BLOT)	500mL	00-2019
TMB Stabilized Chromagen	25mL	SB01
TMB Stabilized Chromagen	1L	SB02
TMB Substrate Solution	100mL	N301
Chromogenic AP substrates		
1-Step NBT/BCIP Substrate Solution	250mL	34042
1-Step NBT/BCIP plus Suppressor Substrate Solution	100mL	34070
BCIP Substrate Powder (5-bromo-4-chloro-3'-indolyphosphate p-toluidine)	1g	34040
BCIP-T	1g	R0821
BCIP-T	5g	R0822
BCIP/NBT KIT	1,000mL	00-2209
NBT	1g	R0841
NBT	5g	R0842
NBT Substrate Powder (nitro-blue tetrazolium chloride)	1g	34035
NBT/BCIP Reagent Kit	1 kit	N-6547
Novex AP Chromogenic Substrate (BCIP/NBT)	250mL	WP20001

Product	Quantity	Cat. No.
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 125mL	WP20005
Pierce ECL Plus Western Blotting Substrate	100mL	32132
Pierce ECL Plus Western Blotting Substrate	300mL	32132X3
Pierce ECL Plus Western Blotting Substrate	25mL	32134
Pierce ECL Western Blotting Substrate	500mL	32106
Pierce ECL Western Blotting Substrate	50mL	32109
Pierce ECL Western Blotting Substrate	250mL	32209
SuperSignal West Dura Extended Duration Substrate	100mL	34075
SuperSignal West Dura Extended Duration Substrate	200mL	34076
SuperSignal West Dura Substrate Trial Kit	20mL	37071
SuperSignal West Femto Maximum Sensitivity Substrate	100mL	34095
SuperSignal West Femto Maximum Sensitivity Substrate	200mL	34096
SuperSignal West Femto Substrate Trial Kit	20mL	34094
SuperSignal West Pico Chemiluminescent Substrate	100mL	34077
SuperSignal West Pico Chemiluminescent Substrate	1L	34078
SuperSignal West Pico Chemiluminescent Substrate	500mL	34080
SuperSignal West Pico Chemiluminescent Substrate	200mL	34087
SuperSignal West Pico Trial Kit	50mL	34079
SuperSignal West Pico Complete Mouse IgG Detection Kit	1.5L	34081
SuperSignal West Pico Complete Rabbit IgG Detection Kit	1.5L	34084
SuperSignal West Pico Mouse IgG Detection Kit	500mL	34082
SuperSignal West Pico Rabbit IgG Detection Kit	500mL	34083

Ordering information

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)	50mL	**T2145**
CDP-Star Substrate (0.25 mM Ready-To-Use)	100mL	**T2146**
CDP-Star Substrate (0.25 mM Ready-To-Use)	250mL	**T2147**
CDP-Star Substrate (0.25 mM Ready-To-Use) with Nitro-Block-II Enhancer	100mL	**T2218**
CDP-Star Substrate (0.4 mM Ready-To-Use) with Emerald-II Enhancer, Size A	100mL	**T2216**
CDP-Star Substrate (0.4 mM Ready-To-Use) with Sapphire-II Enhancer, Size A	100mL	**T2214**
CDP-Star Substrate (12.5 mM Concentrate)	1mL	**T2304**
CDP-Star Substrate (12.5 mM Concentrate)	2mL	**T2305**
CDP-Star Substrate (12.5 mM Concentrate)	5mL	**T2306**
CDP-Star Substrate (12.5 mM Concentrate)	10mL	**T2307**
CDP-Star Substrate (12.5 mM Concentrate)	20mL	**T2308**
CDP-Star Substrate (12.5 mM Concentrate)	50mL	**T2309**
CDP-Star Substrate (12.5 mM Concentrate)	200mL	**T2310**
CSPD Substrate (0.25 mM Ready-To-Use)	50mL	**T2141**

