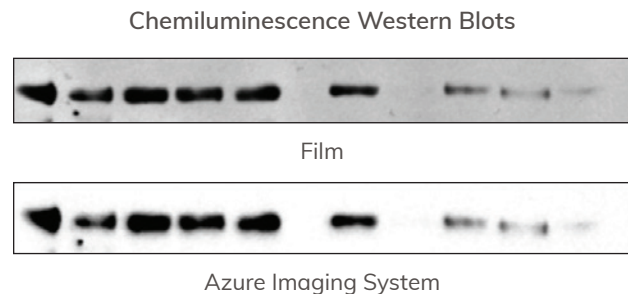


**Link to FREE
substrate samples
inside!**

PLAN YOUR EXPERIMENT AND CHOOSE YOUR DETECTION METHOD

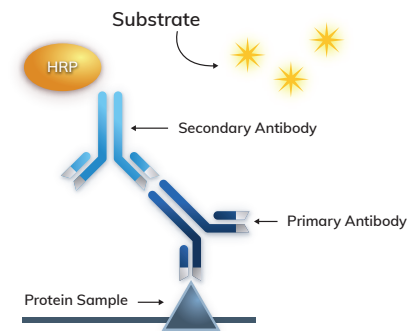
CHEMILUMINESCENT WESTERN BLOTTING

The most common method for Western blot detection is chemiluminescence. Detection by chemiluminescence is relatively sensitive and works well for detecting individual or low abundance proteins.



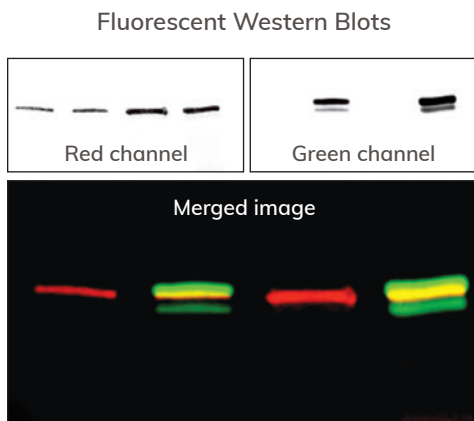
How it works

In chemiluminescent Western blots, a primary antibody is used to detect your protein of interest, followed by a species-specific secondary antibody to detect the primary antibody. The secondary antibody is conjugated to horseradish peroxidase (HRP), which catalyzes the oxidation of luminol to produce a product that emits light (luminescence). The light given off can be captured by film and developed in a darkroom, or it can be detected by a CCD camera using a digital imager.



FLUORESCENT WESTERN BLOTTING

Fluorescence Western blotting is beneficial when your experiment requires quantitation, particularly between different proteins in the same sample. Fluorescent methods are especially useful for detection of post-translationally modified versions of proteins, since the difference in molecular weight is often not enough for clear resolution on an average gel.



