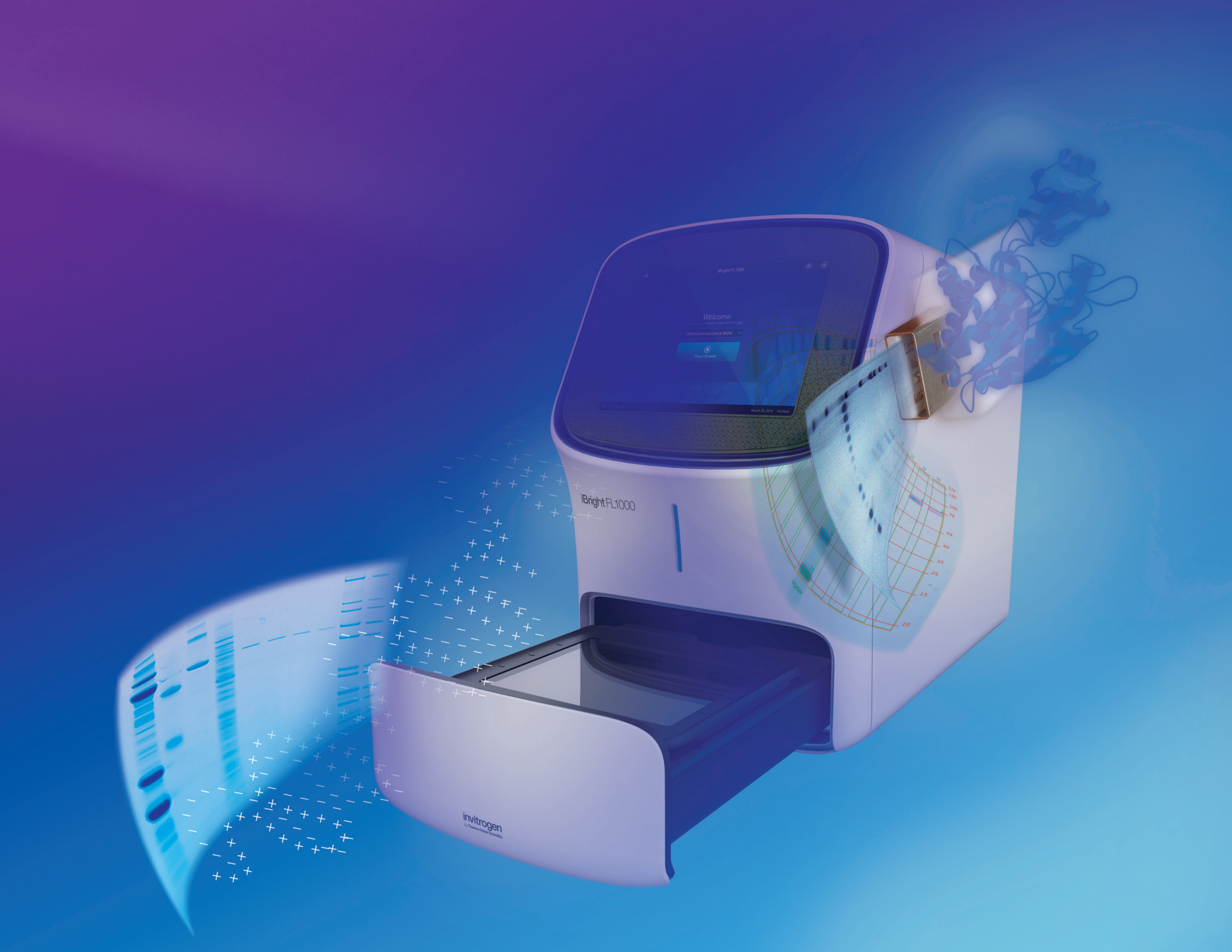
western blotting

thermoscientific invitrogen

Complete, innovative western workflow solutions

ThermoFisher SCIENTIFIC


iBright FL1000
Welcome
Chemiluminescence blots
Open Drawer
iBright FL1000
invitrogen

Contents

Separate. Transfer. Detect.

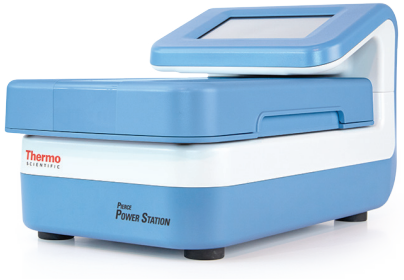
Forget your past western blotting frustrations. Now you can get the tools designed to improve the quality of your western blot data while simultaneously reducing your time and effort. For each of the three steps of the western workflow, we offer high-performance tools and technologies to make the process quick and easy.

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

Separate

- **Invitrogen™ Mini Gel Tank** for convenient electrophoresis: a versatile tank compatible with more than 180 gels, with innovative side-by-side design for clear visualization and faster sample loading
- **Invitrogen™ Bolt™ and NuPAGE™ Bis-Tris Gels** for optimal separation of a broad molecular weight range of proteins under denaturing conditions: offers preserved protein integrity with a neutral-pH buffering system
- **Invitrogen™ Novex™ Tris-Glycine Mini Gels (WedgeWell™ format)** based on traditional Laemmli chemistry and enabling sample load volumes of up to 60 µL
- **Invitrogen™ SureCast™ Gel Handcast System** for protein gel casting: 100% leak-free,* with glass plates that are up to 20 times more durable compared to other suppliers' plates**

  * Restrictions apply. For full details, go to **thermofisher.com/surecastterms**
  ** Based on internal testing.
- **Thermo Scientific™ Pierce™ Power Stainer** for rapid, electrophoretic Coomassie staining of proteins in polyacrylamide gels, typically in 6–11 minutes
- **Protein ladders** available in a broad range of prestained and unstained ready-to-use formats for consistent band migration and intensity during gel electrophoresis and western blotting



Transfer

- **Invitrogen™ Mini Blot Module** for seamless transfer in the Mini Gel Tank: requires less methanol-based transfer buffer than other commercially available transfer systems
- **Thermo Scientific™ Pierce™ Power Blotter** designed specifically for rapid semi-dry transfer of 10–300 kDa proteins from polyacrylamide gels to nitrocellulose or PVDF membranes, typically in 5–10 minutes
- **Invitrogen™ iBlot™ 2 Dry Blotting System** for self-contained, reproducible, and flexible gel transfer in only 7 minutes: compatible with multiple gel chemistries, membrane types, and gel sizes

Detect

- **Invitrogen™ iBind™ or iBind™ Flex Western System** for automated western processing: requires no power source or battery. Just load your solutions and allow the sequential lateral flow technology to work for you
- **Invitrogen™ primary and secondary antibodies** for reproducible western blot analysis: purchase with confidence knowing we stand behind the quality of our antibodies with the Invitrogen™ Antibody Performance Guarantee*
- **Chemiluminescent HRP substrates** for excellent performance in western blotting: Thermo Scientific™ Pierce™ ECL, Pierce™ ECL Plus, and SuperSignal™ chemiluminescent substrates enable high sensitivity, long signal duration, strong signal intensity, and low background
- **Invitrogen™ iBright™ Imaging Systems** for stunningly easy gel and western blot imaging

* Terms and conditions apply; for complete details go to thermofisher.com/antibody-performance-guarantee

Separate

The first step of the western workflow process is the separation of proteins. We offer several options for protein separation, including precast gels, reagents, and accessories for pour-your-own gels, ladders, electrophoresis gel tanks, and power supplies.

For a complete listing of all available protein gel electrophoresis products, visit **thermofisher.com/separate**

Precast gels

Precast gels offer convenience, speed, and consistency. We offer precast gels in four different chemistries and a wide variety of percentages, gradients, and sample well configurations. The choice of whether to use one chemistry or another depends on the abundance of the protein you're separating, the size of the protein, and your downstream application.

Four gel chemistry options to fit your protein separation needs:

- Bis-Tris gel chemistry for broad-range, low-abundance protein separation or for downstream applications requiring high protein integrity, such as posttranslational modification analysis, mass spectrometry, or sequencing
- Tris-glycine gel chemistry for broad-range, high-abundance protein separation
- Tris-acetate gel chemistry for high molecular weight protein separation, up to 500 kDa
- Tricine gel chemistry for low molecular weight protein and peptide separation

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

Pour-your-own gels

The **SureCast Gel Handcast System** is designed for 100% leak-free protein gel casting. The SureCast system is fully compatible with our Mini Gel Tank.

