

# WESTERN DETECTION



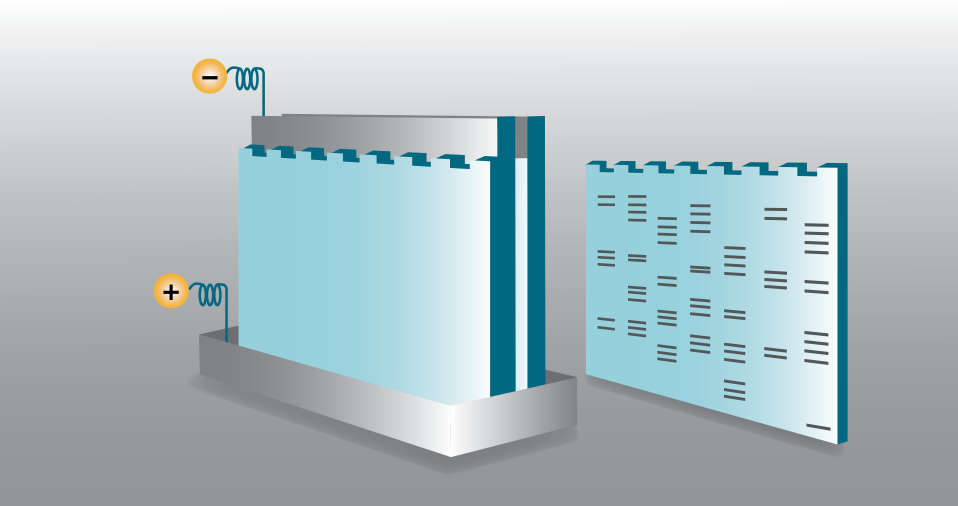
Unique solutions for  
your unique protein

Customize your western blot tools to  
optimize your outcomes

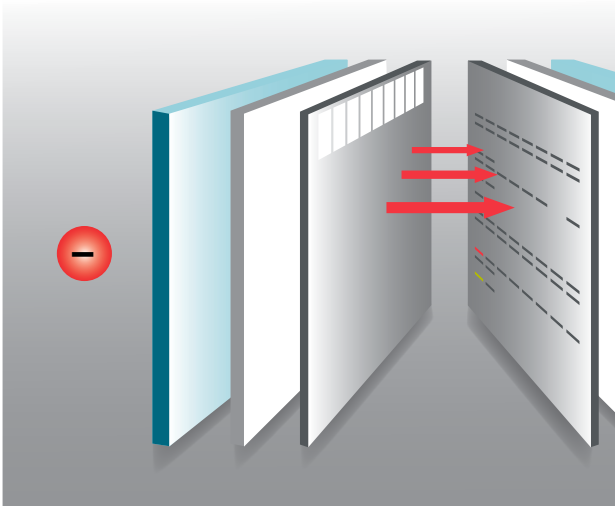


**ThermoFisher**  
SCIENTIFIC

Find a comprehensive suite of solutions for every step of the **western blotting workflow** in order to help you obtain high-quality, publishable results with minimal time and effort.



Separate



Transfer

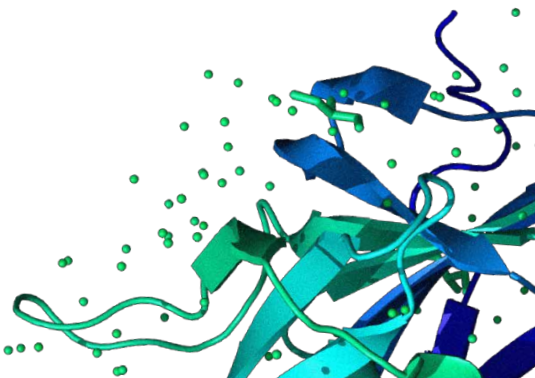


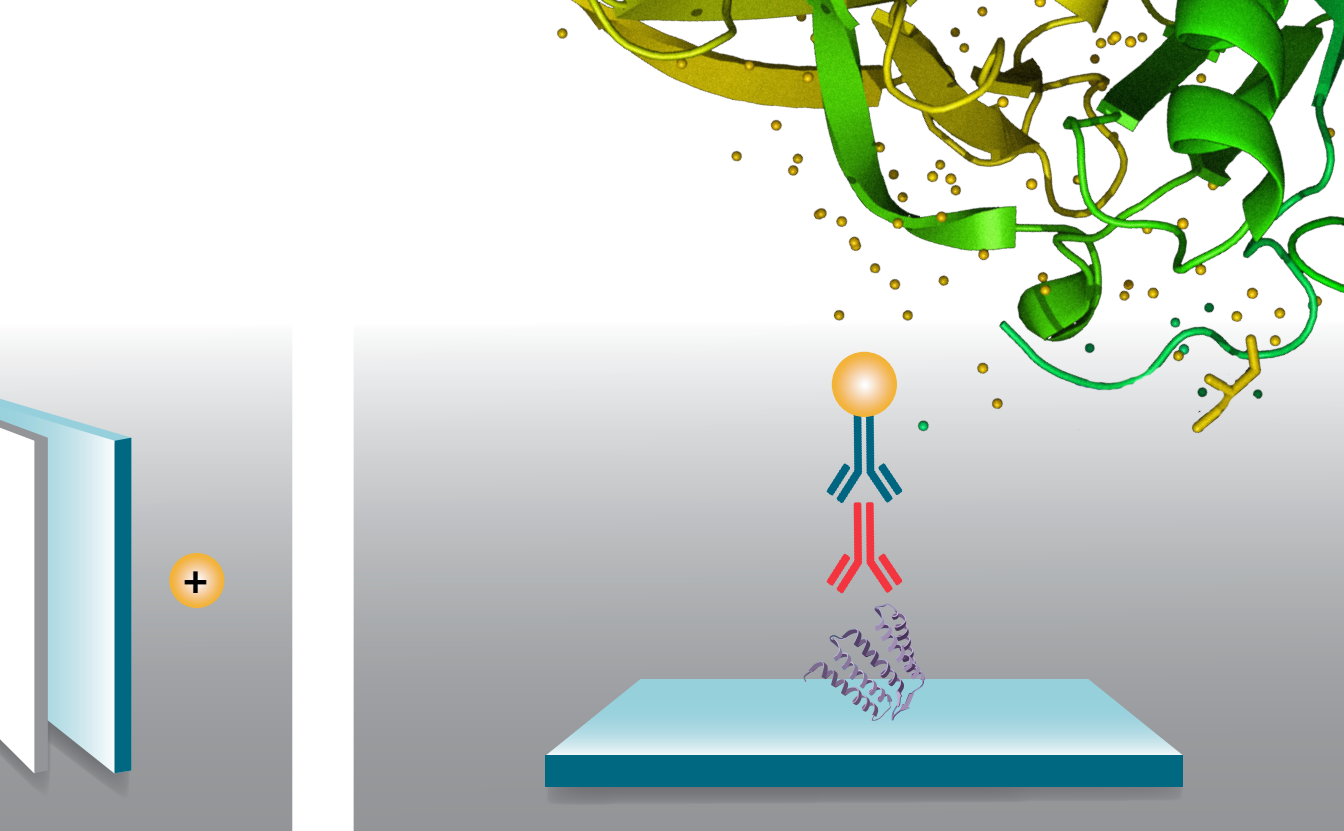
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See a complete listing of all available products at [thermofisher.com/western](https://www.thermofisher.com/western)





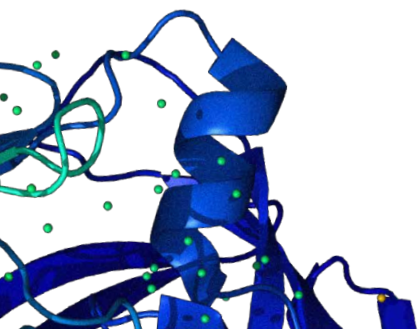
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Western blot and gel imaging systems  
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Fluorescent secondary antibodies  
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Stripping buffers

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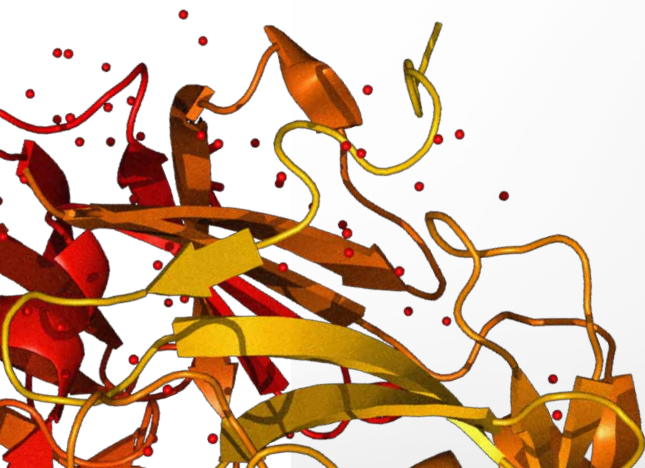
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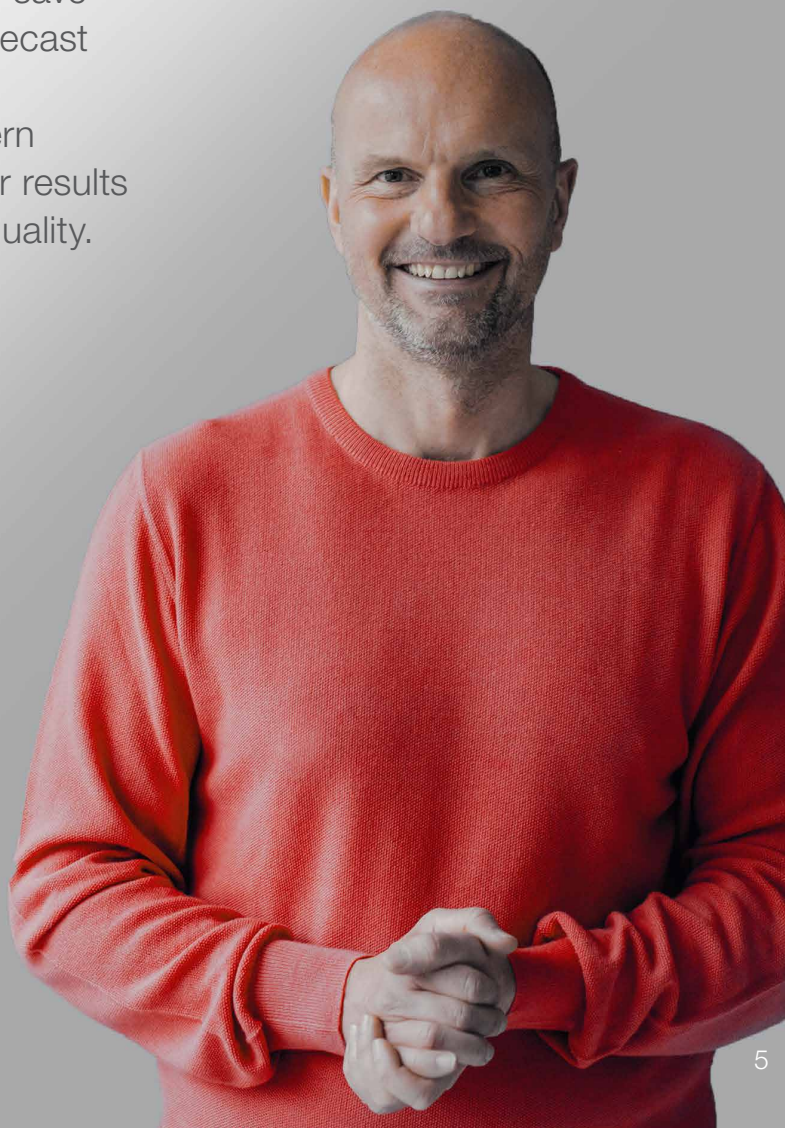
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# High-efficiency western blotting

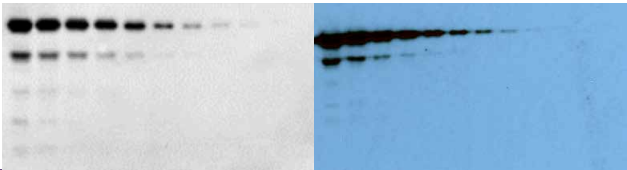
We know your time is precious. We can help you get to your western blot results faster with our time-efficient products. The **Invitrogen™ iWestern™ workflow** features innovative, modern solutions and is designed to save you time and effort. From precast gels to automated detection systems, we have the western blotting products that deliver results quickly, without sacrificing quality.



# High-efficiency western blotting

## iWestern workflow

At the core of the **iWestern workflow bundle** are four innovative products optimized to deliver the performance you expect from us. These products work together to help you achieve exceptional western blotting results with minimal hands-on time.



| Western steps                       | iWestern               | Manual                    |
|-------------------------------------|------------------------|---------------------------|
| Sample prep/<br>gel electrophoresis | 50 min                 | 90 min                    |
| Transfer                            | 7 min                  | 75 min                    |
| Blocking                            | 180 min                | 60 min                    |
| Primary incubation                  |                        | 720 min                   |
| Washes                              |                        | 60 min                    |
| Secondary incubation                |                        | 60 min                    |
| Substrate incubation                | 5 min                  | 5 min                     |
| Target detection                    | 2 min                  | 5 min                     |
| Total time                          | 244 min (approx. 4 hr) | 1,075 min (approx. 18 hr) |

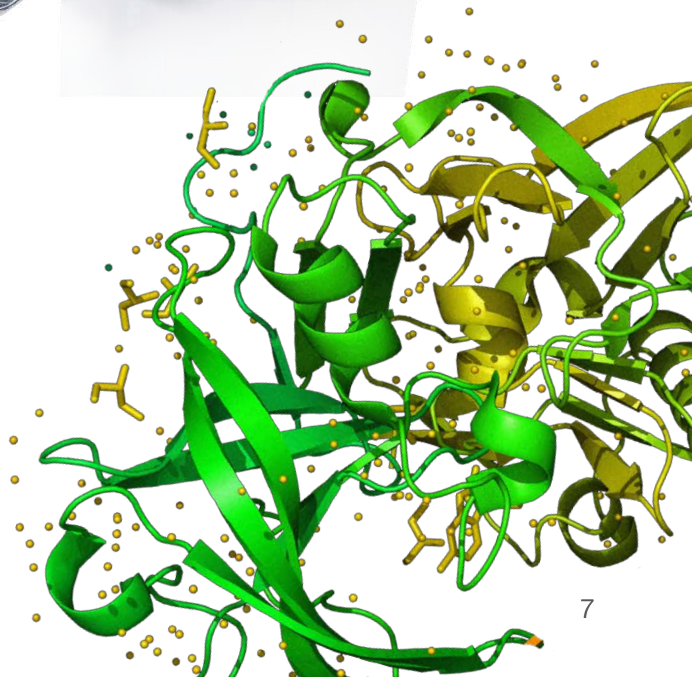
**Compare workflow and data obtained using the iWestern workflow bundle and manual western blotting.** The iWestern workflow enables the detection of HDAC1 in HeLa lysate in approximately 4 hr vs. 18 hr using the manual western workflow. Two-fold serial dilutions (starting with 20 µg of HeLa lysate) were loaded and separated on Tris-glycine gels. The proteins were transferred to nitrocellulose membranes and detected using Thermo Scientific™ SuperSignal™ West Pico PLUS chemiluminescent substrate.

