

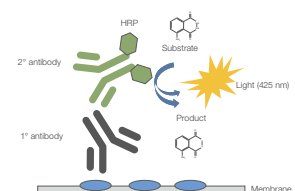
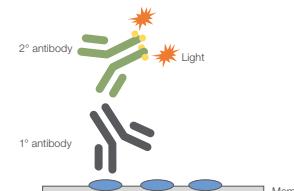


Western blotting

Maximize the outcome of your western blots

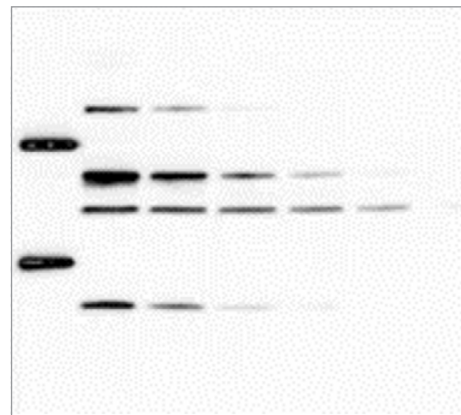
Equip your lab with both chemiluminescent and fluorescent detection capabilities

Chemiluminescent and fluorescent detection technologies have become widely recognized as the preferred methods for protein detection in western blotting. Delve into the key benefits of chemiluminescent and fluorescent detection, and uncover the products that are specifically engineered to enhance each type of detection. Take your protein detection to new heights with the right product combination.

	Chemiluminescent detection	Fluorescent detection
Working principle	Indirect signal 	Direct signal from fluorophore emission 
Sensitivity	Excellent	Good, but may require increased secondary antibody concentration
Linear dynamic range	++	+++
Signal stability	Hours	Weeks/months
Secondary antibody	HRP-conjugated	Fluorescent dye-conjugated
Consistency and reproducibility	Good	Excellent
Detection method	X-ray films, digital imager	Digital imager
Additional considerations	Detecting multiple target proteins requires stripping and reprobing	- Secondary antibodies with non-overlapping emission maxima need to be selected for multiplex experiments - Specialized low-fluorescence reagents and membranes required for optimal results - Longer exposure times can produce high background
Best use	- Single target - Low-abundance proteins - Qualitative or quantitative western blotting	- Multiple targets - Quantitative western blotting - Streamlined total protein normalization

Chemiluminescent detection

Chemiluminescent detection provides excellent flexibility and the highest level of sensitivity. Multiple exposures can be used to enhance the signal-to-noise ratio and improve the chances of detecting proteins at extremely low concentrations. The western blot can be stripped and reprobed to optimize the detection of the target protein or visualize another protein.



HRP-conjugated secondary antibodies

Horseradish peroxidase (HRP) is a commonly used enzyme label for secondary antibodies in western blotting. When used with a chemiluminescent substrate, HRP-conjugated secondary antibodies emit light that can be captured by an imaging instrument. Our portfolio of Invitrogen™ HRP-conjugated secondary antibodies offers a wide range of host and target species to help meet your needs.

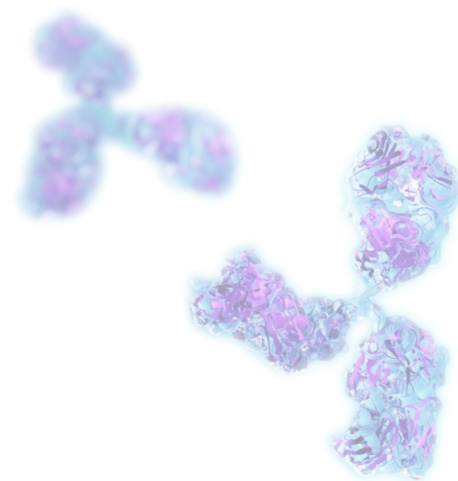
Polyclonal HRP-conjugated secondary antibodies

Highly sensitive; many antibodies can bind epitopes on the primary antibody

Invitrogen™ Superclonal™ recombinant HRP-conjugated secondary antibodies

Precise, accurate detection and multiple-epitope coverage; stable long-term supply and lot-to-lot consistency due to recombinant format

Learn more: thermofisher.com/hrp



Popular secondary antibodies for chemiluminescent detection

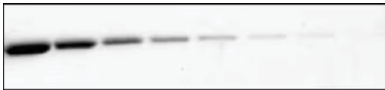
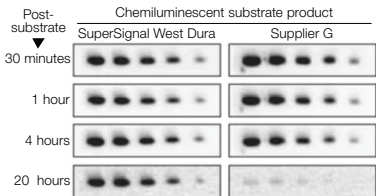
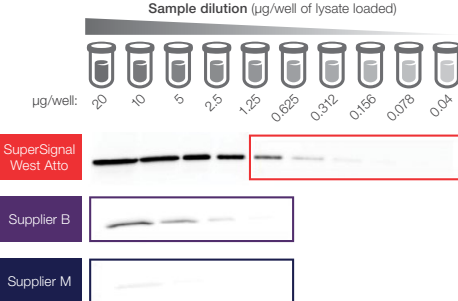
Description	Cat. No.	Price
Goat anti-Rabbit IgG, HRP	31460	
Goat anti-Mouse IgG, HRP	31430	
Goat anti-Rat IgG, HRP	31470	
Goat anti-Human IgG Fc, HRP	31413	
Mouse anti-Human IgG1 Fc, HRP	A10648	

Search all [hrp-conjugated antibodies](#) for western detection

Learn more: thermofisher.com/hrp

Thermo Scientific™ SuperSignal™ substrates for chemiluminescent detection

Select the right substrate for your protein detection needs.