How it works

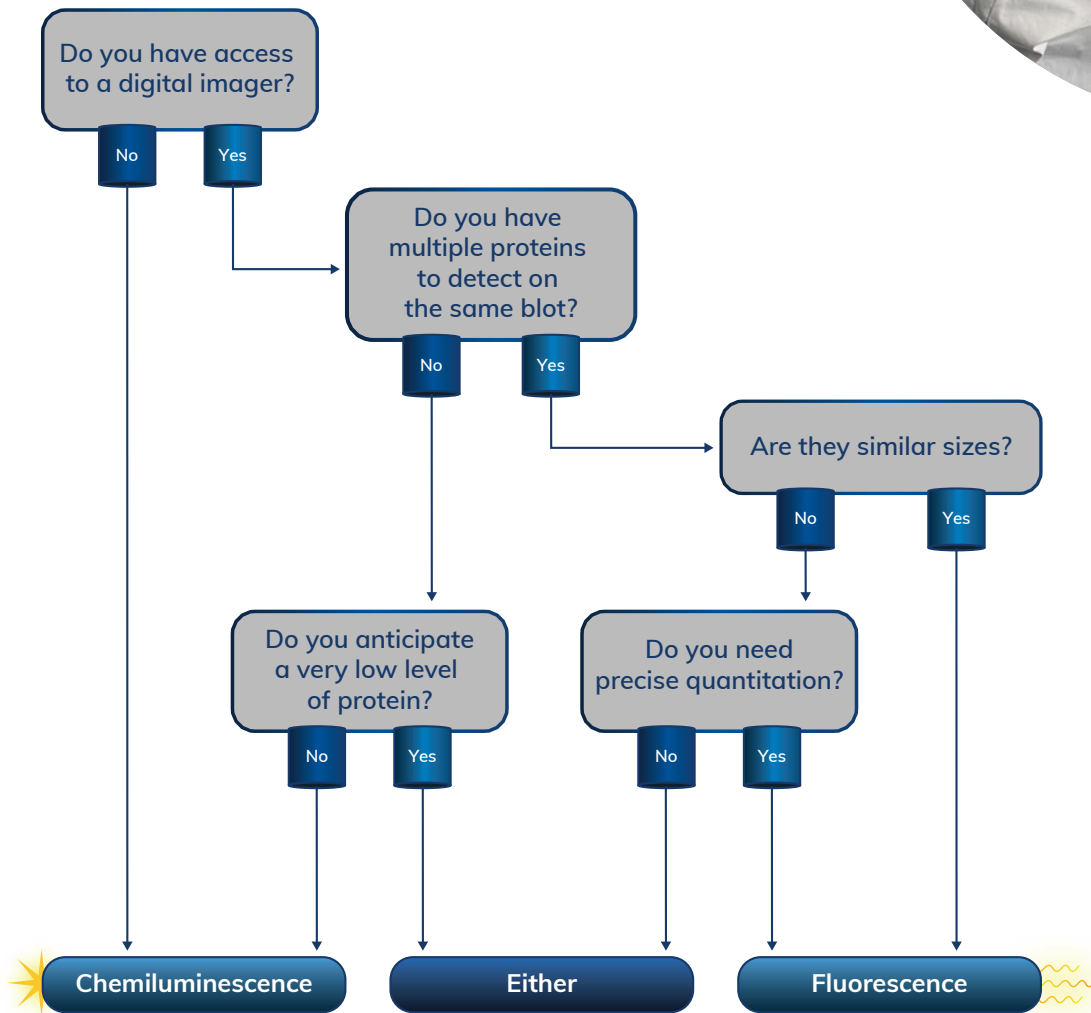
In fluorescent Western blots, the secondary antibody is conjugated to a dye molecule that can undergo fluorescence—meaning, it will give off light of a specific wavelength when excited with light of a shorter wavelength. Fluorophores are available in the visible or infrared (IR) spectrum.

Because different fluorophores can give off light in different ranges, fluorescent Western blotting can be used to multiplex—meaning, different target proteins can be visualized on the same blot at the same time.

With fluorescence, you have the option of using secondary antibodies conjugated to fluorophores, or you can label your primary antibodies with fluorophores of your choice to perform direct Westerns.



CHEMILUMINESCENT OR FLUORESCENT WESTERN BLOTTING?



COMMON APPLICATIONS

Chemiluminescence

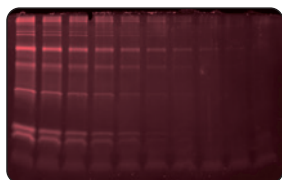
- Checking protein purification
- Presence or absence of one protein in a lysate
- Detecting low abundance proteins

Fluorescence

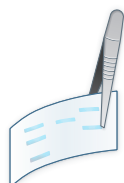
- Analyzing multiple proteins
- Posttranslational modifications
- Quantitative comparisons

RESOLVE YOUR PROTEINS BY ELECTROPHORESIS AND TRANSFER TO A MEMBRANE

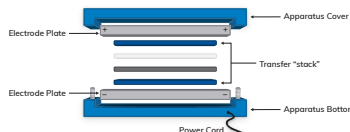
RUN GEL



SELECT MEMBRANE



TRANSFER PROTEINS



PREPARE FOR BLOT



Is Western blotting required?