Benefits offered by the SureCast Gel Handcast System include:

- **Leak-free design**—gels that are more usable, less wasted time
- **Superior glass plate durability**—up to 20 times more durable compared to other suppliers' plates
- **Unique tilt feature**—helps minimize spillage when pouring acrylamide solutions
- **Simple assembly of casting components**—uses a single-motion, load-and-lock mechanism

Use Invitrogen™ SureCast™ Handcast Reagents as well as other popular polyacrylamide gel casting reagents.

SureCast Handcast Reagents
SureCast Stacking Buffer and Resolving Buffer

Invitrogen™ SureCast™ Stacking Buffer and Resolving Buffer are pouches of dry-blend powder, each sufficient to make 500 mL of stacking or resolving buffer.

Benefits include:

- **Convenient pouches of dry-blend powder**—dissolve contents of a single packet in water and the buffer is ready to use
- **Time- and space-saving**—no weighing, no calculations, no pH adjustment, and no need to stock individual components
- **Long shelf life**—stocking and storage as dry powder eliminates concerns about long-term stability of stock solutions

Invitrogen™ SureCast™ Acrylamide Solution, 40%

SureCast Acrylamide Solution can be used to prepare single-percentage and gradient gels using the SureCast Gel Handcast System or other handcast systems.

Features include:

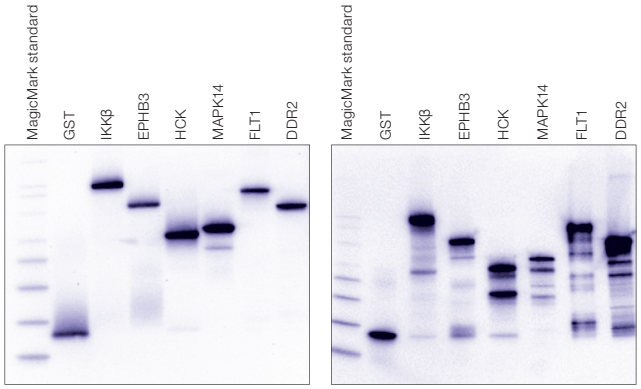
- Room-temperature storage
- Long shelf life
- High purity
- Safer alternative to powdered acrylamide
- Concentrated to enable a broader range of gel percentages to cast

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

Invitrogen™ Bolt™ Bis-Tris Plus gels are precast polyacrylamide gels designed for optimal separation of a broad molecular weight range of proteins under denaturing conditions. The high-capacity, WedgeWell design accommodates more sample volume. Bolt gels are designed to deliver western performance superior to that of Tris-glycine–based gels.

- **Preserved protein integrity**—neutral-pH formulation minimizes protein modifications
- **High sample–volume capacity**—WedgeWell design allows detection of proteins in very dilute samples or visualization of low-abundance proteins
- **Better band quality and band volume**—Bolt Bis-Tris Plus gel chemistry is designed to deliver sharp, straight bands with higher band volume
- **High lot-to-lot consistency**—coefficient of variation (CV) of only 2% for Rf values (migration)
- **Optimized run conditions**—separate your proteins using constant voltage in approximately 35 minutes

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



Bolt Bis-Tris Plus gel **Bio-Rad TGX gel**

Bolt Bis-Tris Plus mini gels help provide better western blotting results. A western blot of a Bolt gel shows clean, sharp protein signals corresponding to only full-length proteins, whereas a western blot of a Bio-Rad™ TGX™ gel shows multiple low molecular weight degradation products. Protein kinases implicated in cancer (IKKβ, EPHB3, HCK, MAPK14, FLT1, and DDR2) were analyzed on a Bolt Bis-Tris Plus gel and a Bio-Rad TGX Tris-Glycine gel. The purified kinases (50 ng each), along with Invitrogen™ MagicMark™ XP Western Protein Standard and purified recombinant GST protein, were loaded on a 10-well, 4–12% Bolt gel and a 10-well, 4–20% Bio-Rad TGX gel. The samples were separated and transferred to 0.45 µm PVDF membranes using the respective manufacturers' protocols. Immunodetection was performed using an anti-GST antibody and Invitrogen™ WesternBreeze™ chemiluminescence detection. The blots were imaged using an LAS-1000 system (FujiFilm).

NuPAGE gels, similar to Bolt gels, but configured with standard wells, simulate the denaturing conditions of the traditional Laemmli system (Tris-glycine SDS-PAGE gels). NuPAGE gels use a unique buffer formulation to maintain a neutral operating pH during electrophoresis, helping to minimize the "smiles" and poor resolution seen with Tris-glycine SDS-PAGE gels. NuPAGE Bis-Tris gels offer:

- **Preserved protein integrity**—neutral-pH formulation minimizes protein modifications or degradation
- **High lot-to-lot consistency**—coefficient of variation (CV) of only 2% for Rf values (migration)
- **A long shelf life**—16 months at room temperature

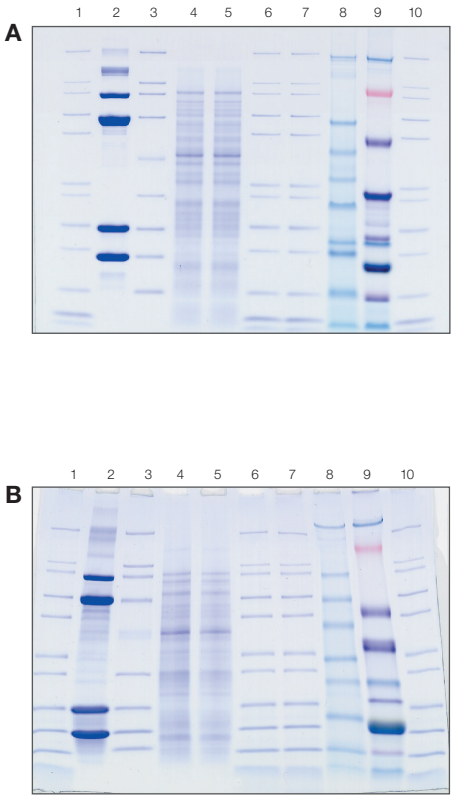
Learn more at **thermofisher.com/nupage**

Novex Tris-Glycine mini gels are polyacrylamide gels based on traditional Laemmli chemistry that enable the use of Laemmli sample and running buffers. Novex Tris-Glycine mini gels provide high-quality performance and separation of a wide range of proteins into well-resolved bands.

Highlights:

- **Wedge-shaped wells**—easily load up to 60 µL of sample without sacrificing gel width or length
- **High performance**—excellent protein band resolution and sharpness
- **Fast run conditions**—quickly separate your proteins using constant voltage in less than 60 minutes
- **Flexible**—Novex Tris-Glycine gels do not contain SDS and can be used to run proteins in native or in denatured form

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



Protein separation using **(A)** a NuPAGE gel and **(B)** a Bio-Rad traditional Tris-glycine gel.

Protein gels welcome packs

Protein gels welcome packs contain the components for outstanding protein separation and are available for each of our protein gels. The typical protein gel welcome pack provides all of the necessary gels, buffers, and reagents you need, as well as our Mini Gel Tank.

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

Protein ladders

We offer a broad range of prestained and unstained protein ladders supplied in a ready-to-use format to facilitate easy protein analysis during gel electrophoresis and western blotting.

Prestained protein ladders are recommended for:

- Approximate determination of molecular weight
- Monitoring the progress of electrophoresis runs
- Estimating the efficiency of protein transfer to the membrane during western blotting

Unstained protein ladders are recommended for:

- Precise determination of target protein molecular weights in any buffer system

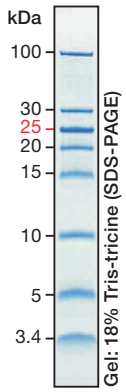
Our protein ladders offer extraordinary value—high quality without the high price.