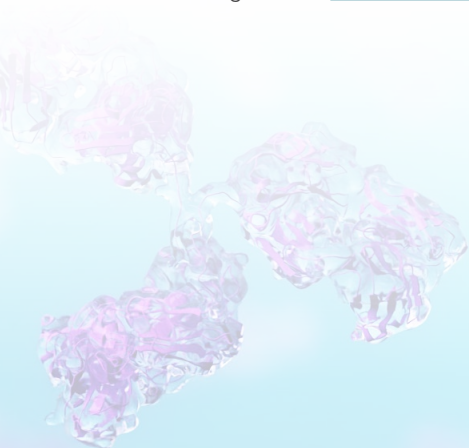
SuperSignal™ West Pico PLUS substrate	SuperSignal™ West Dura substrate	SuperSignal™ West Atto substrate
 Wide dynamic range makes it an excellent choice when you need to detect a broad range of protein levels	 Excellent linearity for quantitative western blotting; the long-lasting signal allows for multiple film or camera exposures	 The ultimate-sensitivity substrate to detect proteins down to the attogram level
 Western blotting detection of His-tagged purified recombinant protein	 Western blotting detection of Hsp86 in HeLa cell lysates	 Western blotting detection of p23 in HeLa cell lysates
Sensitivity		
++	+++	++++
Cat. No.		
34580 (500 mL)	34076 (200 mL)	A38555 (100 mL)

Additional products for chemiluminescent detection

Hand-picked products to set you up for a successful western blotting experiment.

Description	Use for	Cat. No.	Price
StartingBlock Blocking Buffer	Fast blocking buffer for a wide range of antibodies and antibody combinations	37538	
SuperSignal Western Blot Enhancer	3- to 10-fold increased signal intensity and sensitivity	46641	
Restore Western Blot Stripping Buffer	Gentle stripping	21059	
Ponceau S Staining Solution	Evaluating the transfer efficiency of a western blot	A40000279	

Discover reagents for [chemiluminescent western blot detection](#)

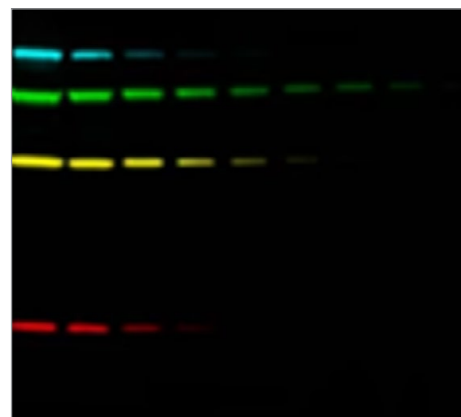


Fluorescent detection

Detect multiple proteins at once and gain a wider understanding of cellular pathways and their responses to drugs and external stimuli. Taking advantage of the broad selection of Invitrogen™ fluorescent dyes is easy with advanced digital imaging systems, like the

Invitrogen™ iBright™ FL1500 Imaging System.

Improve the quality of your data with fast, quantitative western blotting analysis.



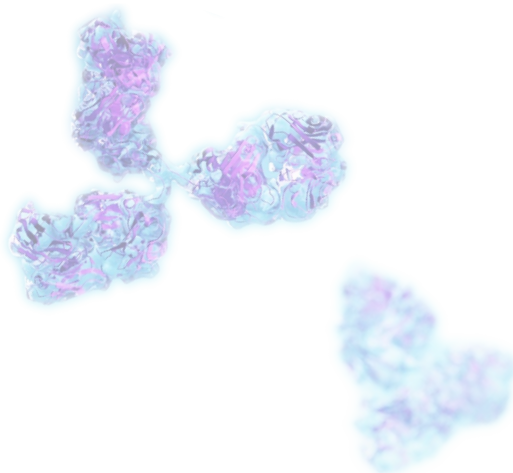
Fluorescent secondary antibodies

Fluorescently labeled secondary antibodies enable simultaneous detection of multiple targets in one blot. Invitrogen™ Alexa Fluor™ and Alexa Fluor™ Plus secondary antibodies offer a wide range of fluorescent dye conjugates, providing excellent multiplexing capabilities. They are available in both polyclonal and Superclonal recombinant formats.