Customize your bundle at [thermofisher.com/iwestern](https://thermofisher.com/iwestern)

## Modernized western blot workflow

The standard bundle contains our innovative iWestern workflow devices, including the necessary consumables and reagents to get started:

- Invitrogen™ Protein Gel Welcome Pack (including Mini Gel Tank)
- Invitrogen™ iBind™ 2 Starter Kit
- Invitrogen™ iBind™ Western Starter Kit
- Invitrogen™ iBright™ FL1500 Imaging System





## Unique chemistries for your unique protein

### Precast gels

Precast gels offer convenience, speed, and consistency. Invitrogen™ precast gels are available in four different chemistries and a wide variety of percentages, gradients, sample-well configurations, and formats. Gel formats include mini gels or the wider midi gel. The choice of which chemistry to use depends on the abundance and size of the protein, and your downstream application.

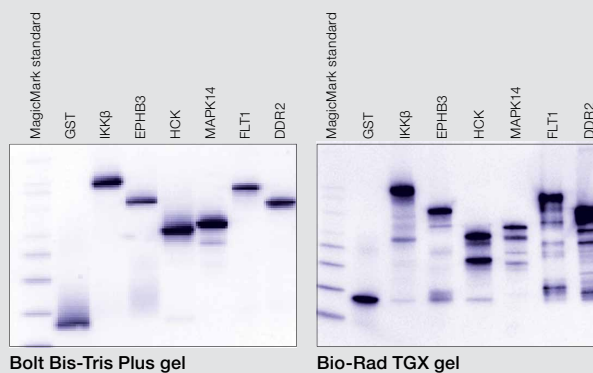
Available gel chemistries:

- **Tris-glycine chemistry** for broad-range, high-abundance protein separation
- **Bis-Tris chemistry** for broad-range, low-abundance protein separation or for applications requiring high protein integrity, such as posttranslational modification analysis, mass spectrometry, or sequencing
- **Tris-acetate chemistry** for high molecular weight protein separation
- **Tricine chemistry** for low molecular weight protein separation

### Load more with optimal separation

Invitrogen™ Bolt™ Bis-Tris Plus mini gels are precast polyacrylamide gels designed for optimal separation of a broad molecular weight range of proteins under denaturing conditions. 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, for publication-quality results the first time
- **High sample-volume capacity**—WedgeWell™ design with up to 60  $\mu$ L sample capacity allows easier loading and detection of proteins in very dilute samples or visualization of low-abundance proteins
- **High lot-to-lot consistency**—coefficient of variation (CV) of only 2% for  $R_f$  values (migration)



**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.

- **Better band quality and band volume**—Designed to deliver sharp, straight bands with higher band volume

Explore protein gel options at [thermofisher.com/proteingels](https://www.thermofisher.com/proteingels)



# Convenient and intuitive electrophoresis tank

## Mini Gel Tank

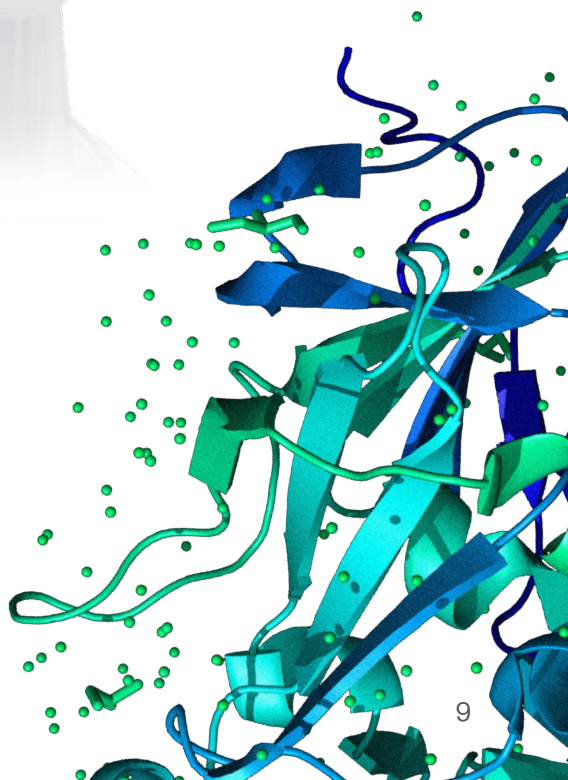
The **Invitrogen™ Mini Gel Tank** is designed for more intuitive use and convenience compared to traditional electrophoresis tanks.

- **Versatile**—compatible with all Invitrogen™ precast or handcast mini gels
- **Simultaneous visualization of both gels**—streamlined, side-by-side tank configuration
- **Easy sample loading**—with forward-facing well configuration
- **Less running buffer required**—two separate gel chambers; you only need to load sufficient buffer for each gel



**Protein Gel Welcome Packs** are available for each of our protein gels. Each welcome pack provides all of the necessary gels, buffers, and reagents you need to get started, as well as the Mini Gel Tank. Find out more at [thermofisher.com/proteingelwelcome](http://thermofisher.com/proteingelwelcome)

Find out more at [thermofisher.com/minigeltank](http://thermofisher.com/minigeltank)





## Fast protein transfer

### iBlot2 Gel Transfer Device



#### The Invitrogen™ iBlot™ 2 Gel Transfer Device

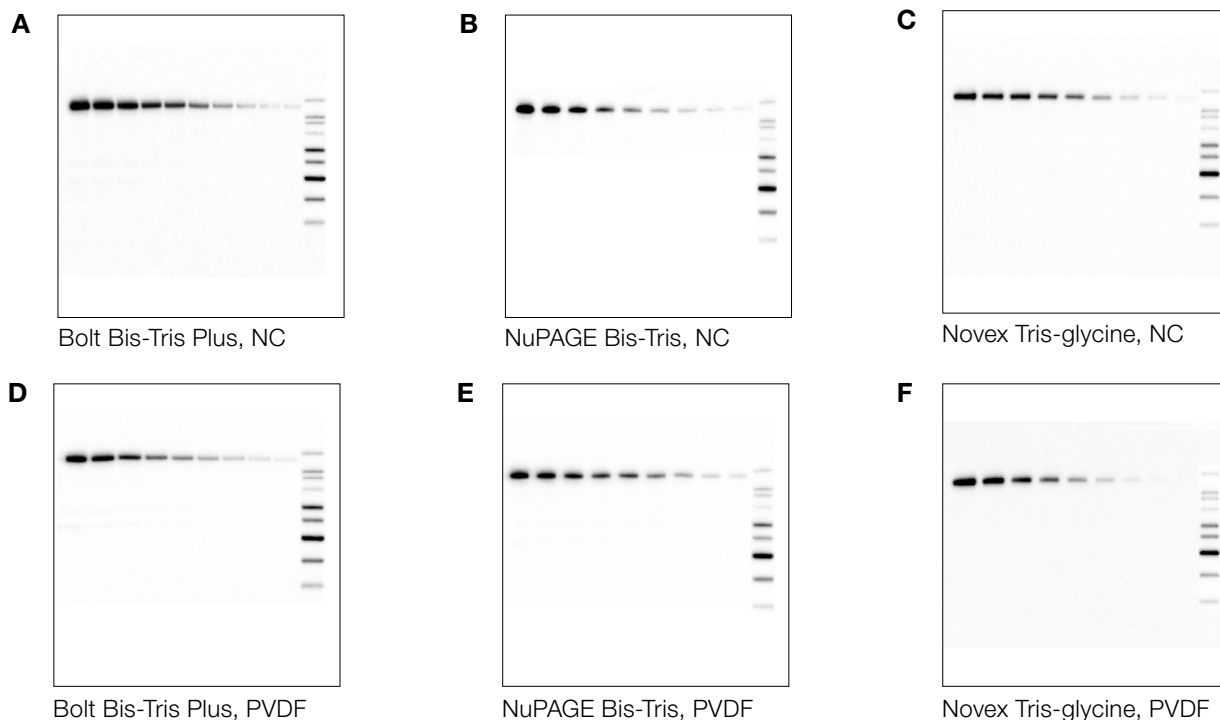
is our premium western blot transfer device, delivering high performance and convenience. A unique, innovative system, the iBlot 2 device utilizes optimized, preassembled transfer stacks with transfer buffer incorporated into gel matrices, so there's no need to prepare messy transfer buffers. Just insert your gel and go.

- **Engineered for rapid transfer**—the short distance between electrodes, along with high field strength and current, reduces transfer time to just 7 min

#### iBlot 2 Starter Kit (Cat. No. IB21001S)

- **Minimal preparation and cleanup**—transfer stacks streamline transfer setup and teardown
- **Convenient**—touchscreen interface, preprogrammed and optimized transfer protocols, and prepackaged ready-to-use stacks for transferring midi and mini blot formats (1 midi blot or up to 2 mini blots at a time)

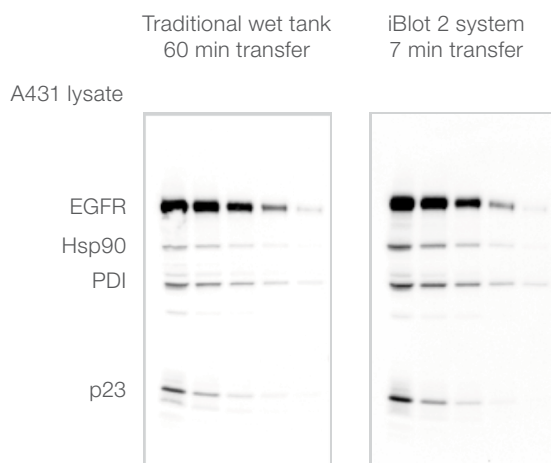
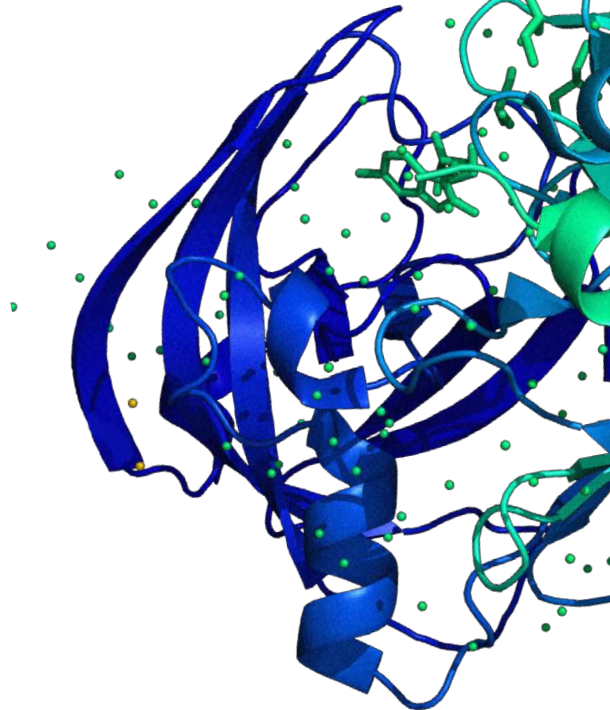
Find out more at [thermofisher.com/iblot2](https://thermofisher.com/iblot2)



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

## Get high-efficiency transfers in a fraction of the time

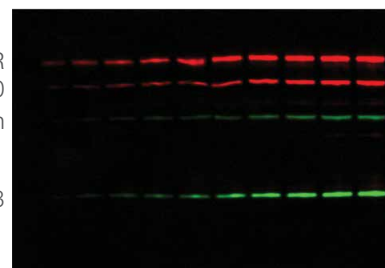
A successful western blot experiment relies on the quality of transfer of proteins from your gel to the blotting membrane. Quality of transfer is dependent on transfer efficiency and consistency. Traditional wet transfer offers high efficiency, but at a cost of time and hands-on effort. The iBlot 2 system offers the same high quality of transfer but with significant improvements in speed and convenience.



**Chemiluminescent detection: high transfer efficiencies achieved using the iBlot 2 Dry Blotting System.** A431 lysate was separated on Invitrogen™ Novex™ Tris-glycine gels and transferred to nitrocellulose using a traditional wet tank system or the iBlot 2 Gel Transfer Device. The membrane was probed for EGFR, Hsp90, PDI, and p23 and detected using Thermo Scientific™ SuperSignal™ West Dura Extended Duration Substrate.

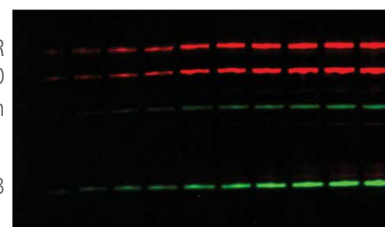
Traditional wet tank  
60 min transfer

EGFR  
Hsp90  
Tubulin  
p23



iBlot 2 system  
7 min transfer

EGFR  
Hsp90  
Tubulin  
p23



**Multiplex fluorescent detection: high transfer efficiencies achieved using the iBlot 2 Dry Blotting System.** A431 lysate was separated on NuPAGE Bis-Tris gels and transferred to nitrocellulose using a traditional wet tank system or the iBlot 2 Gel Transfer Device. The membrane was probed for EGFR, Hsp90, tubulin, and p23.