- **Performance**—sharp protein bands and consistent migration patterns enable easy molecular weight determination
- **Convenient**—protein ladders are ready to load, with no heating required
- **Reliable**—exceptional lot-to-lot consistency and reproducibility

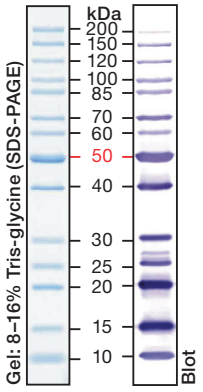
Protein ladders

	MW range	Product	No. of proteins	Range
Unstained	Low	PageRuler Unstained Low Range Protein Ladder	8	3.4–100 kDa
	Broad	PageRuler Unstained Protein Ladder	14	10–200 kDa
	High	NativeMark Unstained Protein Standard	8	20–1,200 kDa
Prestained	Low	PageRuler Prestained Protein Ladder	10	10–170 kDa
	Broad	PageRuler Plus Prestained Protein Ladder	9	10–250 kDa
	High	HiMark Prestained Protein Standard	9	30–460 kDa
Multicolor prestained	Broad	Spectra Multicolor Broad Range Protein Ladder	10	10–260 kDa
	High	Spectra Multicolor High Range Protein Ladder	8	40–300 kDa
Other	Western	iBright Prestained Protein Ladder	12	11–250 kDa
		MagicMark XP Western Protein Standard	9	20–220 kDa
	Specialty	PageRuler Prestained NIR Protein Ladder	10	11–250 kDa
		BenchMark Fluorescent Protein Standard	7	11–155 kDa
		BenchMark His-tagged Protein Standard	10	10–160 kDa
		IEF Marker 3–10	13	3–10 pI

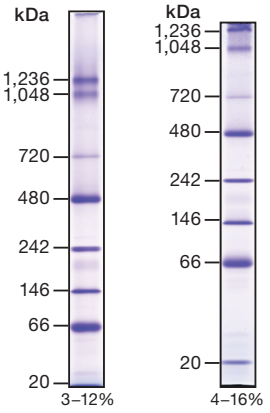
Learn more at **thermofisher.com/proteinladders**



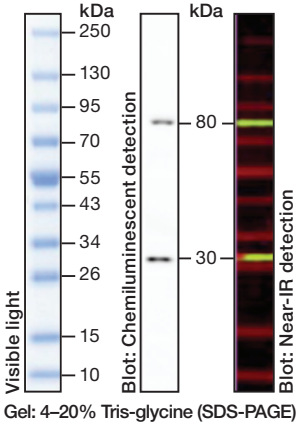
Thermo Scientific™ PageRuler™ Unstained Low Range Protein Ladder
Cat. No. PI26632



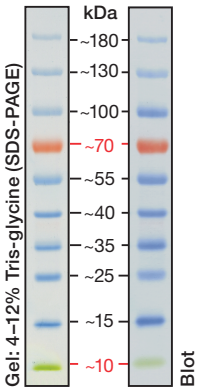
Thermo Scientific™ PageRuler™ Unstained Protein Ladder
Cat. No. PI26614



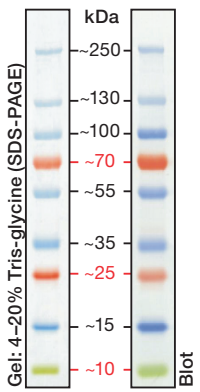
Invitrogen™ NativeMark™ Unstained Protein Standard
Cat. No. LC0725
NativePAGE Bis-Tris Gels



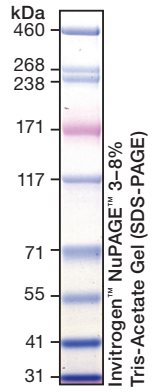
Invitrogen™ iBright™ Prestained Protein Ladder
Cat. No. LC5615



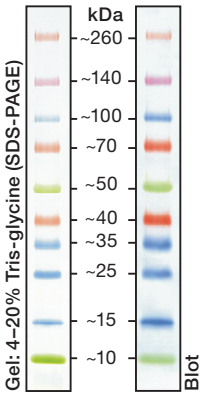
Thermo Scientific™ PageRuler™ Prestained Protein Ladder
Cat. No. PI26616



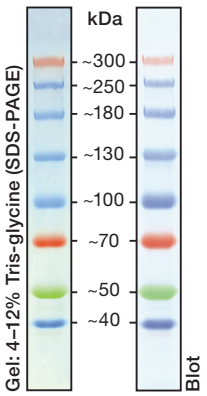
Thermo Scientific™ PageRuler™ Plus Prestained Protein Ladder
Cat. No. PI26619



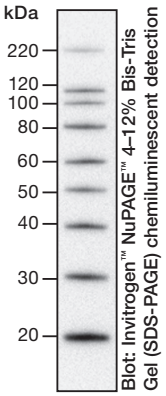
Invitrogen™ HiMark™ Prestained Protein Standard
Cat. No. LC5699



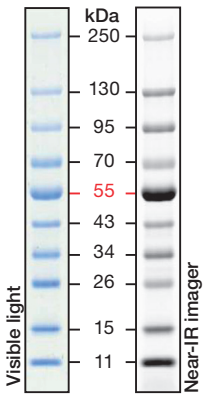
Thermo Scientific™ Spectra™ Multicolor Broad Range Protein Ladder
Cat. No. PI26634



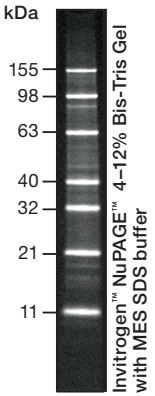
Thermo Scientific™ Spectra™ Multicolor High Range Protein Ladder
Cat. No. PI26625



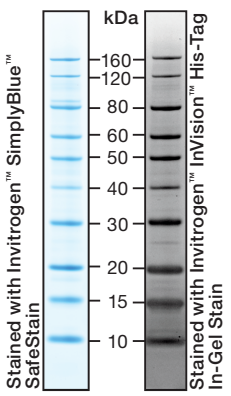
Invitrogen™ MagicMark™ XP Western Protein Standard
Cat. No. LC5602



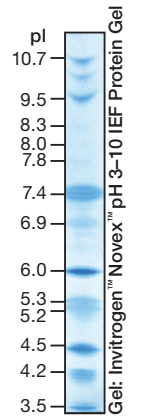
Thermo Scientific™ PageRuler™ Prestained NIR Protein Ladder
Cat. No. PI26635
4–20% Tris-glycine (SDS-PAGE)



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



Invitrogen™ BenchMark™ His-tagged Protein Standard
Cat. No. LC5606
NuPAGE 4–12% Bis-Tris Gel with MES SDS buffer



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

PowerEase power supplies

The Invitrogen™ PowerEase™ 90W Power Supply is designed specifically for mini-gel electrophoresis. The straightforward, intuitive interface makes the powering of gel runs a simple and easy process. In addition, the PowerEase 90W Power Supply features:

- Constant voltage or current settings
- Built-in timer for walk-away gel electrophoresis
- Output jacks that are compatible with most electrophoresis devices

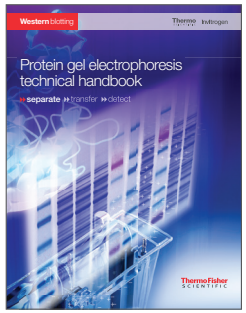
The Invitrogen™ PowerEase™ 300W Power Supply is a fully programmable power supply designed for high-throughput gel electrophoresis. This power supply easily accommodates the running and transferring of 8 mini gels and accommodates up to 10 user-defined programs for your most common electrophoresis runs.

Each program can include up to 10 steps, for precise control over electrophoresis conditions. In addition, the PowerEase 300W Power Supply features:

- Constant voltage, current, or power settings
- Built-in timer for walk-away gel electrophoresis
- Up to 10 custom programs with 10 steps each
- Four sets of output jacks that are compatible with most electrophoresis devices

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

Download the Protein Gel Electrophoresis Technical Handbook to access comprehensive, easy-to-understand information, including technical data, protocols, and troubleshooting tips.



Go to **thermofisher.com/pagehandbook**

Gel tanks

The Mini Gel Tank is designed for more intuitive use and convenience compared to traditional electrophoresis tanks.

- **Versatile**—compatible with NuPAGE, Bolt, or Tris-glycine gels
- **Easy sample loading**—with forward-facing well configuration
- **Simultaneous visualization of both gels**—streamlined, side-by-side tank configuration
- **Simplified monitoring of prestained protein markers**—with white tank stand
- **Less running buffer required**—two separate gel chambers, so you only need to load sufficient buffer for each gel to the specified fill line

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

The **Invitrogen™ XCell4 *SureLock*™ Midi-Cell** allows simultaneous vertical electrophoresis of 1–4 midi gels without leaking, enabling consistent performance. It uses proprietary technology to make electrophoresis easier and more reliable, and is designed to dissipate heat effectively and evenly to enable high-resolution results when using Invitrogen™ midi gels.