Some applications don't require western blotting and can be done by directly staining proteins in the gel after separation.

Applications

Checking for a single band after protein purification.

AzureRed Fluorescent Protein Stain (AC2124) qualitatively stains gels for fluorescent imaging systems.

Choosing your membrane

The membrane provides a material that binds proteins as they move out of the gel matrix. The two common materials are nitrocellulose and PVDF.

Nitrocellulose (AC2106–AC2107)

- For low molecular weight proteins
- Typically used for chemi-applications
- Not ideal for stripping

PVDF (AC2105, AC2108–AC2109)

- High binding capacity is great for low-expression proteins
- Typically used for fluorescence (less background autofluorescence)
- High protein retention for stripping and reprobing

Setting up a good transfer

Proper transfer set-up involves a "sandwich" where gel and membrane are laid in direct contact between blotting paper, flanked by electrode plates. The transfer buffer allows current to flow between electrodes to carry proteins from gel to membrane.

Blotting paper (filter paper) provides consistent pressure so no gaps appear between membrane and gel while remaining absorbent enough for transfer buffer to penetrate the layers.

Azure Pre-cut Blotting Paper (AC2110–AC2111) adds efficiency to this sandwich set-up.

Azure Transfer Buffer (AC2127) is a proprietary formula designed for quick, high quality wet transfer and sensitive western blotting.

Before blotting

Verifying that total protein was sufficiently transferred from gel to the membrane is important when optimizing a transfer setup, condition, or experiment.

Ponceau staining can be used to visualize all protein on the membrane. After, the membrane can be destined for immediate western blotting.

Azure Ponceau Stain (AC2125) can ensure efficient transfer of proteins onto nitrocellulose or PVDF membranes.

Azure Opaque Incubation Trays (AC2120–AC2123) offer protection for light-sensitive membrane incubation. The surface is smoothed to prevent scratches and damage to membranes.

Azure Clear Incubation Trays (AC2150–AC2153) allow protection of your blots, with a smoothed surface to prevent membrane damage and transparency for easy monitoring.

BLOCK MEMBRANE AND PROBE FOR PROTEINS OF INTEREST WITH OPTIMIZED ANTIBODIES

BLOCK & WASH



Blocking the membrane

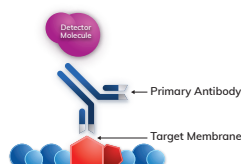
Blocking is important to prevent nonspecific background signals from interfering with your data.

Protein Free Blocking Buffer (AC2112) works best for purified proteins & antibodies with high cross reactivity with protein based blockers.

Fluorescent Blocking Buffer (AC2190) works best for cell lysates and low quality primary antibodies that require protein blockers.

Chemi Blot Blocking Buffer (AC2148) works best for chemiluminescent Western blots.

PRIMARY



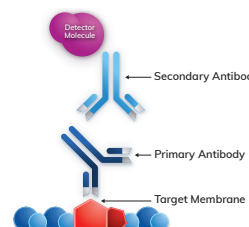
Probe with antibody

Make sure concentrations for new antibodies are optimized.

Have a validated primary antibody? Try a direct Western by labeling your primary antibody with fluorescent dye.

AzureSpectra Labeling Kit (AC2185–AC2189) allows quick and easy labeling of primary antibodies—see more on page 7.

WASH & SECONDARY



Washing after incubations

It is essential to wash excess antibody off the membrane after each incubation to prevent background signal. For fluorescent blots, make sure to rinse off excess wash buffer containing detergent, since it can fluoresce.