## Revolutionary western blot processing—no shakers, trays, or timers required

### Automated iBind Western Systems

**Invitrogen™ iBind™ Western Systems** are automated western blot processing platforms. Simply load primary antibody, secondary antibody, and wash solutions, and then walk away. In less than three hours, the blot is ready for final detection. iBind Western Systems offer:

- **Flexibility**—pick the system that matches your throughput; process 1 mini or midi blot, 2 mini blots, or 6 vertically cut strips using the same or different conditions
- **Antibody savings**—use up to 80% less primary antibody
- **Load and go**—the system processes solutions using sequential lateral flow technology, with no batteries, shakers, trays, or timers required
- **Reproducibility**—automated blot processing enables improved blot-to-blot consistency



**iBind Western Starter Kit (Cat. No. SLF1000S)**

#### iBind Western Device

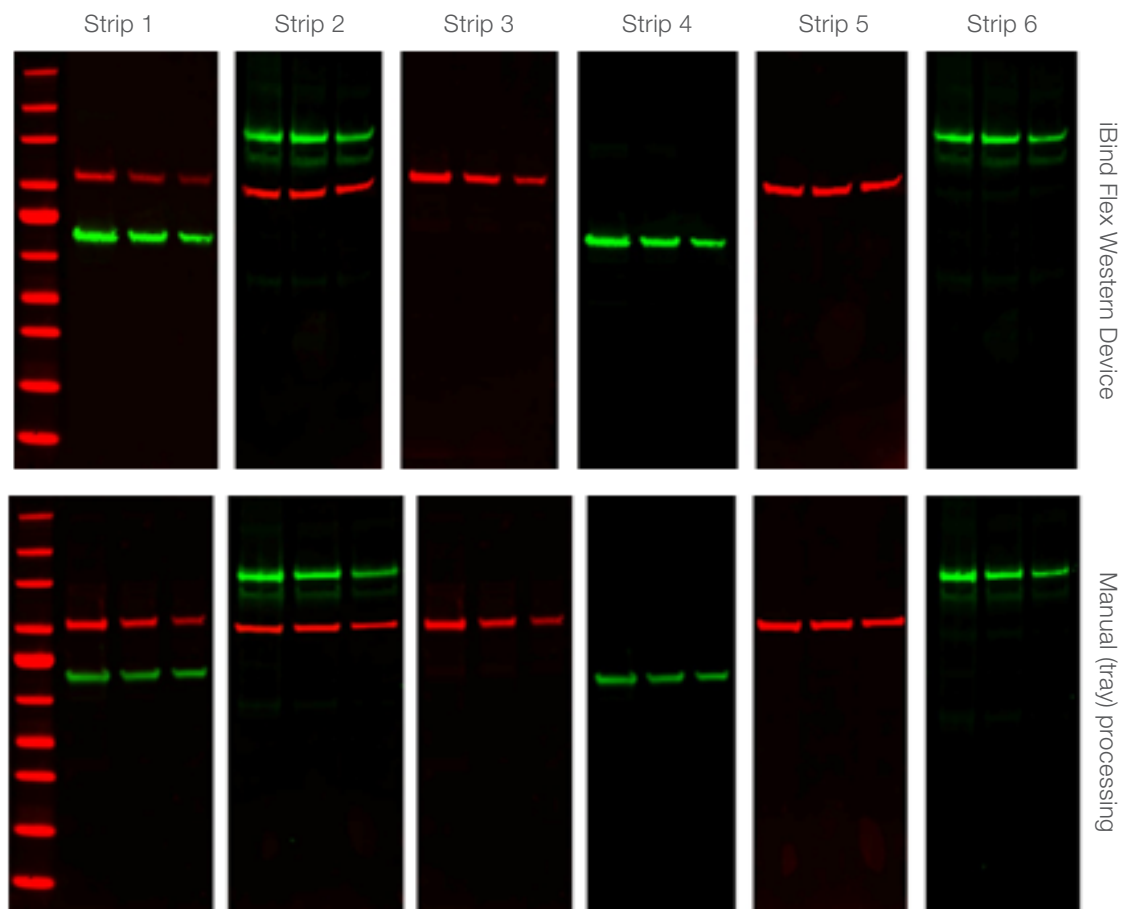
#### iBind Flex Western Device



|                                 |     |     |
|---------------------------------|-----|-----|
| Mini blot (8 x 8 cm), single    | Yes | Yes |
| Mini blot (8 x 8 cm), dual      | No  | Yes |
| Midi blot (13 x 8 cm)           | No  | Yes |
| Vertically cut strips (up to 6) | No  | Yes |

Watch a video demonstration at [thermofisher.com/ibind](http://thermofisher.com/ibind)

## Excellent western performance compared to manual blotting



**Fluorescence detection with the iBind Flex Western Device.** Comparison of strip 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 3-lane strips. Get more information about the target proteins and antibodies used at [thermofisher.com/ibind](https://thermofisher.com/ibind).



## Stunningly easy western blot imaging

### iBright Imaging Systems

#### Simplified western blot and gel imaging

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

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



#### 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 onboard data analysis provide a smooth imaging experience
- **Five-channel fluorescent blotting**—multiplex with the five fluorescence channels of the iBright FL1500 model; capture up to four proteins in a single blot for more meaningful and representative experiments
- **Compliance support**—all models offered with 21 CFR Part 11 compliance support software packages to set up and control security, audit, and e-signature settings

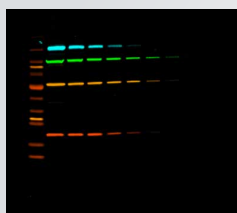
Capture your data faster and more easily than ever before.  
Find out more at [thermofisher.com/ibright](https://thermofisher.com/ibright)

## The core applications you need and the specialty applications you want

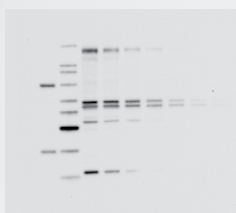
iBright Imaging Systems offer up to five imaging modes to support your multiple applications. Efficiently and easily capture data from protein gels, nucleic acid gels, chemiluminescent western blots, fluorescent western blots, and more.

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

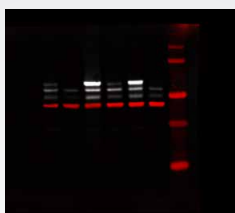
## Example imaging applications



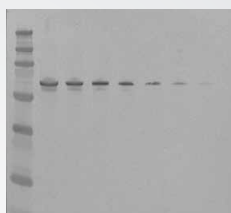
Fluorescent western blots



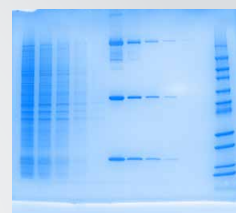
Chemiluminescent western blots



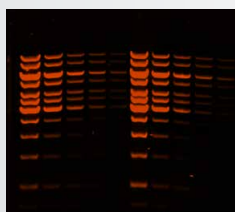
Combined fluorescent and chemiluminescent western blots



Colorimetric western blots



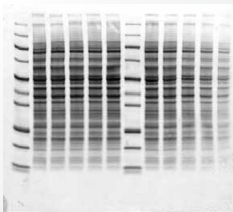
Colorimetric stained protein gels



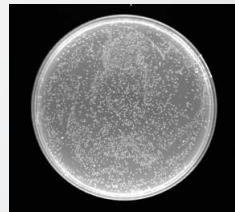
Fluorescent stained nucleic acid gels



Fluorescent stained protein gels



Colorimetric membrane stains



Colony plates

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

## Ordering information

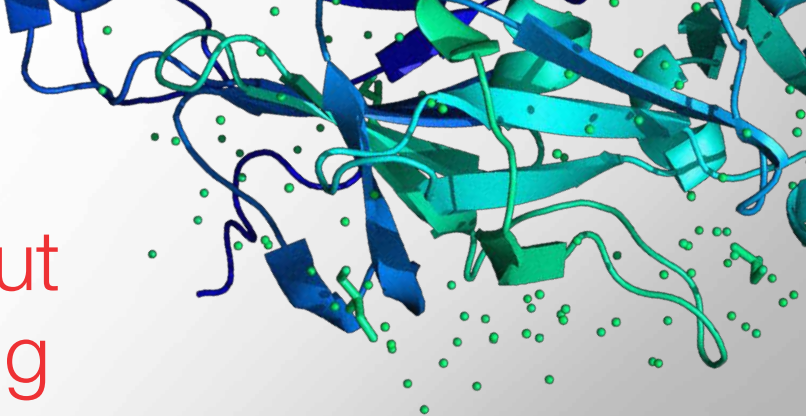
| Separate                                           | Quantity     | Cat. No.   |
|----------------------------------------------------|--------------|------------|
| Mini Gel Tank                                      | 1 unit       | A25977     |
| Mini Gel Tank and Blot Module Set                  | 1 set        | NW2000     |
| Bolt Bis-Tris 4–12%, 10-well Welcome Pack          | 1 kit        | NW0412A    |
| Bolt Bis-Tris 4–12%, 15-well Welcome Pack          | 1 kit        | NW0412B    |
| Transfer                                           | Quantity     | Cat. No.   |
| iBlot 2 Gel Transfer Device                        | 1 device     | IB21001    |
| iBlot 2 Starter Kit                                | 1 kit        | IB21001S   |
| 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    |
| Bolt Welcome Pack with iBlot 2 Dry Blotting System | 1 kit        | NW0412AIB2 |
| Detect                                             | Quantity     | Cat. No.   |
| 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   |
|                                                    | 1 device     | SLF2000    |
| iBind Flex Western Device                          | 2 devices    | SLF20002PK |
|                                                    | 10 cards     | SLF2010    |
| iBind Flex Cards                                   | 40 cards     | SLF2010X4  |
|                                                    | 1 kit        | SLF2020    |
| iBind Flex Solution Kit                            | 4 kits       | SLF2020X4  |
| iBright CL750 Imaging System                       | 1 instrument | A44116     |
| iBright CL1500 Imaging System                      | 1 instrument | A44240     |
| iBright FL1500 Imaging System                      | 1 instrument | A44241     |

Find out more at [thermofisher.com/iwestern](https://thermofisher.com/iwestern)

# High-throughput western blotting

## Processing many samples or blots?

We offer products that are designed for your high-throughput needs. From our precast midi gels that process up to 26 samples per gel to commonly used western blot buffers in larger pack sizes, we have your high-throughput western blot needs covered.



# Running or transferring from 1 to 16 gels—we have a power supply that can support your power needs

## PowerEase power supplies

The **Invitrogen™ PowerEase™ Touch 350W Power Supply** brings a high level of convenience to your electrophoresis experiments. With a bright LCD touchscreen interface, you can enter in custom programs, or use the preprogrammed protocols for Invitrogen™ protein gels and gel transfers. It can run up to 8 midi gels or transfer up to 4 midi gels simultaneously.



Each program can include up to 20 steps, for precise control over electrophoresis conditions. In addition, the PowerEase Touch 350W Power Supply features:

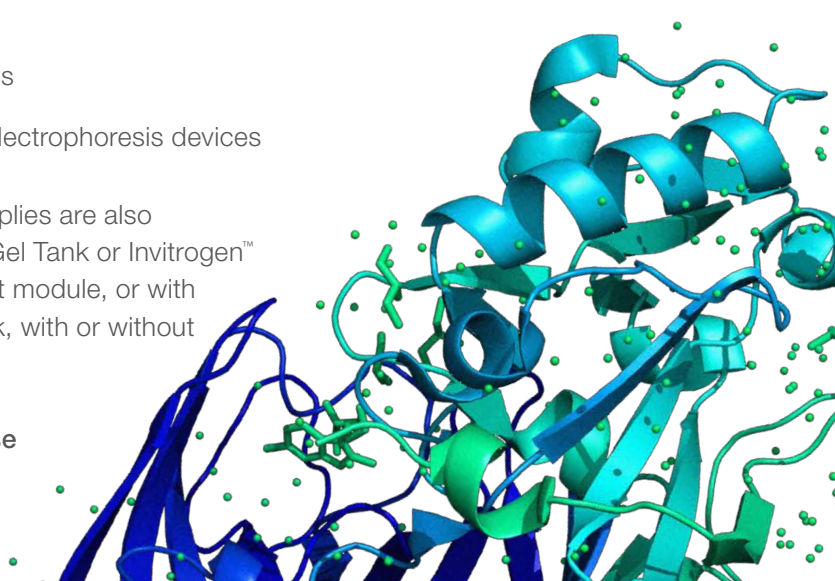
- Constant voltage, current, or power settings
- Built-in timer for walk-away gel electrophoresis
- Storage for up to 100 custom programs with 20 steps each
- Four sets of output jacks that are compatible with most electrophoresis devices

The **Invitrogen™ PowerEase™ Touch 120W 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 120W Power Supply features:

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

PowerEase Touch 120W and 350W Power Supplies are also available in money-saving bundles with a Mini Gel Tank or Invitrogen™ XCell SureLock™ Mini-Cell, with or without a blot module, or with an Invitrogen™ SureLock™ Tandem Midi Gel Tank, with or without a blot module.

Find out more at [thermofisher.com/powerease](https://thermofisher.com/powerease)





## Load up to 26 samples

### Precast midi gels

**Invitrogen™ precast midi gels** are made with high-purity, strictly quality-controlled reagents to provide top-quality, consistent protein separation. Midi gels have a wider format (8 cm x 13 cm), designed for your higher-throughput electrophoresis needs.

#### Precast midi gel options

1. The Invitrogen™ NuPAGE™ SDS-PAGE Midi Gel System is a revolutionary neutral-pH, discontinuous SDS-PAGE system. The neutral environment (pH 7.0) during electrophoresis results in maximum stability of both proteins and gel matrix, providing better band resolution than other gel systems. This system has two gel formulations: NuPAGE Bis-Tris and NuPAGE Tris-acetate midi gels.
2. The Invitrogen™ Novex™ Tris-Glycine Plus Midi Gel System is based on the Laemmli system, with minor modifications for maximum performance in the precast format with extended shelf life and fast run times.



**Both midi gel systems are available in a variety of acrylamide concentrations and well formats.**

## Welcome packs to help you get started

Invitrogen midi gels are available in welcome packs, bundled with gels, buffers, and ladders, as well as a SureLock Tandem Midi Gel Tank at no extra cost.

- Find out more at [thermofisher.com/proteingels](https://thermofisher.com/proteingels)



## Run or transfer up to 2 Invitrogen midi gels in the same tank

### Electrophoresis chamber systems

The **SureLock Tandem Midi Gel Tank** was designed for easy and consistent vertical protein gel electrophoresis of 1 or 2 Invitrogen midi gels. When paired with the Invitrogen™ SureLock™ Midi Transfer Module, this tank performs efficient, 30-minute, room-temperature, wet protein transfers. Separate chambers make it easy to run just one gel or transfer, allowing you to save on buffer and limit methanol waste and disposal costs. Save the time, planning, and mess required with other systems to pre-chill buffers, freeze ice packs, or prepare ice baths. Convenient, precut membranes and filter papers are available to speed up your work.



The SureLock Tandem Midi Gel Tank is available separately or in a variety of product bundles for built-in savings. Welcome packs are available with the SureLock Tandem Midi Blot Module and PVDF or nitrocellulose membranes, and Invitrogen™ SureLock™ Tandem Transfer Tray.

## Simultaneous electrophoresis of up to four midi gels

### XCell4 SureLock Midi-Cell system

The **Invitrogen™ XCell4™ SureLock™ Midi-Cell** system allows simultaneous vertical electrophoresis of 1–4 midi gels, enabling high throughput. It uses unique 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/surelocktandem](https://thermofisher.com/surelocktandem)



## Stay stocked and save more

### Bulk reagents

We offer large pack sizes of our most popular buffers and ladders to meet your high-throughput electrophoresis needs.