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

Pierce Power Stainer

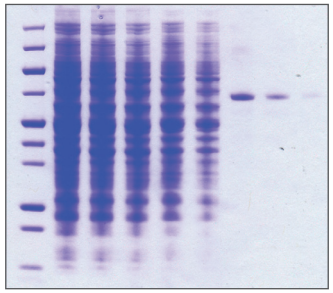
The Pierce Power Stainer is designed for rapid Coomassie staining of proteins in up to two mini polyacrylamide gels, and subsequent removal of unbound stain from the gel in a single step.

Traditional Coomassie staining techniques require a 1-hour to overnight staining step and a separate destaining step(s) for desired results. The Pierce Power Stainer, when used with Thermo Scientific™ Pierce™ Power Staining kits, enables efficient protein staining and gel destaining, typically in about 10 minutes, producing results equivalent to or better than traditional Coomassie staining techniques.

- **Fast**—Coomassie staining and destaining of proteins in about 10 minutes
- **Convenient**—simultaneously stain and destain 1 or 2 mini gels or 1 midi gel
- **Reliable performance**—enables staining results that are equivalent to traditional staining techniques
- **Easy-touch programming**—intuitive LCD touch-screen interface includes preprogrammed protocols

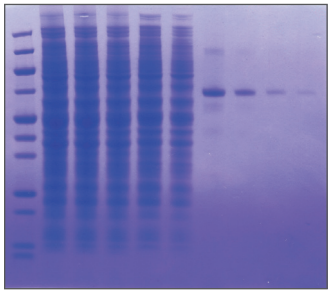
The Pierce Power Stainer enables rapid Coomassie staining of proteins.

Pierce Power Stainer



Total time: 11 minutes

Conventional Coomassie stain



Total time: 230 minutes to overnight

Coomassie stain solution: 45% methanol, 10% acetic acid, 0.25% Coomassie R-250
Coomassie destaining solution: 30% ethanol, 5% acetic acid

Conventional manual Coomassie protein gel staining process

Total time: 230 minutes to overnight

Novel Coomassie staining using the Pierce Power Stainer device

Total time: 11 minutes

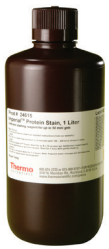
Learn more at **thermofisher.com/powerstainer**

Protein stains

Once protein bands have been separated by electrophoresis, they can be visualized using different methods of in-gel detection. Whether you just need a quick visual confirmation or require a highly sensitive stain to detect low-abundance proteins, we offer a variety of easy-to-use, effective protein stains for in-gel detection.

Check out our comprehensive collection of stains and choose the protein stain most suitable for you:

Protein stains			
	Coomassie staining	**Silver staining**	**Fluorescent protein staining**
Sensitivity	25 ng	0.5 ng	0.5 ng
Ease of use	+++	+	+
Mode of action	In acidic buffer conditions, Coomassie stain binds to basic and hydrophobic residues of proteins, changing from dull reddish-brown to intense blue.	Silver ions interact and bind with carboxylic acid groups (Asp and Glu), imidazole (His), sulfhydryls (Cys), and amines (Lys). Silver ions are reduced to metallic silver, resulting in a brown-black color.	Most fluorescent stains involve simple dye-binding mechanisms rather than chemical reactions that alter protein functional groups.
Detection	Visual	Visual	Compatible imaging system
Compatibility with downstream applications	Mass spectrometry (MS)– and sequencing-compatible	Certain formulations are MS-compatible	Most stains are MS-compatible
Products	**Value:** PageBlue Protein Staining Solution	**Value:** Pierce Silver Stain Kit	**Value:** SYPRO Red Protein Gel Stain
	Performance: SimplyBlue SafeStain	**Performance:** SilverXpress Silver Staining Kit	**Performance:** SYPRO Orange Protein Gel Stain
	Premium: Imperial Protein Stain	**Mass spec:** Pierce Silver Stain for MS	**Premium:** SYPRO Ruby Protein Gel Stain



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

Transfer

After proteins have been separated by gel electrophoresis, the next step in the western workflow is to transfer the proteins to a nitrocellulose or PVDF membrane.

We offer several protein transfer options: wet, semi-dry, and dry electroblotting.

Find out more about all of the options at **thermofisher.com/transfer**

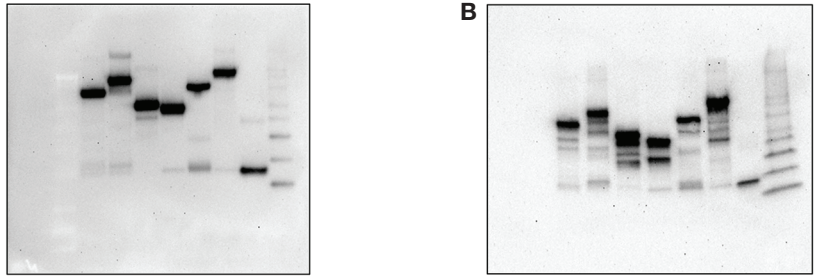
Run and transfer gels in one tank

The Mini Blot Module is a wet-transfer device for use with the Mini Gel Tank. The tank accommodates one blot module per chamber, or two blot modules total with the side-by-side layout. This affordable, leak-resistant module requires less transfer buffer than other transfer systems, and the constant resistance across the blotting electrodes helps ensure uniform field strength for highly efficient western transfers.

- **Unique gasket seal**—helps prevent buffer leakage so there is less mess during setup of your western transfer
- **½-inch buffer chamber**—requires only half the volume of methanol-based transfer buffer
- **Standard 60-minute transfer protocol**—accelerates your western workflow so you can get results fast

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

A western blot of a Bolt gel shows clean, sharp protein signals corresponding to only full-length proteins, whereas a western blot of a Bio-Rad TGX gel shows multiple low molecular weight degradation products. Protein kinases implicated in cancer (IKKB, HCK, EPHB3, MAPK14, FLT1, and DDR2) were analyzed on **(A)** a Bolt Bis-Tris Plus gel and **(B)** a Bio-Rad TGX Tris-glycine gel. Protein samples were prepared for electrophoresis according to each manufacturer's protocol. The purified kinases (50 ng each) as GST fusion proteins, along with Invitrogen™ MagicMark™ XP Western Protein Standard and purified recombinant GST, were loaded in a Bolt 4–12% gel and a Bio-Rad TGX 4–20% gel. The samples were separated and transferred to PVDF membranes using the Mini Blot Module for the Bolt gels or on the Bio-Rad transfer system. Blot detection was performed using an anti-GST antibody and an Invitrogen™ WesternBreeze™ Chemiluminescence Detection Kit. The membranes were then imaged using an LAS-1000™ system (Fujifilm) with an exposure time of 1 minute.

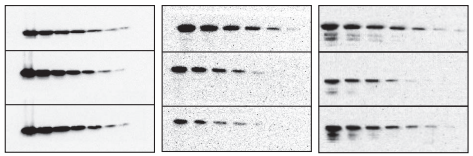


The Pierce Power Blotter is designed for rapid semi-dry transfer of 10–300 kDa proteins from polyacrylamide gels to nitrocellulose or PVDF membranes, typically in 5–10 minutes, when used with Thermo Scientific™ Pierce™ 1-Step Transfer Buffer.

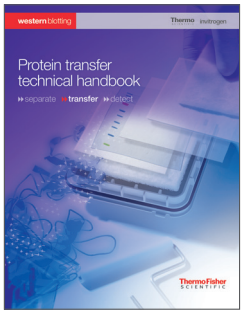
The Pierce Power Blotter features an integrated power supply optimized to deliver consistent, high-efficiency protein transfer when used with precast or homemade gels (SDS-PAGE) and nitrocellulose or PVDF membranes. The Thermo Scientific™ Pierce™ Power Blot cassette enables the simultaneous transfer of up to four mini gels or two midi gels.

- **Integrated power supply**—seamless operation between control unit and cassette provides consistent high-efficiency protein transfer
- **Easy-touch programming**—access preprogrammed transfer methods or create, save, and run customized transfer methods
- **Flexible gel formats**—transfer two midi gels or four mini gels simultaneously

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

Low (<25 kDa)	Medium (25–150 kDa)	High (>150 kDa)	Molecular weight range
Precise Protein Gel (Tris-glycine)	NuPage 4–12% Bis-Tris Gel	NuPage 4–12% Bis-Tris Gel	Gel type
Nitrocellulose	Nitrocellulose	PVDF	Membrane
Cyclophilin B (21 kDa)	PLK-1 (67 kDa)	mTOR (289 kDa)	Target protein and size
			Pierce Power Blotter 10 minutes
			Conventional semi-dry 1 hour
			Conventional tank overnight

The Pierce Power Blotter allows rapid transfers of low, medium, and high molecular weight proteins.