Alexa Fluor secondary antibodies: 15 dyes available for western blotting and a variety of host and target species

Alexa Fluor Plus secondary antibodies: 6 dyes available for western blotting; designed to help detect low-abundance targets with up to 5.8 times higher signal-to-noise compared to traditional Alexa Fluor secondary antibodies

Trial sizes are available for many [Alexa Fluor Plus 488](#) and [Alexa Fluor Plus 647](#) secondary antibodies.



Popular secondary antibodies for fluorescent detection

Description	Cat. No.	Price
Donkey anti-Rabbit IgG, Alexa Fluor Plus 647	A32795	
Goat anti-Human IgG, Alexa Fluor 647	A21445	
Goat anti-Human IgG, Alexa Fluor 546	A21089	
Goat anti-Rat IgG, Alexa Fluor 568	A11077	
Goat anti-Mouse IgG, Alexa Fluor Plus 594	A32742	
Donkey anti-Rat IgG, Alexa Fluor Plus 647	A48272	

Search all [fluorescent secondary antibodies](#) for western blotting

Learn more: thermofisher.com/alexafuor

Products for optimal fluorescent detection

Improve signal-to-noise ratio with products validated for fluorescent detection.

Product	Features	Cat. No.
Fluorescent Compatible Sample Buffer	Formulated without bromophenol blue or other dyes that interfere in the fluorescent channels, but still provides a visible dye front	LC2570
iBright Prestained Protein Ladder	Ten prestained and two unstained proteins featuring IgG binding sites that interact with your secondary antibody	LC5615
Low-Fluorescence PVDF Transfer Membranes	Low-autofluorescence membrane for reduced background fluorescence and improved signal-to-noise ratio	22860
Blocker FL Fluorescent Blocking Buffer	Designed to reduce cross-reactivity and produce high signal-to-noise ratios	37565
Restore Fluorescent Western Blot Stripping Buffer	Formulated to allow removal of primary and near-infrared (IR) dye-labeled secondary antibodies from western blots transferred on PVDF	62300

Discover [fluorescent western blot detection reagents](#)



Invitrogen™ No-Stain™ Protein Labeling Reagent for total protein quantitation

In-gel and on-membrane total protein labeling, for fast, reproducible, and accurate quantitative western blotting. Compatible with chemiluminescence- or fluorescence-based detection.

Primary antibodies

Primary antibodies play a crucial role in western blotting as they specifically recognize and bind to the protein, facilitating the detection and visualization of the protein on the blot.

1. Clonality:

- **Polyclonal antibodies** recognize multiple epitopes, offering increased sensitivity
- **Monoclonal antibodies** offer lot-to-lot consistency and extensive characterization
- **Recombinant antibodies** provide consistent production and can be modified for desired characteristics; they offer engineered versatility, allowing for chimeric switching and adaptation into multiplex formats

2. Multiplexing considerations:

- Conjugated primary antibodies simplify the use of multiple host primary antibodies but may reduce signal intensity; careful dye selection and consideration of antibody sensitivity are necessary
- Unconjugated primary antibodies provide flexibility in target selection and experimental setup; when using secondary antibodies for western blotting multiplexing, careful selection of primary antibodies is crucial to minimize cross-reactivity

3. Advanced application verification:

Invitrogen™ products are subject to advanced verification to confirm antibody specificity, considering target properties like biological function and sample-type influence on epitope exposure. Additionally, we verify the functionality of the primary antibody in western blotting to help ensure correct target binding. Learn more about the [Invitrogen™ antibody verification process for western blotting](#).

Learn more: [primary antibodies for western blotting](#)

Popular primary antibodies for western blotting detection

Epitope-tag antibodies

Target	Description	Cat. No.	Price
GFP	GFP Monoclonal Antibody (3E6)	A11120	
HA	HA Tag Monoclonal Antibody (2-2.2.14)	26183	
Flag	DYKDDDDK Tag Monoclonal Antibody (FG4R)	MA1-91878	

Explore all [epitope-tag antibodies](#)

Housekeeping protein antibodies

Target	Description	Cat. No.	Price
Beta actin	beta Actin Loading Control Monoclonal Antibody (BA3R)	MA5-15739	
GAPDH	GAPDH Loading Control Monoclonal Antibody (GA1R)	MA5-15738	

Explore all [loading control antibodies](#)

Positive control protein

The Invitrogen™ Positope™ Control Protein is designed to be used as a positive control in western blotting for a variety of antibodies. It is a 53 kDa highly purified (>95%) recombinant protein that contains seven epitope tags: Invitrogen™ Xpress™ tag, thioredoxin, HisG, GFP, c-myc, V5, and His(C-term).

Description	Cat. No.	Price
Positope Control Protein	R90050	

Additional resources



Learning resources

[Quantitative western blotting](#)
[Western blotting learning center](#)



Protocols

[Protocols for western blotting with chemiluminescent detection](#)
[Protocols for western blotting with fluorescent detection](#)
[Application verification testing protocol for western blotting](#)



Experiment planning and troubleshooting tools

[Fluorescence SpectraViewer](#)
[Western blot antibody dilution calculator](#)
[Western blotting troubleshooting](#)

Learn more at thermofisher.com/westernblot

invitrogen