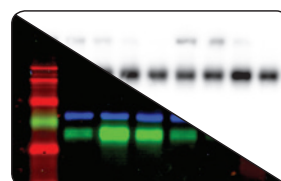
Blot Washing Buffer (AC2113) works well for chemiluminescent HRP substrates and visible fluorescent blots.

Fluorescent Blot Washing Buffer (AC2145) works best with NIR fluorescent blots.

Azure HRP secondary antibodies (AC2114–AC2119, AC2149) offer a range of species specificities.

Fluorescent secondary antibodies (AC2128–AC2139, AC2156–71) come in visible and near IR fluorophores.

DETECT



Chemiluminescence detection

Choose your HRP substrate:

Radiance (AC2101) produces a strong, long-lasting signal with extremely low background.

Radiance Plus (AC2103) is the most sensitive HRP substrate and allows you to detect bands not visualized with other substrates.

ChemiWriter ECL (AC2146) adsorbs to nitrocellulose and PVDF membranes, and reacts with HRP substrates to produce chemiluminescence.

Blot Development Folders (AC2126) protect your blot during imaging and keep it wet for longer lasting signal.

Fluorescence detection

Background Quenching Sheets (AC2144, AC2147) reduce background autofluorescence when imaging your fluorescent (or chemi) blot.

NEED TO REPROBE?

HRP Stripping buffer (AC2154) efficiently removes antibodies from your blot.

Need help getting started with fluorescent Western blotting? Check out the demo kit, page 8.

AzureSpectra Demo Kit (AC2172–AC2179)



Scan for more information about our free substrate samples

DETECT YOUR PROTEINS OF INTEREST BY CHEMILUMINESCENCE OR FLUORESCENCE

AZURE IMAGING SYSTEMS

The Azure Imaging Systems offers 6 unique Western blot imaging systems. Select which one fits your applications now, and learn more about upgrading later when your needs change.