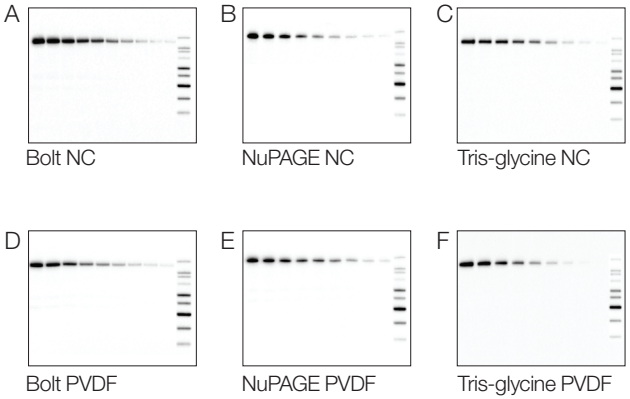


Download our Protein Transfer Technical Handbook for practical information on improving protein transfer efficiency and getting better-quality western blot results.

Go to **thermofisher.com/transferhandbook**

The iBlot 2 Dry Blotting System is designed to deliver reproducible protein transfer in 7 minutes. The iBlot 2 system offers exceptional transfer efficiency, convenience, and speed, producing crisp and clear bands that remain sharp and straight.

- Compatible with multiple gel chemistries (Bis-Tris, Tris-glycine, and Tris-acetate) and membrane types (PVDF and nitrocellulose)
- Flexible gel formats: transfer one midi or two mini gels simultaneously
- Touch-screen interface for ease of use
- Prepackaged, ready-to-use transfer stacks available for easy setup



Membranes processed on the iBlot 2 Dry Blotting System show consistent transfer across various protein gel chemistries to both nitrocellulose (NC) and PVDF membranes. Total cell extracts from A431 cells were transferred to NC membranes from 4–12% Bolt, 4–12% NuPAGE, and 4–20% Tris-glycine precast gels **(A–C)**, and also to PVDF membranes from the same types of gels **(D–F)**, using the iBlot 2 Dry Blotting System.

Thermo Scientific™ Pierce™ Reversible Protein Stain kits for membranes are rapid and sensitive alternatives to Ponceau S stain for protein detection on nitrocellulose or PVDF membranes after transfer from polyacrylamide gels.

These kits for membrane staining use a nondestructive, reversible, reliable, and sensitive method to stain and detect proteins on nitrocellulose and PVDF membranes. The lower limit of detection with this method is 25–50 ng per band (at least five times more sensitive than traditional Ponceau S staining). The staining protocols are simple, quick, and result in turquoise-blue bands that do not fade and are easily photographed for future reference.

Learn more at **thermofisher.com/iblot2**

Detect

The last step in the western workflow is detection. In this step, primary antibodies specific to the protein of interest bind to the protein on the membrane. Secondary antibodies conjugated to horseradish peroxidase (HRP) or alkaline phosphatase (AP) are then added, and they bind to the primary antibody to allow for visualization of the protein bound to the membrane. We offer thousands of primary and secondary antibodies, along with buffers and substrates for use in western blot analysis. In addition, the revolutionary iBind Western System provides automated convenience for primary and secondary antibody binding as well as all wash steps. To complete the detection step, we offer iBright Imaging Systems, featuring push-button optimized exposure and advanced automated features.

Find out more at **thermofisher.com/detect**

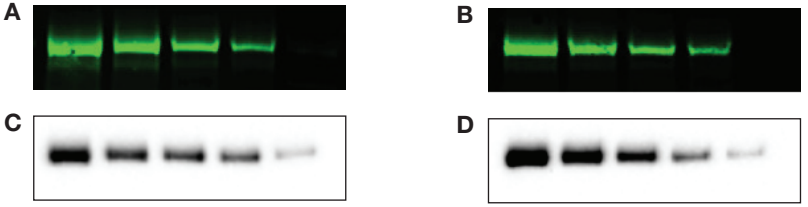
The original notebook-size, automated western blot processing device

The iBind Western System is an automated western blot processing platform. Simply load primary antibody, secondary antibody, and wash solutions, and then walk away. In less than 3 hours, the blot is ready for final detection.

- **Antibody savings**—use up to 80% less primary antibody
- **Load and go**—processes solutions using sequential lateral flow technology, with no batteries, shakers, trays, or timers required
- **Reproducibility**—automated blot processing enables improved blot-to-blot consistency

Watch a video demonstration at **thermofisher.com/ibind**

Phospho-EGFR



Manual processing

iBind Western Device process with 80% less primary antibody than manual method

The iBind Western System enables excellent western blot results with less primary antibody. Proteins in an A431 cell extract were separated using the Mini Gel Tank electrophoresis system and transferred to PVDF or NC membranes using the iBlot 2 Dry Blotting System. The blots were probed with an anti–phospho-EGF receptor [Tyr1068] (1H12) mouse monoclonal antibody (1:1,000 dilution, using 2 µL antibody for the iBind device method and 10 µL antibody for the manual method). **(A, B)** A goat anti-mouse conjugate was used as a secondary antibody. **(C, D)** A peroxidase-conjugated goat anti–mouse IgG (H+L) secondary antibody was used.

Automated blot processing workflow

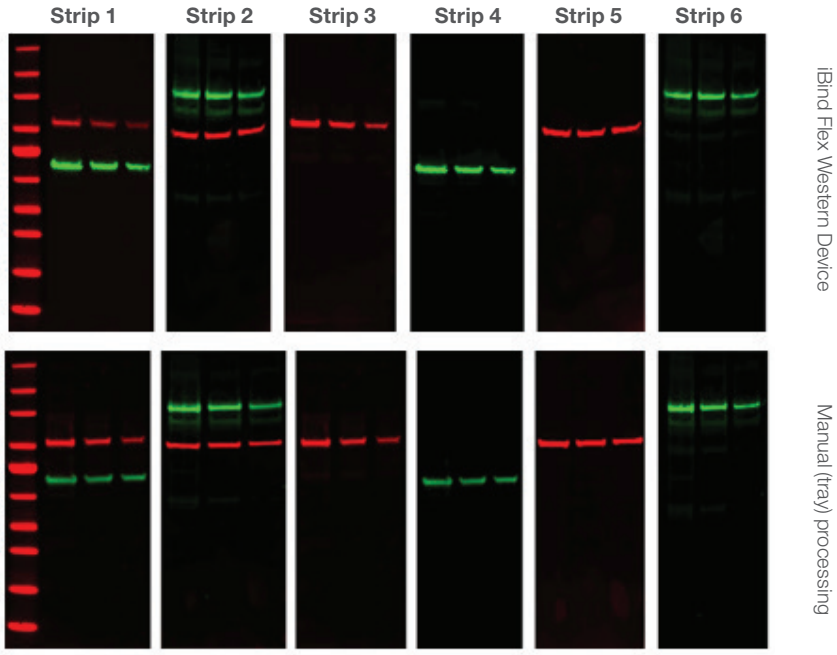
Load iBind card and transferred membrane → Load primary Ab, secondary Ab, and wash solutions → Incubate 3 hr

iBind Flex Western Device

Flexible, automated western blot processing

The Invitrogen™ iBind™ Flex Western Device offers the same automated blot processing technology as the original iBind Western System, but comes with interchangable wells. These interchangable wells provide blot-format flexibility and enable the use of different primary and secondary antibody conditions in a single experiment.

- **Flexibility**—process up to one midi blot, two mini blots, or six vertically cut strips using the same or different conditions
- **Load and go**—processes solutions using sequential lateral flow technology, with no batteries, shakers, trays, or timers required
- **Antibody savings**—use up to 80% less primary antibody than with traditional tray-based incubation steps for western blotting
- **Reproducibility**—automated blot processing enables improved blot-to-blot consistency



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 4–12%, 10-well gels with MES SDS running buffer, rapid-dry transfer to nitrocellulose membrane using the iBlot 2 system and then cutting each into three-lane strips. Final imaging was performed using a fluorescence imaging instrument.
Samples and lanes were as follows:
Lane 1: Thermo Scientific™ PageRuler™ Prestained NIR Protein Ladder (3 µL)
Strip 1: Phosphorylated Akt cell extract (15 µg, 7.5 µg, 3.75 µg) and Elk-1 fusion protein (150 ng, 75 ng, 37.5 ng)
Strip 2: HeLa cell extract (30 µg, 15 µg, 7.5 µg)
Strip 3: Phosphorylated Akt cell extract (15 µg, 7.5 µg, 3.75 µg)
Strip 4: Elk-1 fusion protein (150 ng, 75 ng, 37.5 ng)
Strip 5: HeLa cell extract (30 µg, 15 µg, 7.5 µg)
Strip 6: HeLa cell extract (30 µg, 15 µg, 7.5 µg)

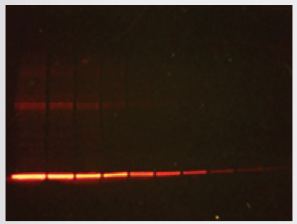
For for more information about the target proteins and antibodies used, go to **thermofisher.com/ibindflex**

Multicolor western analysis

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™ and Alexa Fluor™ 680/790 antibodies are detected on standard membranes with high sensitivity and minimal background signal or scatter. Detect both strong and weak signals at the same time with a >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.