#### Protein electrophoresis running buffers available in 5 L

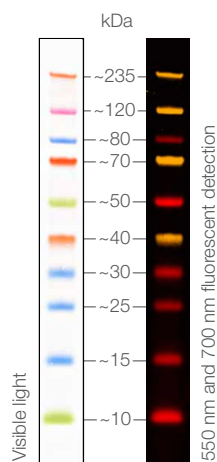
- Invitrogen™ Novex™ Tris-Glycine SDS Running Buffer
- Invitrogen™ NuPAGE™ MES SDS Running Buffer
- Invitrogen™ NuPAGE™ MOPS SDS Running Buffer

#### Protein stains (3.5 L)

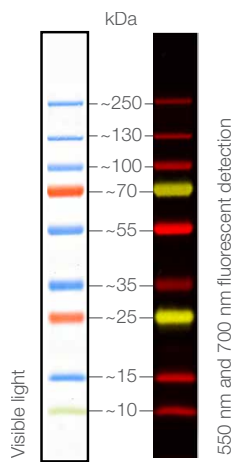
- Thermo Scientific™ GelCode™ Blue Stain Reagent
- Invitrogen™ SimplyBlue™ SafeStain



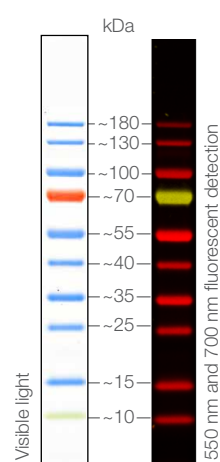
#### Protein ladders available in larger pack sizes (10 x 250 µL)



Thermo Scientific™ Spectra™  
Multicolor Broad Range  
Protein Ladder



Thermo Scientific™ PageRuler™  
Plus Prestained Protein Ladder,  
10 to 250 kDa



Thermo Scientific™ PageRuler™  
Prestained Protein Ladder,  
10 to 180 kDa

## Flexible protein transfer

### Power Blotter Transfer System

The **Invitrogen™ Power Blotter System** features interchangeable blotting cassettes to suit your required throughput, and multiple transfer-stack consumable choices. Designed for rapid 5–10 min transfer of proteins from polyacrylamide gels to nitrocellulose or PVDF membranes, the Power Blotter utilizes an integrated power supply, LCD touchscreen, and preprogrammed, optimized transfer protocols.

- **Versatile**—can be used with do-it-yourself, ready-to-build, or ready-to-use transfer stacks
- **Efficient**—high transfer efficiency for a broad range of protein sizes
- **High throughput**—transfer up to 4 mini or 2 midi gels simultaneously with the Power Blotter XL System

#### Designed to grow around your lab's needs

The Power Blotter platform offers options based on throughput requirements. Both cassettes are interchangeable with the Invitrogen™ Power Blotter Station power supply and control base.



**Power Blotter XL Welcome Pack**  
(Cat. No. PB0113)

#### Power Blotter System

Transfer 1–2 mini-size gels or  
1 midi-size gel



#### Power Blotter XL System

Transfer 1–4 mini-size gels or  
2 midi-size gels



#### The Power Blotter offers high transfer efficiency for a broad range of protein sizes

| Apparatus and consumables | Power Blotter with Select Transfer Stacks | Power Blotter with Pre-cut Membranes and Filters and Power Blotter 1-Step Transfer Buffer |
|---------------------------|-------------------------------------------|-------------------------------------------------------------------------------------------|
| Transfer time             | 5–10 min                                  | 5–10 min                                                                                  |
| Transfer efficiency       |                                           |                                                                                           |

**Invitrogen™ Power Blotter Select Transfer Stacks and Power Blotter Pre-cut Membranes and Filters efficiently transfer high, medium, and low molecular weight proteins.** Western blot analysis of several targets (KLH, EGFR, Hsp90, PDI, and cyclophilin B protein) was performed by loading serially diluted HeLa cell lysate with KLH spike (starting at 7.5 µg HeLa lysate, 7.5 µg KLH spike per well, serially diluted 2:3) onto Bolt 4–12% Bis-Tris Plus gels. Proteins were transferred in 7 min using a Power Blotter Select Transfer Stack (left, Cat. No. PB5310 and PB3310) or Power Blotter Pre-cut Membranes and Filters (right, Cat. No. PB9320 and PB7320), probed with target-specific primary antibodies and fluorescently conjugated secondary antibodies. Images were captured using automatic exposure on an iBright FL1000 Imaging System.

Find out more at [thermofisher.com/powerblotter](http://thermofisher.com/powerblotter)



## Automated western blot processing

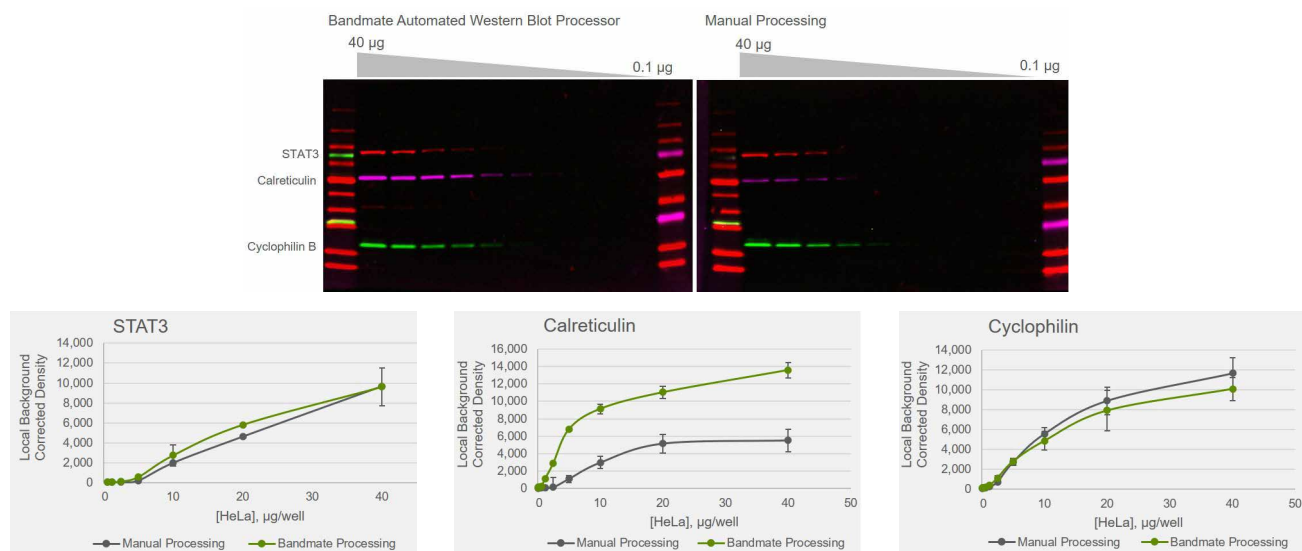
### Bandmate Automated Western Blot Processor

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

The Bandmate Automated Western Blot Processor offers:

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

Explore more at [thermofisher.com/bandmate](https://thermofisher.com/bandmate)



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



## Ordering information

| Separate                                                                  | Quantity    | Cat. No. |
|---------------------------------------------------------------------------|-------------|----------|
| PowerEase Touch 350W Power Supply (115 VAC)                               | 1 unit      | PS0350   |
| PowerEase Touch 120W Power Supply (115 VAC)                               | 1 unit      | PS0120   |
| PowerEase Touch 350W Power Supply (230 VAC)                               | 1 unit      | PS0351   |
| PowerEase Touch 120W Power Supply (230 VAC)                               | 1 unit      | PS0121   |
| XCell4 <i>SureLock</i> Midi-Cell                                          | 1 unit      | WR0100   |
| SureLock Tandem Midi Gel Tank                                             | 1 unit      | STM1001  |
| Novex Tris-Glycine SDS Running Buffer                                     | 5 L         | LC26755  |
| NuPAGE MES SDS Running Buffer                                             | 5 L         | NP000202 |
| NuPAGE MOPS SDS Running Buffer                                            | 5 L         | NP000102 |
| Spectra Multicolor Broad Range Protein Ladder                             | 10 x 250 µL | 26623    |
| PageRuler Plus Prestained Protein Ladder                                  | 10 x 250 µL | 26620    |
| PageRuler Prestained Protein Ladder, 10 to 180 kDa                        | 10 x 250 µL | 26617    |
| SimplyBlue SafeStain                                                      | 3.5 L       | LC6065   |
| GelCode Blue Stain Reagent                                                | 3.5 L       | 24592    |
| Transfer                                                                  | Quantity    | Cat. No. |
| Power Blotter Welcome Pack                                                | 1 kit       | PB0112   |
| Power Blotter XL Welcome Pack                                             | 1 kit       | PB0113   |
| Power Blotter Station                                                     | 1 unit      | PB0010   |
| Power Blotter Cassette                                                    | 1 cassette  | PB0002   |
| Power Blotter Cassette XL                                                 | 1 cassette  | PB0003   |
| Power Blotter System                                                      | 1 system    | PB0012   |
| Power Blotter XL System                                                   | 1 system    | PB0013   |
| Power Blotter Select Transfer Stacks, nitrocellulose, mini                | 10 stacks   | PB3210   |
|                                                                           | 40 stacks   | PB3240   |
| Power Blotter Select Transfer Stacks, PVDF, mini                          | 10 stacks   | PB5210   |
|                                                                           | 40 stacks   | PB5240   |
| Power Blotter Select Transfer Stacks, nitrocellulose, regular size        | 10 stacks   | PB3310   |
|                                                                           | 40 stacks   | PB3340   |
| Power Blotter Select Transfer Stacks, PVDF, regular size                  | 10 stacks   | PB5310   |
|                                                                           | 40 stacks   | PB5340   |
| Power Blotter Pre-cut Membranes and Filters, nitrocellulose, mini         | 20 stacks   | PB7220   |
| Power Blotter Pre-cut Membranes and Filters, PVDF, mini                   | 20 stacks   | PB9220   |
| Power Blotter Pre-cut Membranes and Filters, nitrocellulose, regular size | 20 stacks   | PB7320   |
| Power Blotter Pre-cut Membranes and Filters, PVDF, regular size           | 20 stacks   | PB9320   |
|                                                                           | 250 mL      | PB7100   |
| Power Blotter 1-Step Transfer Buffer (5X)                                 | 1 L         | PB7300   |
| Detect                                                                    | Quantity    | Cat. No. |
| Bandmate Automated Western Blot Processor                                 | 1 system    | BW1000   |

# Multiplex western blotting

Multiplex fluorescent western blotting affords the ability to clearly evaluate multiple protein targets on a single blot. It can save time, reduce cost, and improve the efficiency of your data generation and collection. Choosing products uniquely designed for fluorescent western blotting is key to generating clean, specific results.

Explore our comprehensive product solutions for multiplex western blotting from sample preparation to data collection. These products provide accurate, quantitative results and stable signals to help you optimize each step, avoid pitfalls, and achieve your best outcomes.

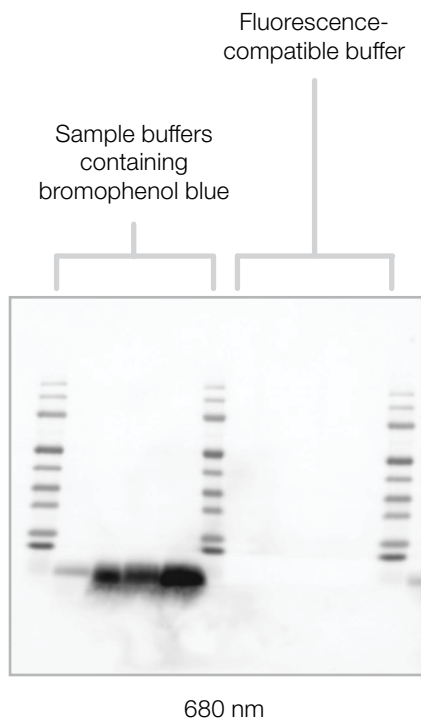


## Fluorescence-compatible sample buffers

Sample buffers containing bromophenol blue will fluoresce and can contribute to increased background. While the dye front may be run off the gel prior to transfer or cut from the membrane after transfer to avoid background fluorescence signal, it is ideal to use fluorescence-compatible sample buffers without bromophenol blue, such as **Invitrogen™**

### Fluorescent Compatible Sample Buffer.

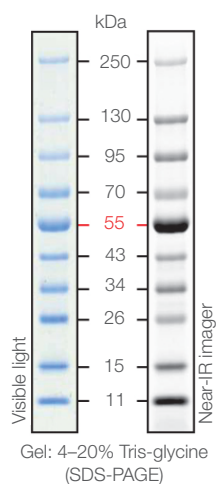
- **Nonreducing**—ready to use for nonreducing SDS-PAGE, or add the preferred type and amount of reducing agent (e.g., DTT) to produce reducing conditions
- **Lane marker dyes**—visible dye front to monitor run
- **Convenient**—stable at room temperature, enabling storage at the bench where electrophoresis is performed



**HeLa lysates prepared with Fluorescent Compatible Sample Buffer or with Novex Tris-Glycine SDS Sample Buffer.** Samples were separated on a Tris-glycine gel and transferred to a nitrocellulose membrane. Image was captured using the appropriate filter sets for Near-IR dyes at 680 nm.

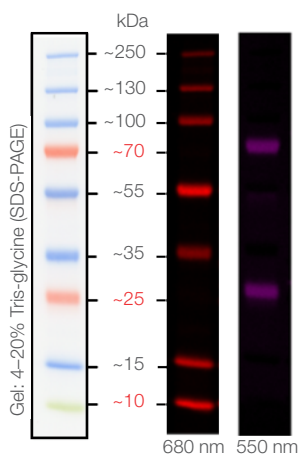
## Prestained fluorescent ladders

During western blotting, prestained and fluorescent protein ladders can be used for approximate determination of molecular weight, monitoring the progress of electrophoresis runs, and/or estimating the efficiency of protein transfer to the membrane during western blotting. We offer several prestained and fluorescent protein ladders supplied in a ready-to-use format to facilitate easy protein analysis during gel electrophoresis and fluorescent western blotting.