AZURE 600



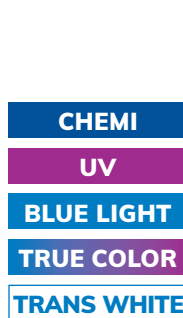
AZURE 500



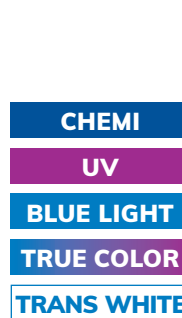
AZURE 400



AZURE 300



AZURE 280

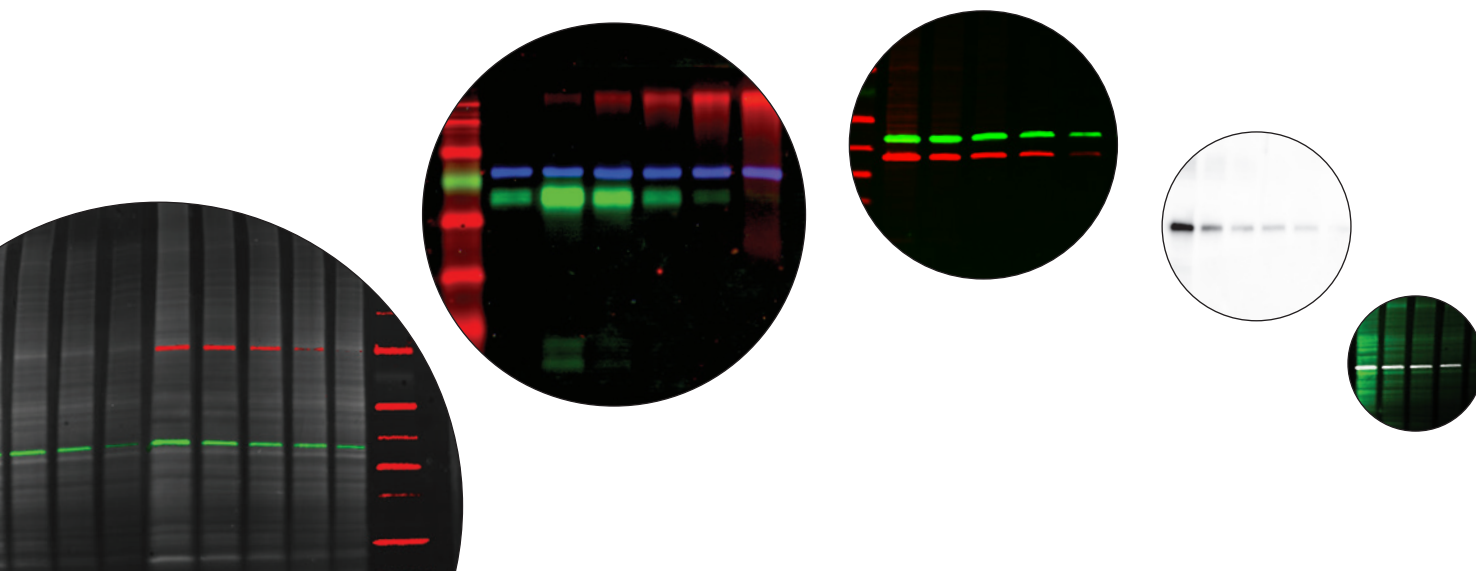


AZURE 200



Upgrades available for chemiluminescence, RGB fluorescence and NIR fluorescence.

Q Module—Upgrade the Azure 500 and Azure 300 to include a green fluorescence Q module to add a dedicated channel for total protein normalization in quantitative Western blots.



ANALYZE YOUR DATA WITH EASY-TO-USE SOFTWARE TO GET THE ANSWERS YOU NEED

AZURESPOT PRO ANALYSIS

AzureSpot Pro helps you interpret your data easily and accurately. Analyze your gels and blots in either manual or automatic modes.

Automatically detect bands, even on distorted gels, and apply your selected method of background correction.

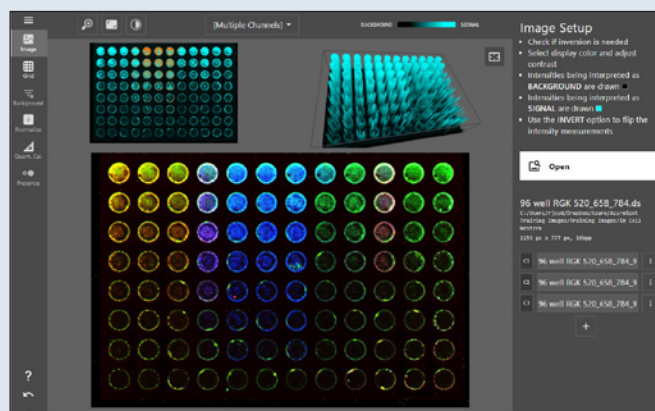
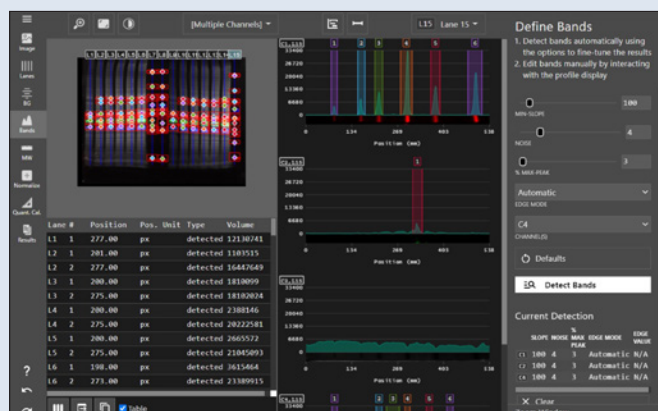
Visualize intensity plots by lane (sample) and channel (probe) to get detailed information about your proteins of interest.

The integrated tutorial helps you get the most information out of your analyses. Generate a report of your experiment or export your data to other applications (e.g. Excel) for further analysis.