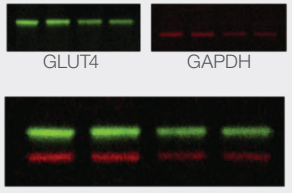
- Simple, quantitative western blots
- Uses existing benchtop equipment
- Wide linear dynamic range
- Multiplexing capability

Single-color fluorescence



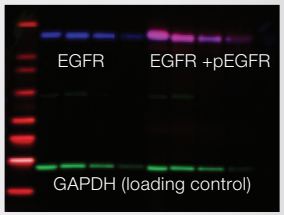
Transilluminator with ethidium bromide amber filter–with WesternDot 625

2-color fluorescence



Near IR imaging system with Alexa Fluor 680 and Alexa Fluor 790 secondary antibodies

3-color fluorescence



Standard gel imaging system with WesternDot 800–, WesternDot 585–, and WesternDot 655–labeled secondary antibodies*

* Visit **thermofisher.com/westerndot** for more information.

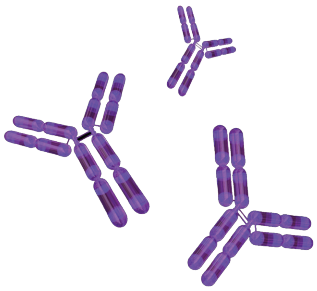
Antibodies

Get the right antibodies for western blot detection

We have an extensive selection of high-quality conjugated and unconjugated secondary antibodies that can be used for the fluorescent, colorimetric, and chemiluminescent detection of primary antibodies in western blotting and many other applications. These include highly cited research antibodies, many of which are conjugated to a broad range of dyes and enzymes.

- Invitrogen™ Alexa Fluor™ and Alexa Fluor™ Plus dyes
- Classic fluorescent dyes (FITC, RPE, APC)
- Enzyme conjugates (HRP, AP)

To find the right antibody for your research needs, go to **thermofisher.com/antibodies**



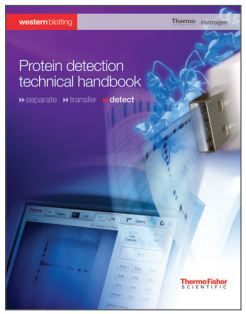
Reagents for manual western detection

The traditional manual blot probing procedure includes a series of essential steps before the addition of the detection substrate, as shown in the figure below. The target protein on the membrane is then detected by X-ray film or CCD imaging systems. At this point, the blot can be stripped and reprobed, if necessary.

Blocking → Primary Ab incubation → Wash → Secondary Ab incubation → Wash → Incubation with substrate → Target detection → Stripping (if necessary)

We offer a wide range of ready-to-use western blotting reagents, including blocking buffers, wash buffers, detergents, membrane-stripping buffers, and western blot signal enhancers. Our blocking buffers include traditional protein blocking agents, such as BSA, casein, and milk, as well as exclusive blocking buffers, such as Thermo Scientific™ SuperBlock™, StartingBlock™, and Pierce™ Protein-Free Blocking Buffers, for efficient blocking in western blotting and other immunoassay detection methods. Our wash buffers include pouches of preblended powder mixtures of commonly used buffers, such as PBS and TBS for western blotting; simply add water to dissolve and they're ready for use.

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

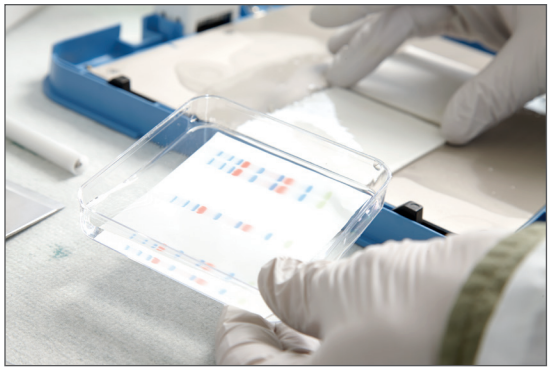


Download our Protein Detection Technical Handbook to explore our complete line of western detection products.

Go to **thermofisher.com/detecthandbook**

We offer nitrocellulose and PVDF transfer membranes, available in rolls and as precut sheets and X-ray film for chemiluminescence and other western blot detection techniques. Our specially formulated membrane-stripping buffers are designed to dissociate and strip primary and secondary antibodies from western blots, so that membranes can be reprobed under alternate conditions or with another antibody to detect a different protein target. Included in our specialty reagents for western blotting is our Thermo Scientific™ SuperSignal™ Western Blot Enhancer that is designed to help increase both signal intensity and sensitivity 3–10-fold compared to a detection performed without it.

Explore our reagents for manual western detection on pages 23–25 to select products most suitable for your western application.



Blocking

Block unreacted sites on the membrane to reduce the amount of nonspecific binding.

We have a complete selection of blocking buffers to improve the sensitivity of your western blot. The proper choice of buffer depends on the antigen and type of enzyme conjugate to be used. With the wide range we offer, you can achieve the highest signal-to-noise ratio possible for your blots.

- Thermo Scientific™ StartingBlock™ Blocking Buffer in PBS (Cat. No. PI37538) and in TBS (Cat. No. PI37542)
- Thermo Scientific™ StartingBlock™ T20 Blocking Buffer (contains 0.05% Tween-20) in PBS (Cat. No. PI37539) or TBS (Cat. No. PI37543)
- Thermo Scientific™ SuperBlock™ Buffer in PBS (Cat. Nos. PI37515 and PI37518) and in TBS (Cat. No. PI37535)
- Thermo Scientific™ SuperBlock™ T20 Blocking Buffer (contains 0.05% Tween-20) in PBS (Cat. No. PI37516) or TBS (Cat. No. PI37536)
- Thermo Scientific™ SuperBlock™ Blocking Buffer—blotting in PBS (Cat. No. PI37517) and in TBS (Cat. No. PI37537)
- Thermo Scientific™ Pierce™ Protein-Free Blocking Buffer (Cat. Nos. PI37570, PI37571, PI37572, and PI37573)

Wash

Remove unbound primary reagents and reduce background.

Our dry buffers and high-purity detergents all serve to enhance your signal-to-noise ratio.

Buffered saline solutions:

- Thermo Scientific™ BupH™ Phosphate Buffered Saline Packs (Cat. No. PI28372)
- Thermo Scientific™ Pierce™ 20X Phosphate Buffered Saline (Cat. Nos. PI28348, PI28358)
- Thermo Scientific™ BupH™ Tris Buffered Saline (Cat. Nos. PI28376, PI28379)
- Thermo Scientific™ Pierce™ Modified Dulbecco's PBS Buffer (Cat. Nos. PI28344, PI28374)
- Thermo Scientific™ Surfact-Amps™ detergents, including:
  - Thermo Scientific™ Tween™-20 Detergent (Cat. No. PI28320)
  - Thermo Scientific™ Tween™-80 Detergent (Cat. No. PI28328)
  - Thermo Scientific™ Triton™ X-100 Detergent (Cat. No. PI28314)
  - NP-40 Detergent (Cat. No. PI28324)

Primary and secondary antibody incubation

We offer thousands of antibodies for over 50 research areas, such as cancer, neuroscience, immunology, and epigenetics. Purchase with confidence knowing that we stand behind the quality of our antibodies with the with the Invitrogen Antibody Performance Guarantee.*

Our secondary antibodies and detection reagents are available in a variety of formats and conjugated types, including HRP, AP, Alexa Fluor, and others.

* Terms and conditions apply. For complete details, go to **thermofisher.com/antibody-performance-guarantee**

Get your antibodies now at **thermofisher.com/antibodies**

Incubation with substrate

Add the detection reagent to your blot.