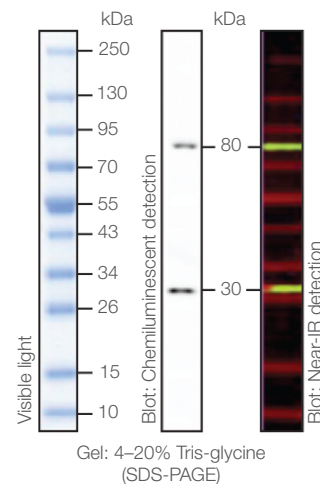
### Thermo Scientific™ PageRuler™ Prestained NIR Protein Ladder

- Ten blue-stained proteins; also fluorophore-labeled to enable direct and near-IR (NIR) fluorescent visualization



### Thermo Scientific™ PageRuler™ Plus Prestained Protein Ladder

- Nine color-stained proteins, providing NIR fluorescence with seven bands, and RGB fluorescence (550 nm channel) with the two orange bands



### Invitrogen™ iBright™ Prestained Protein Ladder

- Ten blue-stained proteins; also fluorophore-labeled to enable direct and NIR fluorescent visualization
- Two unstained proteins (30 kDa and 80 kDa) that enable visualization with chemiluminescent or fluorescent detection

# Fluorescence-friendly transfer membranes

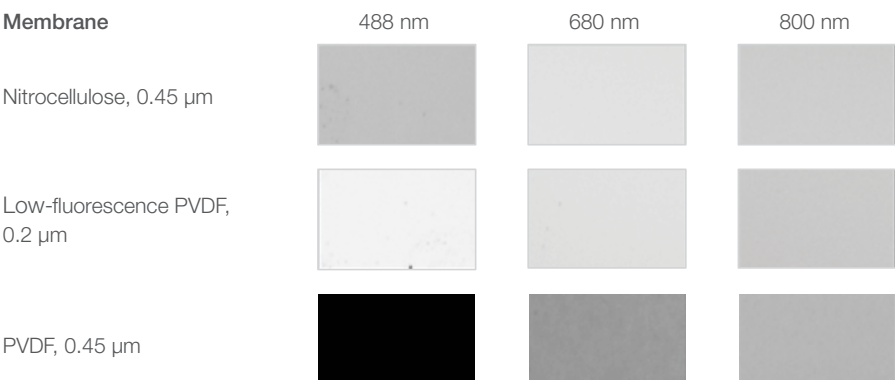
To eliminate a major source of background fluorescence, we recommend using membranes with low autofluorescence, including nitrocellulose and specialty low-fluorescence PVDF membranes.

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



|                       | Mini                                                                | Midi                                                                                  | Roll                                            |
|-----------------------|---------------------------------------------------------------------|---------------------------------------------------------------------------------------|-------------------------------------------------|
| Nitrocellulose        | Nitrocellulose/filter Pre-Cut Blotting Membranes, 0.2 µm pore size  | Nitrocellulose/filter Pre-Cut Blotting Membranes, 0.2 µm pore size, 8.5 cm x 13.5 cm  | Nitrocellulose Membrane, 0.45 µm, 30 cm x 3.5 m |
|                       | Nitrocellulose/filter Pre-Cut Blotting Membranes, 0.45 µm pore size | Nitrocellulose/filter Pre-Cut Blotting Membranes, 0.45 µm pore size, 8.5 cm x 13.5 cm |                                                 |
| Low-fluorescence PVDF | Low-Fluorescence PVDF Transfer Membrane, 0.2 µm, 7 cm x 8.4 cm      |                                                                                       |                                                 |

## Background fluorescence of common membranes



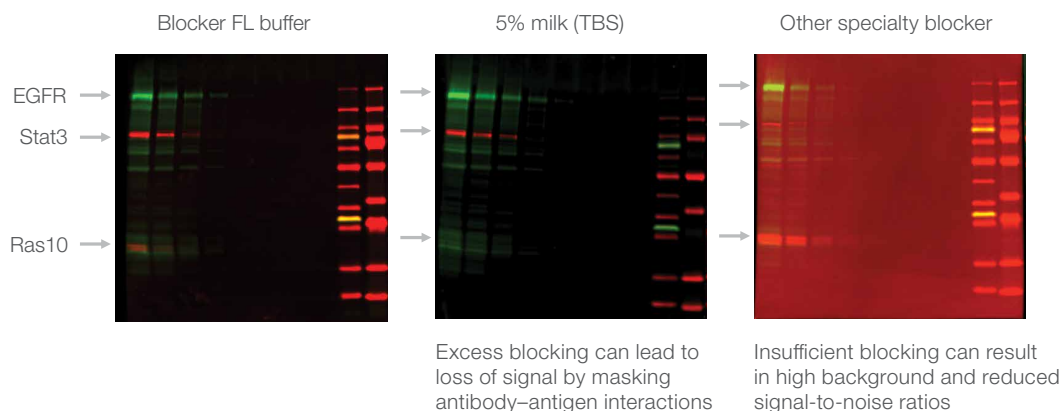
Learn more at [thermofisher.com/membranes](https://thermofisher.com/membranes)





## High-quality, filtered blocking buffers

Particles and contaminants in blocking and wash buffers can settle on membranes and create fluorescent artifacts, so it's important to use high-quality, filtered buffers in fluorescent western blotting. In addition, limit the use of detergents during blocking steps, as common detergents can autofluoresce, possibly increasing nonspecific background.



## Blocker FL Fluorescent Blocking Buffer

**Thermo Scientific™ Blocker™ FL Fluorescent Blocking Buffer** is designed to block excess nonspecific binding sites to help reduce background fluorescence in western blotting applications. Blocker FL buffer helps enhance fluorescent protein detection by increasing the signal-to-noise ratio in blotting applications where background fluorescence may otherwise interfere with signal detection.



Find out more at [thermofisher.com/blockingbuffers](https://thermofisher.com/blockingbuffers)



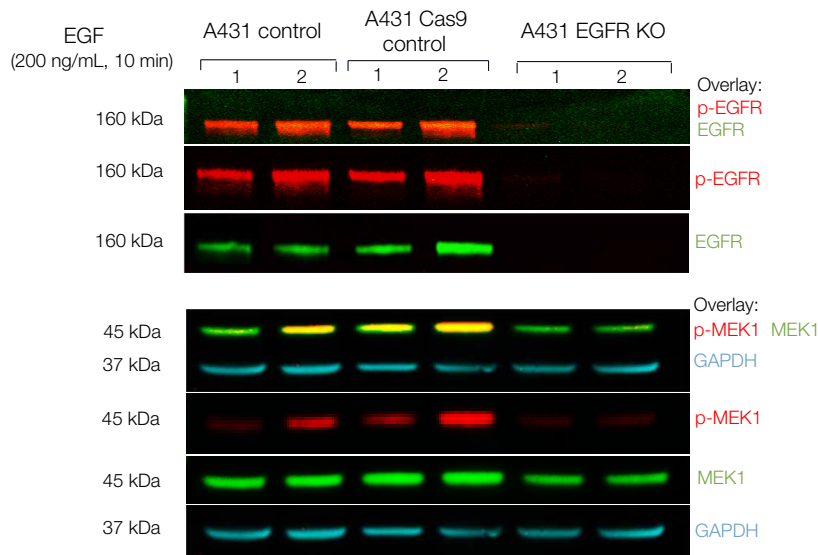
## Fluorescent-labeled antibodies

### Get clean, multiplexed fluorescent western blots with Invitrogen™ cross-adsorbed secondary antibodies

Cross-adsorbed secondary antibodies are polyclonal antibodies that are manufactured with an additional purification step to filter out members that bind to off-target species of immunoglobulin (IgG). The process decreases species cross-reactivity and increases specificity.

We offer a wide range of cross-adsorbed, specific antibody conjugates for target and host species, including Invitrogen™ Alexa Fluor™, Alexa Fluor™ Plus, and classical fluorophores (FITC, TRITC, Cy®3, Cy®5, Cy®5.5, APC, etc.). Alexa Fluor conjugates come in 19 colors and 45 host/target species combinations. Alexa Fluor Plus conjugates come in 6 colors and 6 host/target species combinations.

- **Avoid cross-reactivity**—use our secondary antibodies that are highly cross-adsorbed against serum proteins or IgG of other species
- **Highly purified**—our secondary antibodies are highly purified and manufactured for lot-to-lot consistency
- **Alexa Fluor and Alexa Fluor Plus secondary antibodies**—get distinct fluorescent bands in multiplex experiments due to easy selection of non-overlapping spectra from a variety of conjugates
- **Easy visualization**—clearly detect independent bands in the same lane and blot
- **Sensitive detection**—use Alexa Fluor Plus secondary antibodies for detection of low-abundance proteins



Alexa Fluor  
Secondary Antibody

**Fluorescent western blot analysis of p-EGFR, EGFR, p-MEK1, MEK1, and GAPDH in A431 control, Cas9, and EGFR KO cells.** Targets were detected using Alexa Fluor Plus donkey anti-rabbit 680 and 800 conjugates and Alexa Fluor Plus donkey anti-goat 488 and 555 conjugated secondary antibodies (Cat. No. A32814 and A32816).

Search for your secondary antibodies at [thermofisher.com/secondaries](https://www.thermofisher.com/secondaries)

# Total protein normalization with fluorescent No-Stain Protein Labeling Reagent

## Get accurate total protein normalization

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

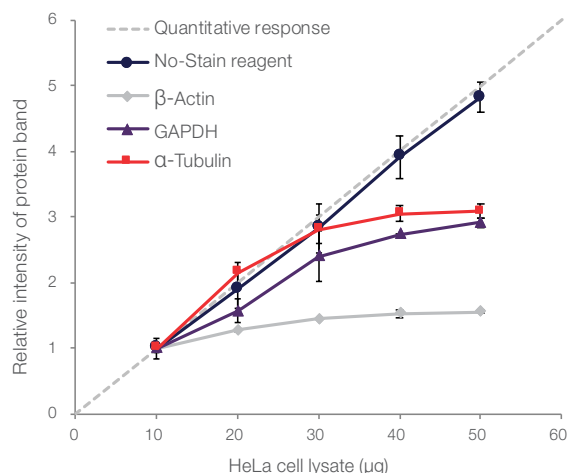
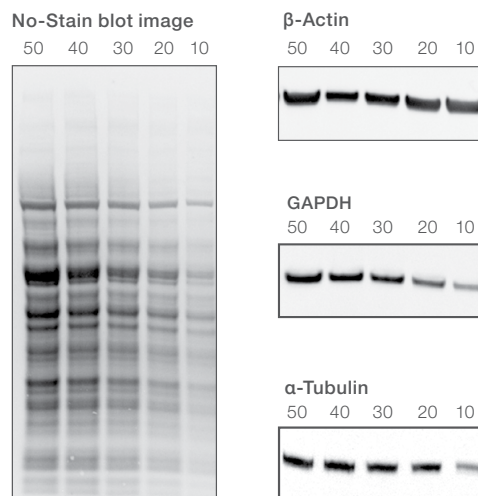
## Features

**Flexible-use labeling format**—use with any protein gel to perform total protein labeling of the membrane posttransfer, or use it as a fast protein stain after electrophoresis of gels you do not intend to transfer

**Easy-to-use and rapid protocol**—mix and incubate with the transferred PVDF or nitrocellulose membrane or gel to label lysine residues; the reaction time is 10 min

**Flexible visualization**—visualize with UV or blue LED transilluminators, or by using imaging systems with fluorescence (~488 nm) light sources, including iBright Imaging Systems

**Accurate total protein normalization**—broad linear range for protein detection of 1–80 µg (total protein load); protein bands are detected down to 20 ng and signal is compatible with antibody detection using chemiluminescence or fluorescence methods



## Total protein normalization with No-Stain Protein Labeling

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

Find out more at [thermofisher.com/no-stain](http://thermofisher.com/no-stain)

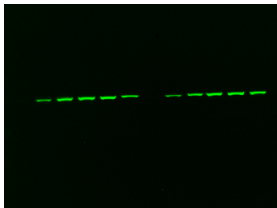


Let your colors show with iBright Imaging Systems

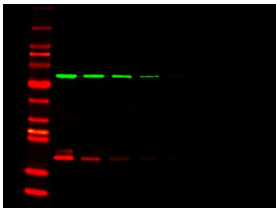
The iBright FL1500 model features five fluorescence channels, permitting up to four-color fluorescent western blot multiplexing—expanding your possibilities for studying multiple proteins in a single blot. Obtain meaningful and representative comparisons to help enhance your experiments. Built-in Smart Exposure™ technology further improves acquisition of multiplex fluorescent western blot data, because signal-to-noise ratios are optimized for each fluorescence channel separately.

Filter sets are pre-installed in iBright FL1500 Imaging Systems for visible light range (RGB) and NIR fluorescent western blotting applications.

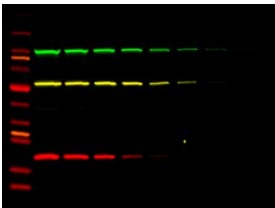
| Example compatible fluorophores       | Excitation channel | Filter range (nm) | Emission channel | Filter range (nm) |
|---------------------------------------|--------------------|-------------------|------------------|-------------------|
| Alexa Fluor Plus 488, Alexa Fluor 488 | EX1                | 455–485           | EM1              | 508–557           |
| Alexa Fluor Plus 555, Alexa Fluor 546 | EX2                | 515–545           | EM2              | 568–617           |
| Alexa Fluor Plus 647, Alexa Fluor 594 | EX3                | 608–632           | EM3              | 675–720           |
| Alexa Fluor Plus 680, Alexa Fluor 680 | EX4                | 610–660           | EM4              | 710–730           |
| Alexa Fluor Plus 800, Alexa Fluor 790 | EX5                | 745–765           | EM5              | 800–850           |



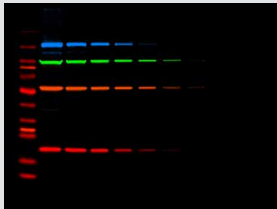
One-color fluorescence blot



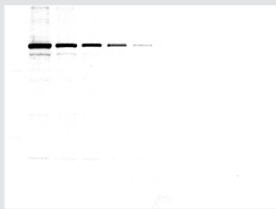
Two-color fluorescence blot



Three-color fluorescence blot



Four-color fluorescence blot



Alexa Fluor Plus 488 dye—RB1 protein with GST and HA tag (134 kDa)



Alexa Fluor 546 dye—calreticulin protein (55 kDa)



Alexa Fluor Plus 680 dye—p23 protein (23 kDa)



Alexa Fluor Plus 800 dye—HSP90 protein (90 kDa)

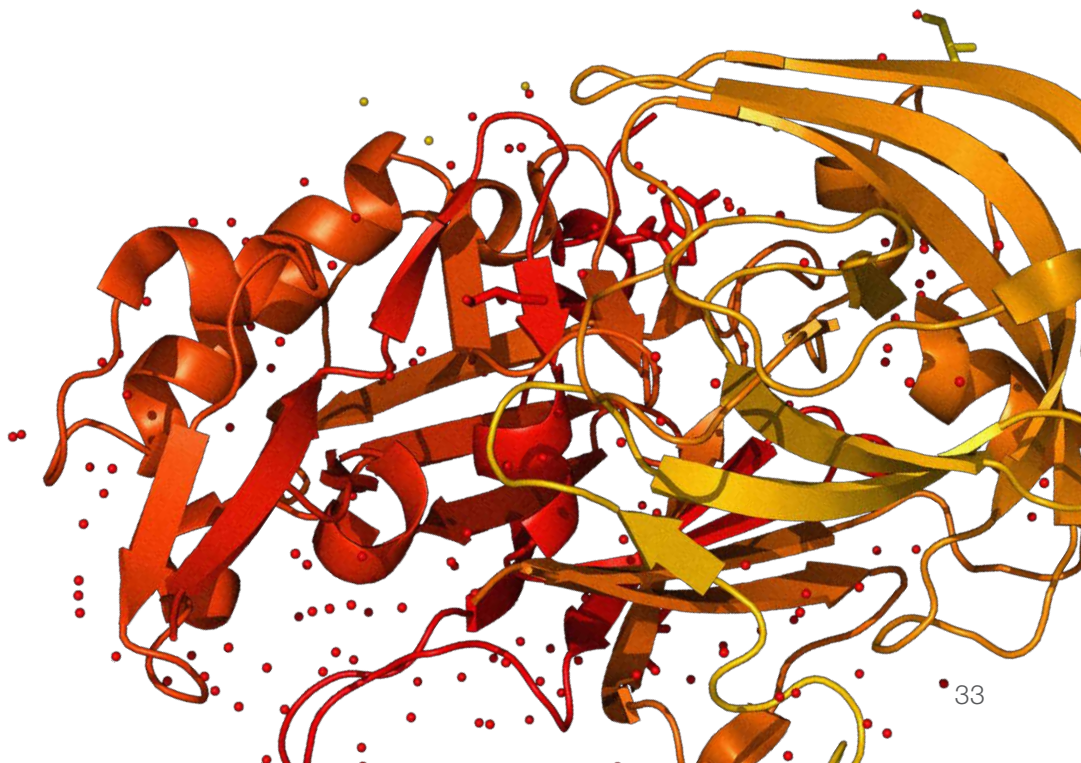


Membrane—iBright prestained marker

Four-color multiplexed fluorescent blot: false color composite top left, and individual channels shown in grayscale as pictured.