Product	Quantity	Cat. No.
CSPD Substrate (0.25 mM Ready-To-Use)	100mL	**T2142**
CSPD Substrate (0.25 mM Ready-To-Use)	250mL	**T2143**
CSPD Substrate (0.25 mM Ready-To-Use) with Nitro-Block Enhancer	100mL	**T2217**
CSPD Substrate (0.4 mM Ready-To-Use) with Emerald-II Enhancer, Size A	100mL	**T2212**
CSPD Substrate (0.4 mM Ready-To-Use) with Sapphire-II Enhancer, Size B	100mL	**T2210**
CSPD Substrate (25 mM Concentrate)	0.5mL	**T2040**
CSPD Substrate (25 mM Concentrate)	2mL	**T2041**
CSPD Substrate (25 mM Concentrate)	5mL	**T2042**
CSPD Substrate (25 mM Concentrate)	10mL	**T2043**
CSPD Substrate (25 mM Concentrate)	25mL	**T2044**
CSPD Substrate (25 mM Concentrate)	1mL	**T2098**
CSPD Substrate (25 mM Concentrate)	100mL	**T2138**
Luminescence Enhancer For Membrane Blotting Assays, Nitro-Block Enhancer	20mL	**T2026**
Luminescence Enhancer For Membrane Blotting Assays, Nitro-Block-II Enhancer	20mL	**T2184**
Novex AP Chemiluminescent Substrate	100mL	**WP20002**
Novex AP Chemiluminescent Substrate Nitro-Block-II Enhancer	5mL	**WP20003**
Novex AP Mouse Chemiluminescent Detection Kit	1 kit	**SLF1021**
Novex AP Rabbit Chemiluminescent Detection Kit	1 kit	**SLF1022**
Western-Star Immunodetection System with Goat Anti-Mouse IgG + IgM AP conjugate	30 blots	**T1046**
Western-Star Immunodetection System with Goat Anti-Rabbit IgG AP conjugate	30 blots	**T1048**

Ordering information

Product	Quantity	Cat. No.
Chemiluminescent detection instruments		
MYECL Imager	1 unit	62236
Blue Filter for MYECL Imager	1 filter	62241
Chemiluminescence Exposure Screen for MYECL Imager	1 screen	62242
Green Filter for MYECL Imager	1 filter	62239
Imaging Reference Target for MYECL Imager	1 target	62267
Orange Filter for MYECL Imager	1 filter	62238
Red Filter for MYECL Imager	1 filter	62240
Touch screen Stylus and Holder for MYECL Imager	1 set	62261
UV Exposure Screen for MYECL Imager	1 screen	62244
UV Transilluminator Bulb, 306 nm, for MYECL Imager	1 bulb	62258
UV Transilluminator Bulb, 365 nm, for MYECL Imager	1 bulb	62255
White Light Conversion Screen for MYECL Imager	1 screen	62243
Power Cord with C/13 Connector, Australia	1 cord	84857
Power Cord with C/13 Connector, China	1 cord	84855
Power Cord with C/13 Connector, Continental Europe	1 cord	84859
Power Cord with C/13 Connector, Japan	1 cord	84856
Power Cord with C/13 Connector, North America	1 cord	84858
Power Cord with C/13 Connector, United Kingdom	1 cord	84860
X-ray film		
CL-XPosure Film, 14 x 17 in. (35 x 43cm)	100 sheets	34099
CL-XPosure Film, 5 x 7 in. (12.5 x 17.5cm)	50 sheets	34088
CL-XPosure Film, 5 x 7 in. (13 x 18cm)	100 sheets	34090
CL-XPosure Film, 7 x 9.5 in. (18 x 24cm)	100 sheets	34089
CL-XPosure Film, 8 x 10 in. (20 x 25cm)	100 sheets	34091
CL-XPosure Film, 8 x 10 in. (20 x 25cm)	50 sheets	34093
CL-XPosure Film, 9.5 x 11.8 in. (24 x 30cm)	100 sheets	34097

Product	Quantity	Cat. No.
Fluorescent detection		
iBind Fluorescent Detection (FD) Solution Kit	1 kit	SLF1019
Fluorescent instruments		
E-Gel Imager System with E-Gel Adaptor	1 each	4466613
E-Gel Imager System with Blue Light Base	1 each	4466612
E-Gel Imager System with UV Light Base	1 each	4466611
E-Gel Imager Adaptor Base	1 each	4466604
E-Gel Imager Blue Light Base	1 each	4466603
E-Gel Imager UV Light Base	1 each	4466602
E-Gel Imager Universal Filter	1 each	4466606
E-Gel Imager Qdot™ 625 Filter	1 each	4466607
E-Gel Imager UV/SYBR Filter	1 each	4466608
E-Gel Imager Quantitation USB Dongle	1 each	4466610
E-Gel Imager Band Excision Kit	1 each	4466605
E-Gel Imager White-Light Conversion Screen	1 each	4473061