Choose the appropriate substrate for your needs from the Pierce ECL and SuperSignal families of chemiluminescent HRP substrates. Our ECL and SuperSignal substrates offer excellent performance in western blotting with longer light emission and stronger signal intensity.

Learn more about our substrates on page 25.

Stripping (if necessary)

Reprobe the blot if needed.

Using our Thermo Scientific™ Restore™ products, you can quickly strip and reprobe, as well as reuse the blot again and again. We help you save time, money, and aggravation in reprobing your blots.

- Thermo Scientific™ Restore™ Western Blot Stripping Buffer (Cat. No. PI21059)
- Thermo Scientific™ Restore™ PLUS Western Blot Stripping Buffer (Cat. No. PI46430)
- Thermo Scientific™ Restore™ Fluorescent Western Blot Stripping Buffer (Cat. Nos. PI62299 and PI62300)

Target detection

Capture and analyze your image.

Our Thermo Scientific™ CL-XPosure™ Film is an affordable, convenient, high-performance clear-blue X-ray film for your chemiluminescent western blot detection needs. For speed and performance, consider digital data acquisition with the iBright Imaging Systems, featuring push-button optimized exposure and advanced automated features. The handy Thermo Scientific™ Pierce™ Background Eliminator Kit (Cat. No. 21065) helps retrieve data from overexposed films.

- Thermo Scientific™ CL-XPosure™ Film (Cat. Nos. PI34089, PI34090, and PI34091)
- iBright Imaging Systems (Cat. Nos. A32749 and A32752)

Chemiluminescent substrates

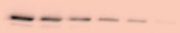
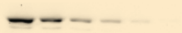
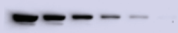
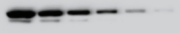
Choose the appropriate chemiluminescent substrate for western blot detection

As with other components in a western blotting system, there are many chemiluminescent substrate choices available. The appropriate substrate selection depends on the detection level (sensitivity) required, the target protein abundance, and the sample availability.

Our chemiluminescent substrates offer:

- **Excellent sensitivity**—five substrates providing picogram- to femtogram-level sensitivity
- **Strong light emission**—longer signal duration allows for multiple exposures
- **High intensity**—signal is twice as intense as other luminescence-based systems
- **Antibody savings**—our substrates are optimized to work with more dilute primary and secondary antibodies

We offer five types of chemiluminescent substrates for western blot detection with HRP:

	Pierce ECL	Pierce ECL 2	SuperSignal West Pico PLUS	SuperSignal West Dura	SuperSignal West Femto
					
Advantage	Same signal, lower price than other entry-level ECL substrates	Same signal and lower price than competing ECL Plus substrates	Excellent sensitivity, intensity, and duration than other ECL substrates in its class	Best for use with imaging equipment	Most sensitive substrate for HRP detection
Detection level	Low to mid picogram	Low picogram	Low picogram to high femtogram	Mid femtogram	Low to mid femtogram
Signal duration	30 min–2 hr	5 hr	Up to 24 hr	24 hr	8 hr
Detection methods	X-ray film, CCD imager	X-ray film, CCD imager, fluorescence imager	X-ray film, CCD imager	X-ray film, CCD imager	X-ray film, CCD imager
Recommended primary and secondary antibody dilutions	1° 1:1K 2° 1:1K–1:15K	1° 1:1K 2° 1:25K–1:200K	1° 1:1K 2° 1:20K–1:100K	1° 1:5K 2° 1:50K–1:250K	1° 1:5K 2° 1:100K–1:500K
Select when:	Target is abundant, sample is abundant, and substrate is for everyday use	Target is less abundant, sample is limited, and for chemifluorescent detection	Target is less abundant, sample is limited, and you need more sensitivity than an entry-level ECL substrate	Target is less abundant, sample is limited, and for CCD image capture	Target is least abundant, sample is precious, and for maximum sensitivity
Value to you	Low cost; easy to switch from other entry-level ECL substrates	Best detection flexibility with chemifluorescent detection option	Best value; works for majority of western blots	Best signal duration	Best sensitivity

For data above: STAT3 detection in HeLa cell lysate (lane 1: 20 µg total protein; lanes 2–6: serially diluted 1:1) was performed using Thermo Scientific™ HRP chemiluminescent substrates. The blots were developed using Invitrogen™ anti-STAT3 Antibody (Cat. No. MA1-13042) and Goat anti-Mouse IgG Secondary Antibody, HRP conjugate (Cat. No. PI31430). Images were captured using the Thermo Scientific™ myECL™ Imager (Cat. No. PI62236).

Don't know where to start? Try Thermo Scientific™ SuperSignal™ West Pico PLUS Chemiluminescent Substrate, designed to work for the majority of westerns.

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

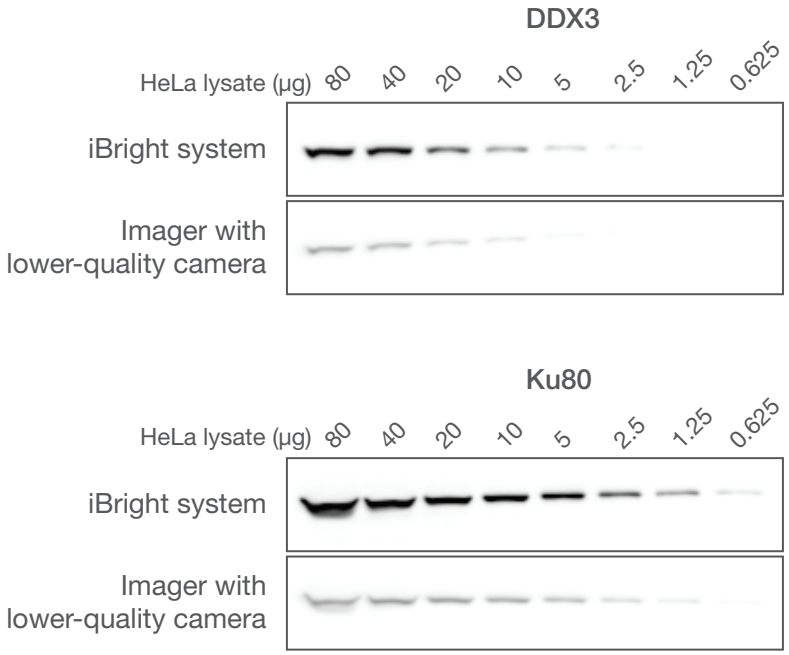
iBright Imaging Systems

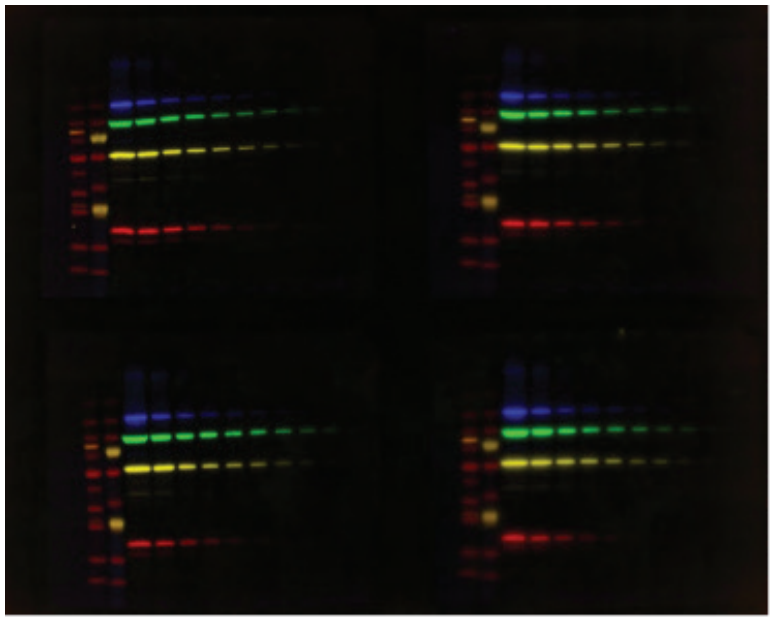
Stunningly easy western blot imaging

Experience an easier time capturing and analyzing data from gels and western blots with the introduction of iBright Imaging Systems. Designed with a streamlined, intuitive interface and workflows, iBright Imaging Systems are easy to use for researchers of all experience levels. Two iBright Imaging System models are available: the iBright CL1000 and the iBright FL1000. The iBright CL1000 model is capable of imaging chemiluminescent western blots in addition to stained protein and nucleic acid gels. The iBright FL1000 model features the same imaging modes as the iBright CL1000 system, yet also offers fluorescent blot imaging capability, in both visible and near-IR channels.