Ordering information

| Separate                                                                            | Quantity   | Cat. No. |
|-------------------------------------------------------------------------------------|------------|----------|
| Fluorescent Compatible Sample Buffer (4X, nonreducing)                              | 5 mL       | LC2570   |
| iBright Prestained Protein Ladder                                                   | 2 x 250 µL | LC5615   |
| PageRuler Plus Prestained Protein Ladder, 10 to 250 kDa                             | 2 x 250 µL | 26619    |
| PageRuler Prestained NIR Protein Ladder                                             | 2 x 250 µL | 26635    |
| Transfer                                                                            | Quantity   | Cat. No. |
| Nitrocellulose/Filter Pre-cut Blotting Membranes, 0.2 µm pore size, (8.3 x 7.3 cm)  | 20 pack    | LC2000   |
| Nitrocellulose/Filter Pre-cut Blotting Membranes, 0.45 µm pore size, (8.3 x 7.3 cm) | 20 pack    | LC2001   |
| Nitrocellulose/Filter Pre-cut Blotting Membranes, 0.2 µm pore size, (8.5 x 13 cm)   | 16 pack    | LC2009   |
| Nitrocellulose/Filter Pre-cut Blotting Membranes, 0.45 µm pore size, (8.5 x 13 cm)  | 16 pack    | LC2006   |
| Nitrocellulose Membrane Roll, 0.45 µm pore size (30 cm x 3.5 m)                     | 1 roll     | 88018    |
| Low-Fluorescence PVDF Transfer Membranes, 0.2 µm pore size, (7 x 8.4 cm)            | 10 sheets  | 22860    |
| Detect                                                                              | Quantity   | Cat. No. |
| Blocker FL Fluorescent Blocking Buffer (10X)                                        | 100 mL     | 37565    |
| No-Stain Protein Labeling Reagent                                                   | 40 rxn     | A44449   |
| No-Stain Protein Labeling Reagent, Trial Size                                       | 10 rxn     | A44717   |
| iBright FL1500 Imaging System                                                       | 1 system   | A44241   |

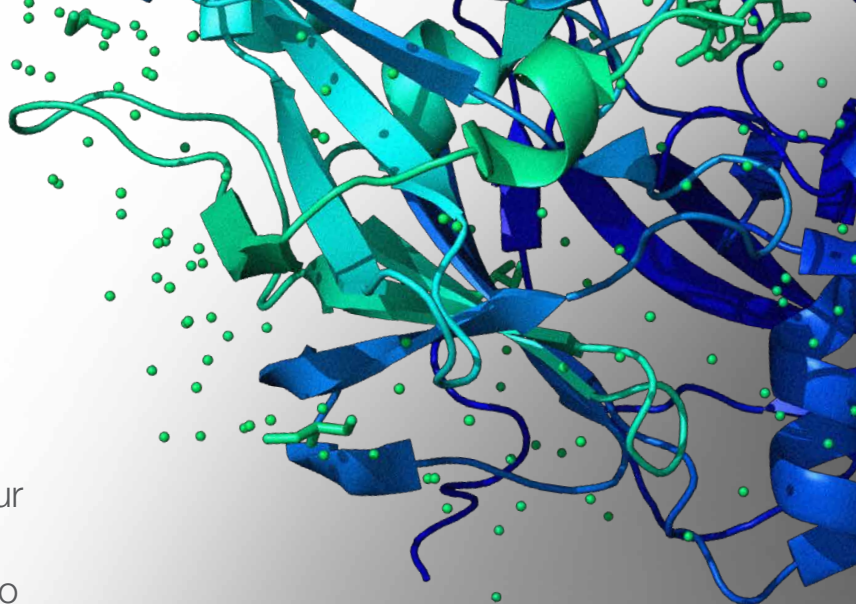




Notes

# Optimizing your western blots

Experiments don't always work—we understand that. Help increase your western blot efficiency by using our western blotting products, optimized to work together for publication-quality blot data. We offer products and solutions, along with tips and tricks to help you optimize your western blots.



# Casting multiple gels because of leaks?



**Pour-your-own gels with a 100% leak-free system\***  
 Pour your own polyacrylamide mini gels easily and confidently with the **Invitrogen™ SureCast™ Gel Handcast System**. The SureCast system is fully compatible with our Mini Gel Tank. Use Invitrogen™ SureCast™ handcasting reagents as well as other popular polyacrylamide gel casting reagents.

\* Terms and conditions apply. For complete details, go to [thermofisher.com/surecastterms](http://thermofisher.com/surecastterms).

Features of the SureCast Gel Handcast System include:

- **Leak-free design**—gels that are more usable, for 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

## Welcome packs

| SureCast Gel Handcast Bundle A                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | SureCast Gel Handcast Bundle B                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|  <p>The Invitrogen™ SureCast™ Gel Handcast Bundle A includes all the hardware and reagents to get started:</p> <ul style="list-style-type: none"> <li>2 SureCast Gel Handcast Stations</li> <li>2 SureCast Glass Plate Sets (2 front, 2 back)</li> <li>2 SureCast Sealing Pads (attached to handcast stations)</li> <li>10 SureCast Gel Spacers</li> <li>1 10-Well Multi-Use Tool</li> <li>1 12-Well Multi-Use Tool</li> <li>1 15-Well Multi-Use Tool</li> <li>6 Gel Combs (2 each: 10-, 12-, 15-well)</li> <li>1 SureCast Stacking Buffer 2-pack</li> <li>1 SureCast Resolving Buffer 2-pack</li> <li>1 SureCast APS (25 g)</li> <li>1 SureCast TEMED (30 mL)</li> <li>1 SureCast Acrylamide Solution, 40% (450 mL)</li> </ul> |  <p>The Invitrogen™ SureCast™ Gel Handcast Bundle B includes all the hardware to get started:</p> <ul style="list-style-type: none"> <li>2 SureCast Gel Handcast Stations</li> <li>2 SureCast Glass Plate Sets (2 front, 2 back)</li> <li>2 SureCast Sealing Pads (attached to handcast stations)</li> <li>10 SureCast Gel Spacers</li> <li>1 10-Well Multi-Use Tool</li> <li>1 12-Well Multi-Use Tool</li> <li>1 15-Well Multi-Use Tool</li> <li>6 Gel Combs (2 each: 10-, 12-, 15-well)</li> </ul> |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Find out more at <a href="http://thermofisher.com/surecast">thermofisher.com/surecast</a>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |

## Avoid poor separation and poor band resolution

### Get optimal separation with the right gel for your target protein

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. For separation of a broad range of proteins, two chemistries—Bis-Tris and Tris-glycine—are well suited. Bis-Tris gel chemistry provides greater sensitivity for protein detection compared to Tris-glycine gel chemistry. Choose Bis-Tris gel chemistry when you have a low abundance



**Protein Gel Welcome Packs are available for each of our protein gels.** Each welcome pack provides all of the necessary gels, buffers, and reagents you need to get started, as well as the Mini Gel Tank. Find out more at [thermofisher.com/proteingelwelcome](https://thermofisher.com/proteingelwelcome)

of protein or when your downstream applications require high protein integrity, such as posttranslational modification analysis, mass spectrometry, or sequencing.

|                     | Bis-Tris                                                                                                               | Tris-glycine                                                                                             | Tris-acetate                                                                                                                         | Tricine                                                          |
|---------------------|------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------|
| Protein sample type | Broad range MW (6–400 kDa)                                                                                             | Broad range MW (6–400 kDa)                                                                               | High range MW (40–500 kDa)                                                                                                           | Low range MW (2.5–40 kDa)                                        |
| Chemistry benefits  | Neutral pH for high-sensitivity applications and reduced protein degradation                                           | Traditional Laemmli-style                                                                                | Transfer and analysis of high molecular weight proteins; neutral pH                                                                  | Transfer and analysis of low molecular weight proteins           |
| Recommended for     | Western blotting, mass spectrometry, posttranslationally modified proteins, dilute samples, and low-abundance proteins | Western blotting, in-gel staining, samples containing detergents and high salt, native PAGE applications | High molecular weight proteins, western blotting, mass spectrometry, posttranslationally modified proteins, native PAGE applications | Low molecular weight proteins, western blotting, in-gel staining |

### Find the right buffers for the best separation

| Gel chemistry | Product                                         | Running buffer                                                                          | Transfer buffer                    |
|---------------|-------------------------------------------------|-----------------------------------------------------------------------------------------|------------------------------------|
| Bis-Tris      | Bolt Bis-Tris Plus gels<br>NuPAGE Bis-Tris gels | Small- to medium-sized proteins:<br>MES SDS buffer                                      | Bolt or NuPAGE transfer buffer     |
|               |                                                 | Medium- to large-sized proteins:<br>MOPS SDS Buffer                                     |                                    |
| Tris-glycine  | Novex Tris-glycine gels                         | <b>Denaturing:</b> Tris-glycine SDS buffer<br><b>Native:</b> Tris-glycine native buffer | Novex Tris-glycine transfer buffer |
| Tris-acetate  | NuPAGE Tris-acetate gels                        | <b>Denaturing:</b> Tris-acetate SDS buffer<br><b>Native:</b> Tris-glycine native buffer | NuPAGE transfer buffer             |
| Tricine       | Novex Tricine gels                              | Tricine SDS buffer                                                                      | Novex Tris-glycine transfer buffer |

Find your protein gel using the Protein Gel Selection Guide at [thermofisher.com/proteingels](https://thermofisher.com/proteingels)

## Choose the right gel percentage

In general, the size of the molecule being separated should dictate the acrylamide or agarose percentage you choose. Use a lower percentage gel to resolve larger molecules and a higher percentage gel to resolve smaller ones. Refer to the gel migration chart (right) to find the gel best suited for your application. As a general rule, molecules should migrate through about 70% of the length of the gel for the best resolution. When protein molecular weights are wide-ranging or unknown, gradient gels are usually the best choice.

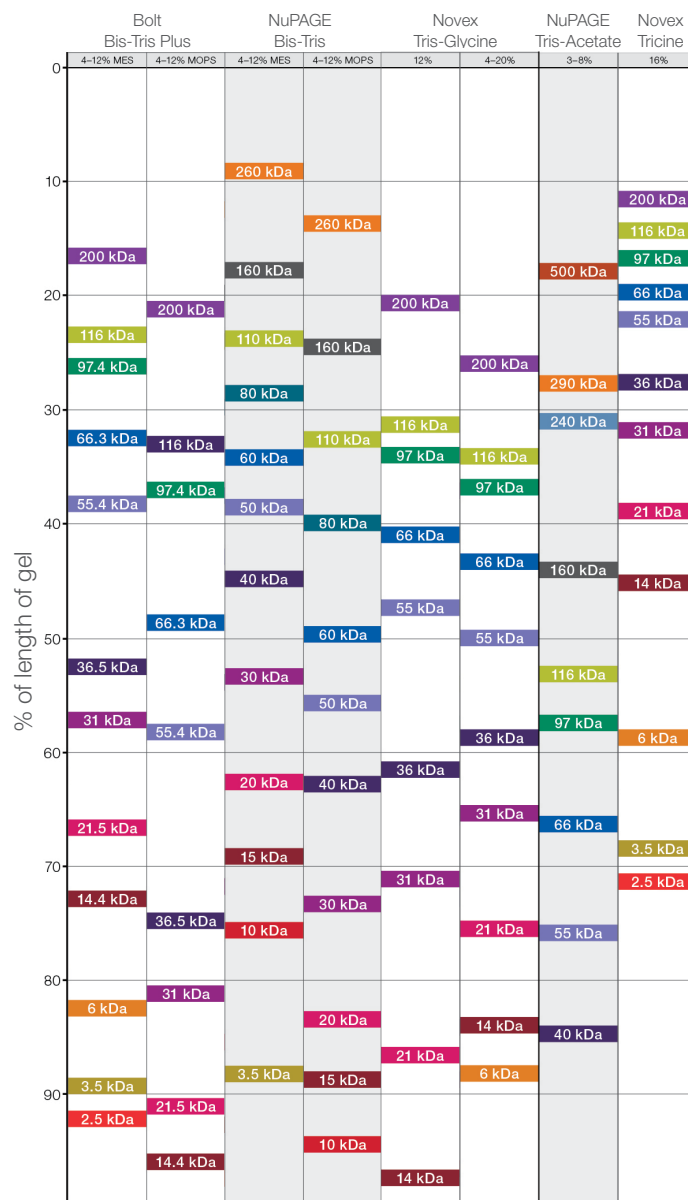
## Improve weak or faint bands

Bolt Bis-Tris Plus and Novex Tris-glycine mini gels feature a high-capacity WedgeWell design that accommodates more sample volume compared to traditional wells. The larger well capacity allows detection of proteins in very dilute samples or visualization of low-abundance proteins. Standard tips can be used when loading.