Ordering information

Product	Quantity	Cat. No.
Specialty Western kits		
Pierce Far-Western Blot Kit for Biotinylated Proteins	220mL	23500
Pierce Fast Western Blot Kit, ECL Substrate, Mouse	1L	35050
Pierce Fast Western Blot Kit, ECL Substrate, Mouse	200mL	35055
Pierce Fast Western Blot Kits, SuperSignal West Dura, Mouse	400mL	35070
Pierce Fast Western Blot Kits, SuperSignal West Dura, Mouse	80mL	35075
Pierce Fast Western Blot Kits, SuperSignal West Dura, Rabbit	400mL	35071
Pierce Fast Western Blot Kits, SuperSignal West Dura, Rabbit	80mL	35076
Pierce Fast Western Blot Kits, SuperSignal West Femto, Mouse	400mL	35080
Pierce Fast Western Blot Kits, SuperSignal West Femto, Mouse	80mL	35085
Pierce Fast Western Blot Kits, SuperSignal West Femto, Rabbit	400mL	35081
Pierce Fast Western Blot Kits, SuperSignal West Femto, Rabbit	80mL	35086
Pierce Fast Western Kit, SuperSignal West Pico, Mouse	800mL	35060
Pierce Fast Western Kit, SuperSignal West Pico, Mouse	200mL	35065
Pierce Fast Western Kit, SuperSignal West Pico, Rabbit	800mL	35061
Pierce Fast Western Kit, SuperSignal West Pico, Rabbit	200mL	35066
O-GlcNAc Western Blot Detection Kit	255mL	24565
Pierce S-Nitrosylation Western Blot Kit	40 reactions	90105
HENS Buffer for Pierce S-Nitrosylation Western Blot Kit	100mL	90106
SuperSignal West HisProbe Kit	685mL	15168
HisProbe-HRP Conjugate	2mg	15165
Clean-Blot IP Detection Kit (HRP)	25 blots	21232
Clean-Blot IP Detection Reagent (HRP)	2.5mL	21230

Product	Quantity	Cat. No.
Strip and reprobe		
Restore Fluorescent Western Blot Stripping Buffer	20mL	62299
Restore Fluorescent Western Blot Stripping Buffer	100mL	62300
Restore PLUS Western Blot Stripping Buffer	30mL	46428
Restore PLUS Western Blot Stripping Buffer	500mL	46430
Restore Western Blot Stripping Buffer	500mL	21059
Restore Western Blot Stripping Buffer	5L	21063
Restore Western Blot Stripping Buffer, Trial Size	30mL	21062
Pierce Background Eliminator Kit	200mL	21065

Western blot detection word search challenge

To participate in the word find challenge, go to **thermofisher.com/detectwordsearch**

D	A	Y	F	B	X	C	S	W	E	X	C	X	P	R	W	D	U
E	U	A	P	L	S	O	U	U	B	R	E	S	T	O	R	E	Y
T	I	E	E	S	U	L	S	A	P	L	P	N	G	C	T	X	S
E	B	A	R	P	B	O	T	L	N	E	O	N	Q	Z	C	I	K
R	I	G	O	X	S	R	R	X	U	T	R	C	X	L	L	M	X
G	N	U	X	P	T	I	I	E	R	M	I	S	K	K	O	A	V
E	D	B	I	Q	R	M	P	U	S	A	I	B	I	E	G	G	G
N	F	U	D	C	A	E	P	X	G	C	Y	N	O	G	R	E	N
T	L	H	A	P	T	T	I	Q	M	U	E	I	O	D	N	R	G
Y	E	U	S	M	E	R	N	C	T	O	W	N	S	L	Y	A	Y
E	X	F	E	U	L	I	G	B	X	Y	A	S	T	Q	O	A	L
F	W	V	C	V	V	C	H	X	H	R	C	O	H	H	E	W	I

Find the following words in the puzzle. Words are hidden →, ↓ and ↘.

ANTIBODY
BLOCKER
COLORIMETRIC
DETERGENT
FLUORESCENT
IBINDFLEX
IMAGER
LUMINOL
PEROXIDASE
RESTORE
STRIPPING
SUBSTRATE
SUPERSIGNAL
XRAY

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