The iBright Imaging Systems provide:

- **Push-button optimized exposure**—Smart Exposure™ acquisition technology for the rapid determination of optimal exposure times—helps minimize the need to repeat exposures to get the desired signal
- **Powerful 9.1 megapixel (MP) camera**—capture crystal-clear images with robust imaging potential
- **Advanced automated features**—automatic sample rotation, auto-zoom, auto-focus, and automatic on-board data analysis provide for a smooth imaging experience
- **5-channel fluorescent blotting**—multiplex with the 5 fluorescent channels of the iBright FL1000 model; capture up to 4 proteins in a single blot for more meaningful and representative experiments





iBright Imaging Systems feature a powerful 9.1 MP camera for greater sensitivity compared to instruments with a lower-resolution camera. Two-fold serial dilutions of HeLa cell lysate (starting at 80 µg/lane) were loaded and run on Novex Tris-glycine gels, transferred, and probed with antibodies against DDX3 or Ku80 proteins. Blots were then probed with relevant HRP-conjugated secondary antibodies, developed with SuperSignal West Pico PLUS chemiluminescent substrate and visualized on the iBright FL1000 Imaging System and another imaging device with a lower-quality, 4.1 MP camera—each with 10-second exposures.

High-throughput blotting experiment with 4 mini blots captured in a single image. Proteins detected with target-specific primary antibodies and the following Invitrogen™ Alexa Fluor™ secondary antibodies: Alexa Fluor 488 (blue), Alexa Fluor 546 (yellow), Alexa Fluor Plus 680 (red), and Alexa Fluor Plus 800 (green). This high degree of multiplexing potential enables one to study multiple proteins in a blot so that more meaningful and representative blotting experiments are possible—combine what could be several blotting experiments into one high-throughput experiment.

Learn more or request your demo today at **thermofisher.com/ibright**

Ordering information

Product	Quantity	Cat. No.
Separate		
Mini Gel Tank	1 unit	A25977
Bolt Welcome Pack, 10-well	1 kit	NW0412A
Bolt Welcome Pack, 15-well	1 kit	NW0412B
NuPAGE Bis-Tris Welcome Pack, 4-12%, 10-well	1 kit	NP032A
NuPAGE Bis-Tris Welcome Pack, 10%, 10-well	1 kit	NP030A
Novex WedgeWell Welcome Pack, 10-well, 10%	1 kit	XP0010A
Novex WedgeWell Welcome Pack, 10-well, 4–12%	1 kit	XP0412A
Novex WedgeWell Welcome Pack, 15-well, 10%	1 kit	XP0010C
Novex WedgeWell Welcome Pack, 15-well, 4–12%	1 kit	XP0412C
SureCast Gel Handcast Bundle A	Multiple	HC1000SR
SureCast Gel Handcast Bundle B	Multiple	HC1000S
SureCast Gel Handcast System	1 casting system	HC1000
SureCast Glass Plates	2 glass plate sets (2 front and 2 back)	HC1000S
SureCast Sealing Pads	2 sealing pads	HC1002
SureCast 10-well Multi-Use Tool	1 multi-use tool	HC1010
SureCast 12-well Multi-Use Tool	1 multi-use tool	HC1012
SureCast 15-well Multi-Use Tool	1 multi-use tool	HC1015
SureCast Gel Spacer	10 spacers	HC1003
SureCast Stacking Buffer (1 L), 2-pack	2 x 500 mL dry packs	HC2112
SureCast Stacking Buffer (2.5 L), 5-pack	5 x 500 mL dry packs	HC2115
SureCast Resolving Buffer (1 L), 2-pack	2 x 500 mL dry packs	HC2212
SureCast Resolving Buffer (2.5 L), 5-pack	5 x 500 mL dry packs	HC2215
SureCast APS	25 g	HC2005
SureCast Acrylamide Solution, 40%	450 mL	HC2040
SureCast TEMED	30 mL	HC2006
MagicMark XP Western Protein Standard	250 µL	LC5602
NativeMark Unstained Protein Standard	5 x 50 µL	LC0725
PageRuler Unstained Low Range Protein Ladder	2 x 250 µL	PI26632
PageRuler Unstained Protein Ladder	2 x 250 µL	PI26614
PageRuler Prestained Protein Ladder	2 x 250 µL	PI26616
PageRuler Plus Prestained Protein Ladder	2 x 250 µL	PI26619
Spectra Multicolor Broad Range Protein Ladder	2 x 250 µL	PI26634
Spectra Multicolor High Range Protein Ladder	2 x 250 µL	PI26625

Ordering information

Product	Quantity	Cat. No.
HiMark Prestained Protein Standard	250 µL	LC5699
iBright Prestained Protein Ladder	2 x 250 µL	LC5615
Bolt Bis-Tris Plus Precast Gels	Varies	Varies
XCell4 *SureLock* Midi-Cell	1 each	WR0100
Novex Tris-Glycine Mini Gels (WedgeWell format)	Varies	Varies
NuPAGE Bis-Tris Precast Gels	Varies	Varies
NuPAGE Tris-Acetate Precast Gels	Varies	Varies
Novex Tris-Glycine Precast Gels	Varies	Varies
PowerEase 90W Power Supply (115 VAC)	1 each	PS0090
PowerEase 300W Power Supply (115 VAC)	1 each	PS0300
Pierce Power Stainer Welcome Pack	1 kit	PI22833SPCL
PageBlue Protein Stain	1 L	PI24620
SimplyBlue SafeStain	1 L	LC6060
Imperial Protein Stain	1 L	PI24615
Pierce Silver Stain	1 L kit	PI24612
SilverXpress Silver Stain	1 kit	LC6100
Pierce Silver Stain for MS	1 L kit	PI24600
SYPRO Orange/Red/Ruby Protein Gel Stains	Varies	Varies
Separate and transfer		
Bolt Welcome Pack with iBlot 2 Dry Blotting System	1 kit	NW0412AIB2
Mini Gel Tank and Blot Module Set	1 kit	NW2000
Transfer		
iBlot 2 Gel Transfer Device	1 device	IB21001
Mini Blot Module	1 unit	B1000
iBlot 2 Transfer Stacks, Nitrocellulose, Regular	10 stacks	IB23001
iBlot 2 Transfer Stacks, Nitrocellulose, Mini	10 stacks	IB23002
iBlot 2 Transfer Stacks, PVDF, Regular	10 stacks	IB24001
iBlot 2 Transfer Stacks, PVDF, Mini	10 stacks	IB24002
Pierce Midi Gel Power Staining Kit	30 pads	PI22839
Pierce Mini Gel Power Staining Kit	60 pads	PI22840
Pierce Reversible Protein Stain for NC/PVDF Membranes	Varies	Varies
Pierce Power Blotter Welcome Pack	1 kit	PI22834SPCL
Separate and transfer		
Bolt Welcome Pack with iBlot 2 Dry Blotting System	1 kit	NW0412AIB2
Mini Gel Tank and Blot Module Set	1 kit	NW2000

Ordering information

Product	Quantity	Cat. No.
Detect		
iBind Western Starter Kit	1 kit	SLF1000S
iBind Western Device	1 device	SLF1000
iBind Cards	10 cards	SLF1010
	40 cards	SLF1010X4
iBind Solution Kit	1 kit	SLF1020
	4 kits	SLF1020X4
iBind Flex Western Starter Kit	1 kit	SLF2000S
iBind Flex Western Device	1 device	SLF2000
	2 devices	SLF20002PK
iBind Flex Cards	10 cards	SLF2010
	40 cards	SLF2010X4
iBind Flex Solution Kit	1 kit	SLF2020
	4 kits	SLF2020X4
iBright CL1000 Imaging System	1 device	A32749
iBright FL1000 Imaging System	1 device	A32752
Invitrogen primary and secondary antibodies for western blot analysis	Over 74,000	Varies
Pierce ECL Substrate	500 mL	PI32106
Pierce ECL 2	100 mL	PI80196
SuperSignal West Pico PLUS Chemiluminescent Substrate	500 mL	PI34580
SuperSignal West Dura Extended Duration Substrate	200 mL	PI34076
SuperSignal West Femto Maximum Sensitivity Substrate	200 mL	PI34096

Notes

Find out more at **thermofisher.com/western**

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