## Maximum loading volumes per well

| Maximum loading volume |                                   |
|------------------------|-----------------------------------|
| Comb type              | 1.0 mm thickness                  |
| 10-well WedgeWell      | 60 µL                             |
| 12-well WedgeWell      | 45 µL                             |
| 15-well WedgeWell      | 35 µL                             |
| 17-well WedgeWell      | 30 µL                             |
| 10-well                | 25 µL                             |
| 12-well                | 20 µL                             |
| 15-well                | 15 µL                             |
| 17-well                | 15 µL                             |
| 12+2 well*             | 45 µL (12 wells), 15 µL (2 wells) |
| 20-well*               | 25 µL                             |
| 26-well*               | 15 µL                             |

\* Midi gel.



Find out more at [thermofisher.com/proteingels](https://thermofisher.com/proteingels)



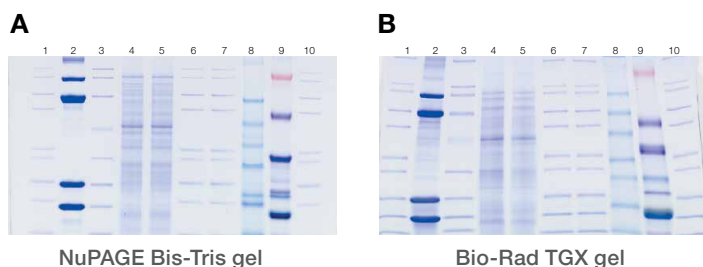
## Frustrated by smiling or uneven bands?

The most widely used gel system for separating a broad range of proteins by SDS-PAGE is the Laemmli system (Tris-glycine). The highly alkaline operating pH of the Laemmli system may cause band distortion, loss of resolution, or artifact bands as a result of protein degradation.

The major causes of poor band resolution with the Laemmli system are:

- Hydrolysis of polyacrylamide at the high pH of the resolving gel, resulting in a short shelf life of 8 weeks
- Chemical alterations such as deamination and alkylation of proteins due to the high pH of the resolving gel
- Reoxidation of reduced disulfides from cysteine-containing proteins, as the redox state of the gel is not constant
- Cleavage of Asp-Pro bonds of proteins when heated at 100°C in Laemmli sample buffer, pH 5.2

**NuPAGE Bis-Tris chemistry with neutral pH minimizes protein distortion during the electrophoresis run.**



**Protein separation using a NuPAGE Bis-Tris gel and a Bio-Rad TGX gel.** Samples were run on (A) a NuPAGE 4–12% Bis-Tris protein gel in MES SDS running buffer or (B) a TGX 4–20% Tris-glycine gel. Note the high resolution of the sample bands in the NuPAGE protein gel. The poorly resolved “fuzzy” bands seen in the other supplier’s gel are a result of reoxidation of some disulfide bonds within the sample, leading to slight changes in migration rates.

Unlike traditional Tris-glycine gels, **NuPAGE and Bolt gels** are Bis-Tris HCl-buffered (pH 6.4) and have an operating pH of about 7.0. The neutral operating pH of the Bis-Tris systems provides the following advantages over the Laemmli system:

- Longer shelf life of 8–16 months due to improved gel stability
- Improved protein stability during electrophoresis at neutral pH, enabling sharper band resolution and accurate results
- Complete reduction of disulfides under mild heating conditions (70°C for 10 minutes) and absence of cleavage of Asp-Pro bonds
- Reduced state of the proteins maintained during electrophoresis and blotting of the proteins when using Invitrogen™ NuPAGE™ and Bolt™ Antioxidant



**Purchase with confidence with our protein gel performance guarantee**

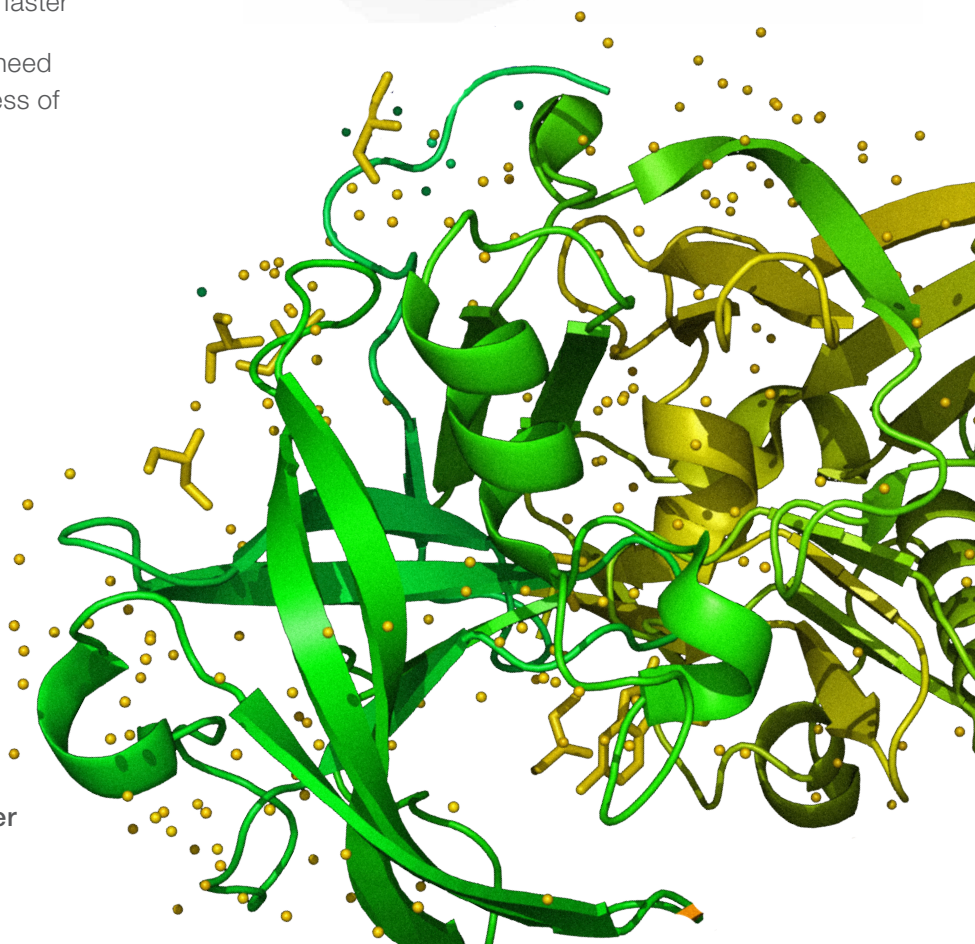
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## Convenient, reliable western wet transfer

Two blot modules fit into the **Mini Gel Tank** and the **SureLock Tandem Midi Gel Tank**. These tanks are designed to make your western transfers simple and easy to perform.

- **Universal module design**—allows modules to fit in either chamber of the tank, simplifying the transfer setup
- **Unique gasket seal**—helps prevent buffer leakage so there is no mess during setup of your western transfer
- **Half-inch buffer chamber**—requires half the typical volume of methanol-based transfer buffers, reducing hazardous waste and disposal cost (220 mL per mini blot; 300 mL per midi blot)
- **Standard 30–60 min transfer protocol**—accelerates your western workflow so you can get results faster
- **Room-temperature transfer**—eliminate the need to prechill buffers and the hassle and messiness of ice baths



## Overcome high background

### Blocking buffers

Before probing for proteins of interest, the remaining binding surface of the membrane must be blocked to prevent nonspecific binding of the antibodies. Otherwise, the antibodies or other detection reagents will bind to any remaining sites on the membrane that initially served to immobilize the proteins of interest. There is no single blocking buffer that can universally work for all western blotting applications. Refer to the table below to choose the optimal blocking buffer for your application.

| Select when ...                                         | Thermo Scientific™ product                    | Blocking agent                                | Highlights                                                                                                                                                                                                                                                                                                                                           | When to use                                                                                                                                                                                                                     | Available formats          |
|---------------------------------------------------------|-----------------------------------------------|-----------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------|
| ... optimizing a new western blot system                | StartingBlock™ Blocking Buffer                | Serum and biotin-free single purified protein | <ul style="list-style-type: none"> <li>• Performs well with a wide range of antibodies and antibody combinations</li> <li>• Compatible with streptavidin systems</li> <li>• Blocks in less than 15 min</li> </ul>                                                                                                                                    | <ul style="list-style-type: none"> <li>• With medium- to high-abundance proteins or strong antibody affinity</li> <li>• Current blocking buffer has high background</li> <li>• Stripping and reprobing western blots</li> </ul> | PBS<br>TBS<br>PBST<br>TBST |
| ... performing fluorescent western detection            | Blocker™ FL Fluorescent Blocking Buffer (10X) | Single purified protein                       | <ul style="list-style-type: none"> <li>• Blocks excess nonspecific binding sites to help reduce background fluorescence</li> <li>• Works with both nitrocellulose and low-fluorescence PVDF membranes</li> <li>• Detergent-free</li> <li>• Blocks in 15–30 min</li> </ul>                                                                            | <ul style="list-style-type: none"> <li>• Imaging and storage of dry fluorescence blots</li> </ul>                                                                                                                               | 10X concentrate            |
| ... you have high background with homemade milk buffers | Pierce™ Clear Milk Blocking Buffer            | Clarified and stabilized milk proteins        | <ul style="list-style-type: none"> <li>• High-performance replacement for homemade milk blocking buffers in western blotting applications</li> <li>• Long shelf life at room temperature</li> </ul>                                                                                                                                                  | <ul style="list-style-type: none"> <li>• Use when high background is seen with nonfat milk</li> </ul>                                                                                                                           | 10X concentrate            |
| ... targeting phosphoproteins                           | Blocker™ BSA                                  | Purified bovine serum albumin                 | <ul style="list-style-type: none"> <li>• 10% solutions of high-quality bovine serum albumin</li> <li>• Single purified protein provides fewer chances of cross-reaction with assay components than serum or milk solutions</li> </ul>                                                                                                                | <ul style="list-style-type: none"> <li>• Use when targeting phosphoproteins</li> <li>• Best to use when storing reused antibodies in blocker</li> </ul>                                                                         | 10X concentrate            |
| ... you need a protein-free blocker                     | Pierce™ Protein-Free Blocking Buffer          | Non-protein blocking compound                 | <ul style="list-style-type: none"> <li>• Helps minimize or eliminate cross-reactivity associated with protein-based blocking buffers</li> <li>• Sample-and-antibody combinations require the elimination of all possible exogenous animal proteins in the assay system to avoid cross-reaction or quenching of the desired probe function</li> </ul> | <ul style="list-style-type: none"> <li>• Use when protein-based blockers cause high background</li> </ul>                                                                                                                       | PBS<br>TBS<br>PBST<br>TBST |

## Light up weak or faint bands

### Get better sensitivity with the right chemiluminescent substrate

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.

### Don't know where to start?

Try Thermo Scientific™ SuperSignal™ chemiluminescent substrate bundles.

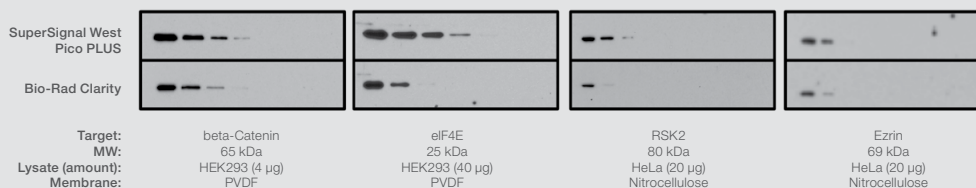


**Thermo Scientific™ SuperSignal™ West Pico PLUS Chemiluminescent Substrate** provides excellent performance, versatility, and economy for routine western blotting needs. It is designed to work for the majority of western blots and recommended for detecting a new protein of interest when western blotting conditions are not yet optimized.

- **Excellent sensitivity**—low-picogram to high-femtogram sensitivity
- **Long signal duration**—up to 24 hours
- **Stable reagent**—8 hour working solution stability, 1 year kit stability at room temperature



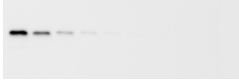
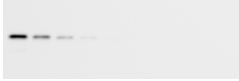
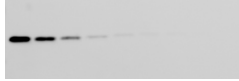
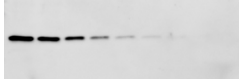
- **Exceptional robustness**—high performance, even outside of the recommended antibody ranges, including the most commonly used 1:5,000 to 1:10,000 secondary antibody dilutions from a 1 mg/mL stock solution



**SuperSignal West Pico PLUS substrate outperforms Bio-Rad™ Clarity™ substrate.** Detection of the indicated targets was performed using 2-fold serial dilutions of HEK293 or HeLa cell lysates. Chemiluminescent detection of indicated target and substrate comparison was performed, following a 5 min incubation with either SuperSignal West Pico PLUS or Bio-Rad Clarity substrate.

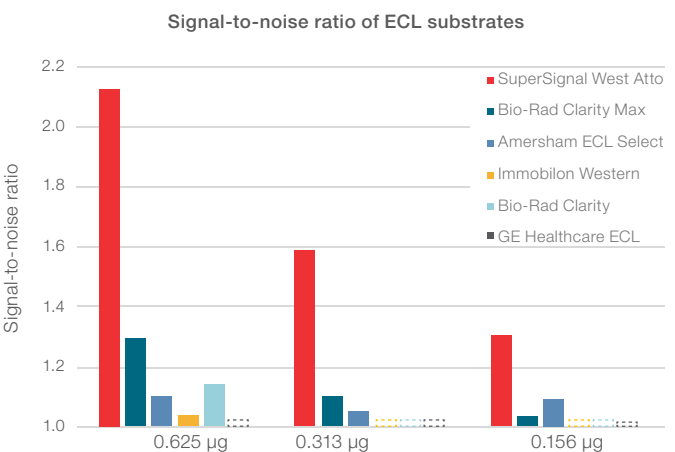
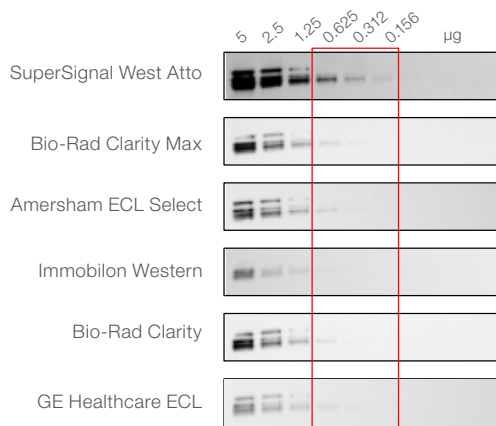
Learn more at [thermofisher.com/chemisubstrates](https://thermofisher.com/chemisubstrates)

### Choose the right SuperSignal substrate for your needs.

|                          | SuperSignal West Pico PLUS                                                                                    | SuperSignal West Dura                                                             | SuperSignal West Femto                                                             | SuperSignal West Atto                                                                                   |
|--------------------------|---------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------|
|                          |                              |  |  |                      |
| <b>Detection level</b>   | Low-picogram to high-femtogram                                                                                | Mid-femtogram                                                                     | Mid- to low-femtogram                                                              | Low-femtogram to high-attogram                                                                          |
| <b>Signal duration</b>   | Up to 24 hours                                                                                                | Up to 24 hours                                                                    | Up to 8 hours                                                                      | Up to 6 hours                                                                                           |
| <b>Antibody dilution</b> | 1°: 1:1K–5K<br>2°: 1:10K–1:100K                                                                               | 1°: 1:1K–50K<br>2°: 1:50K–1:250K                                                  | 1°: 1:5K–100K<br>2°: 1:100K–1:500K                                                 | 1°: 1:1K–1:5K<br>2°: 1:100K–1:250K                                                                      |
| <b>Advantages</b>        | Superior sensitivity and intensity and longer duration than other entry-level ECL substrates                  | Best for use with imaging equipment; long-lasting signal duration                 | Good sensitivity with optimized conditions                                         | Most sensitive substrate for HRP detection with high signal-to-noise ratio                              |
| <b>Select when:</b>      | Routine western blots; working with new protein target when western blotting conditions are not yet optimized | Maximum signal duration is needed; performing sensitive quantitation              | Target is low in abundance/sample is precious; system is optimized                 | Target is very low in abundance/sample is precious; requires maximum sensitivity with less optimization |

**Thermo Scientific™ SuperSignal™ West Atto Ultimate Sensitivity Substrate** provides the highest sensitivity and improved signal-to-noise ratio compared to other commercially available high-performance horseradish peroxidase (HRP) substrates. It is the ideal choice for detection of very low-abundance targets or precious samples that require maximum levels of sensitivity.

- **Ultra-sensitivity**—low-femtogram to high-attogram levels
- **High signal-to-noise ratio**—exhibits superior intensity and sensitivity versus other typical high-performance ECL substrates on the market
- **Excellent signal duration**—up to 6 hours of usable light output when conditions are optimized
- **Stable reagent**—working solution is stable for 48 hours; kit is stable at 2–8°C for 12 months



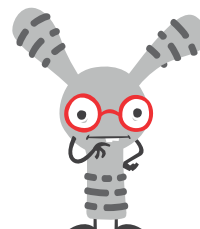


## Blot with confidence with high-quality primary and secondary antibodies

Find an extensive selection of high-quality Invitrogen™ antibodies for western blotting, available in a wide range of targets and hosts. Explore our cross-adsorbed secondary HRP antibodies that help decrease species cross-reactivity and minimize background. Purchase with confidence thanks to our antibody performance guarantee.\*

Find the right antibody for your research needs at [thermofisher.com/antibodies](https://thermofisher.com/antibodies)

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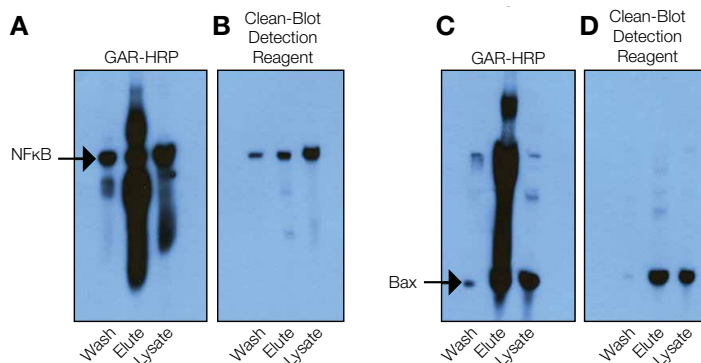
HRP Secondary Antibody

## Avoid interference from IP antibodies

When performing immunoprecipitation (IP), target protein signal can be hindered by the heavy and light chains from IgGs (~50 kDa and 25 kDa). Western blot interference from IgGs results from IP methods that release the antibody with the antigen and use sample types that contain IgGs, such as tissue extracts. Traditional secondary antibodies will detect the denatured and blotted IgGs.

### Thermo Scientific™ Clean-Blot™ IP Detection Reagent

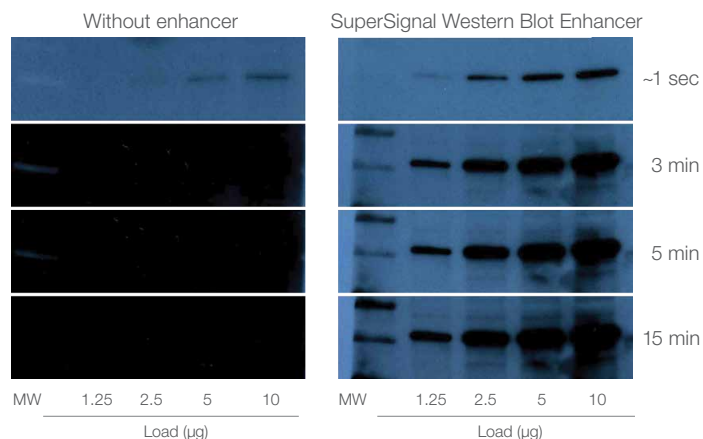
is a horseradish peroxidase conjugate that is optimized for post-IP western blot detection of primary antibodies without interference from denatured IP antibody fragments. The Clean-Blot IP reagent minimizes detection interference from both heavy-chain and light-chain IgG fragments of antibodies by only detecting the native antibodies, providing accurate and specific detection of the target antigen.



**NFκB and Bax were immunoprecipitated from A549 lysate using Protein A/G agarose resin and rabbit anti-NFκB (panels A and B) and rabbit anti-Bax (panels C and D). Panels A and C were detected with goat anti-rabbit HRP, which masked the targets. Panels B and D were detected with the Clean-Blot IP Detection Reagent (HRP), unambiguously revealing the target protein without interference.**

## Low-abundance target or weakly immunoreactive antigens?

When a protein or antigen is difficult to detect because of low abundance or poor immunoreactivity, use **Thermo Scientific™ SuperSignal™ Western Blot Enhancer** to significantly reduce background and to enhance detection of low-abundance and weakly immunoreactive antigens. SuperSignal Western Blot Enhancer increases both signal intensity and sensitivity 3- to 10-fold compared to detection performed using conventional western blotting.



**SuperSignal Western Blot Enhancer reduces background to enable detection of low-abundance targets.**

## Optimize further: reprobe your western blots

Reprobing a western blot saves time and conserves sample while allowing optimization to be performed as needed. These specially formulated 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. **Thermo Scientific™ Restore™ stripping buffers** safely and effectively remove primary and secondary antibodies so blots can be reprobed.

|                    | Restore Stripping Buffer                             | Restore Plus Stripping Buffer                       | Restore Fluorescent Western Blot Stripping Buffer               |
|--------------------|------------------------------------------------------|-----------------------------------------------------|-----------------------------------------------------------------|
| Select when        | Primary antibody is susceptible to stripping buffers | Removing high-affinity primary antibodies           | Performing fluorescent western blots                            |
| Membrane           | Nitrocellulose and PVDF                              | Nitrocellulose and PVDF                             | Use with low-fluorescence PVDF membranes (e.g., Cat. No. 22860) |
| Time of incubation | 15–30 min at 37°C                                    | 5–15 min at RT or 37°C for high-affinity antibodies | 10–20 min at RT                                                 |

## Ordering information

| Separate                                               | Quantity | Cat. No. |
|--------------------------------------------------------|----------|----------|
| SureCast Gel Handcast Bundle A - Hardware and Reagents | 1 kit    | HC1000SR |
| SureCast Gel Handcast Bundle B - Hardware Only         | 1 kit    | HC1000S  |
| Bolt Bis-Tris 4–12%, 10-well Welcome Pack              | 1 kit    | NW0412A  |
| Bolt Bis-Tris 4–12%, 15-well Welcome Pack              | 1 kit    | NW0412B  |
| NuPAGE Bis-Tris 4–12%, 10-well Welcome Pack            | 1 kit    | NP032A   |
| NuPAGE Bis-Tris 10%, 10-well Welcome Pack              | 1 kit    | NP030A   |
| NuPAGE Tris-Acetate 3–8%, 10-well Welcome Pack         | 1 kit    | EA0375A  |
| Novex Tris-Glycine 10%, 10-well Welcome Pack           | 1 kit    | XP0010A  |
| Novex Tris-Glycine 4–12%, 10-well Welcome Pack         | 1 kit    | XP0412A  |
| Novex Tris-Glycine 10%, 15-well Welcome Pack           | 1 kit    | XP0010C  |
| Novex Tris-Glycine 4–12%, 15-well Welcome Pack         | 1 kit    | XP0412C  |
| Transfer                                               | Quantity | Cat. No. |
| Mini Blot Module                                       | 1 unit   | B1000    |
| Surelock Tandem Midi Blot Module                       | 1 unit   | STM2001  |
| Detect                                                 | Quantity | Cat. No. |
| StartingBlock (TBS) Blocking Buffer                    | 1 L      | 37542    |
| StartingBlock T20 (TBS) Blocking Buffer                | 1 L      | 37543    |
| StartingBlock (PBS) Blocking Buffer                    | 1 L      | 37538    |
| StartingBlock T20 (PBS) Blocking Buffer                | 1 L      | 37539    |
| Blocker FL Fluorescent Blocking Buffer (10X)           | 100 mL   | 37565    |
| Pierce Clear Milk Blocking Buffer (10X)                | 100 mL   | 37587    |
| Blocker BSA (10X) in TBS                               | 125 mL   | 37520    |
| Blocker BSA (10X) in PBS                               | 200 mL   | 37525    |
| Pierce Protein-Free (TBS) Blocking Buffer              | 1 L      | 37570    |
| Pierce Protein-Free T20 (TBS) Blocking Buffer          | 1 L      | 37571    |
| Pierce Protein-Free (PBS) Blocking Buffer              | 1 L      | 37572    |
| Pierce Protein-Free T20 (PBS) Blocking Buffer          | 1 L      | 37573    |
| Pierce ECL Western Blotting Substrate                  | 250 mL   | 32209    |
| Pierce ECL Plus Western Blotting Substrate             | 100 mL   | 32132    |
| SuperSignal West Pico PLUS Chemiluminescent Substrate  | 200 mL   | 34577    |
| SuperSignal West Dura Extended Duration Substrate      | 100 mL   | 34075    |
| SuperSignal West Femto Maximum Sensitivity Substrate   | 100 mL   | 34095    |
| SuperSignal West Atto Ultimate Sensitivity Substrate   | 100 mL   | A38555   |

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### Protein electrophoresis and western blotting education center

Gaining publication-quality results immediately is not exactly the norm when performing western blotting. Access resources to learn about protein gel electrophoresis and western blotting methods, from webinars to quick tips and tricks. Whether you are new to western blotting or an experienced researcher wanting to confirm your knowledge, consider this center to help you get better western results and succeed sooner.



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### Having problems with your western blot?

Count on our technical support scientists for experienced troubleshooting advice.

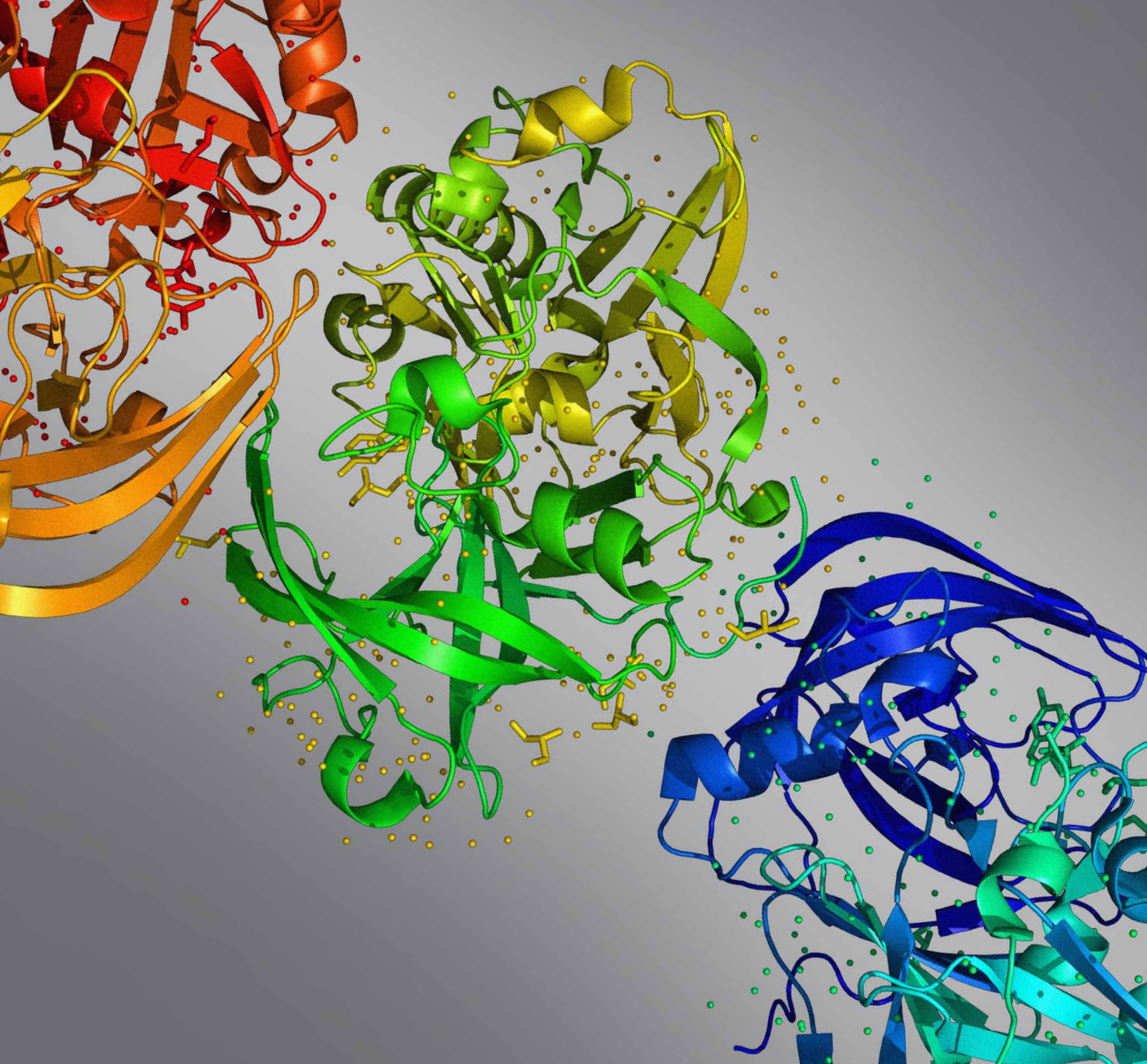
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