Product features

- Integrated tutorial
- Report generator
- Automatic saturated pixel detection
- Analyze multiple blots or plates per image
- Lane creation tools
- Molecular size calibration
- Quantity calibration tool
- Blot and array normalization tool
- Module for easy shape selection/deselection
- Wide range of data fields to display in measurements
- Annotation tools for comments and highlighting of image
- Automatically detect bands
- Correct for background
- Calculate molecular weights
- Quantify fluorescent signals
- Annotate your results
- Visualize multiplex analyses
- Count colonies
- Analyze grids, well-plates and arrays
- Export reports



AZURESPECTRA LABELING KITS

Label your primary or secondary antibody with a fluorescent dye

Fast and simple antibody labeling

- Easy and fast—The fluorescent antibody labeling kit provides the materials necessary to fluorescently label the antibody of your choice in less than 1 hour
- Label primary antibodies—use antibodies that your lab has already validated and optimized. Labeled primary antibodies can then be used for direct Westerns.
- Label secondary antibodies—secondary antibodies can be labeled for traditional indirect Western blot applications
- Choose from 5 dyes—the non-overlapping emission spectra of the fluorophores available for labeling means you can easily perform multiplexing



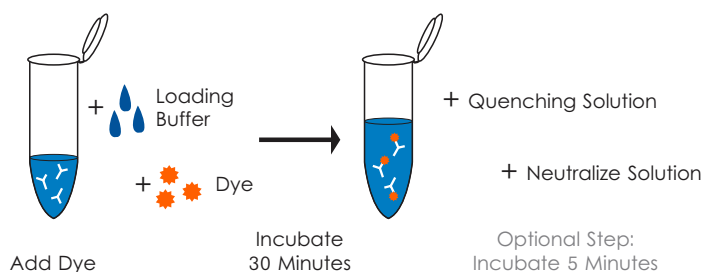
Each kit includes

Sufficient reagents for labeling of 2 x 50 µg of antibody

- Labeling Buffer
- Dye solution
- Quenching solution
- Neutralization buffer
- Two G-25 columns



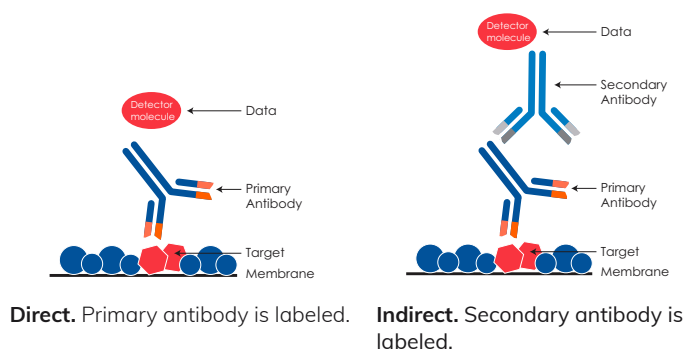
Perform multiplexing assays quick and easily. Fluorescent Western blot imaged with Azure c600. Secondary antibodies were labeled with the AzureSpectra labeling kit: anti-rabbit with AzureSpectra 700, anti-mouse with AzureSpectra 550, and anti-rat with AzureSpectra 800. Primary antibodies used were rat anti-tubulin, rabbit anti-beta actin, and mouse anti-GAPDH.



Simple labeling protocol.



	Excitation max. (nm)	Emission max. (nm)
● Dye-490	491	515
● Dye-550	551	565
● Dye-650	653	672
● Dye-IR700	692	709
● Dye-IR800	783	800



AZURESPECTRA DEMO KIT

New to fluorescent Western blotting?

LET US HELP YOU GET STARTED

Azure's Fluorescent Western blotting Demo Kit contains everything you need to perform single color fluorescent Westerns using your samples and primary antibodies.

What you need

- Your protein sample, resolved by SDS PAGE
- Primary antibody against protein of interest

How to use

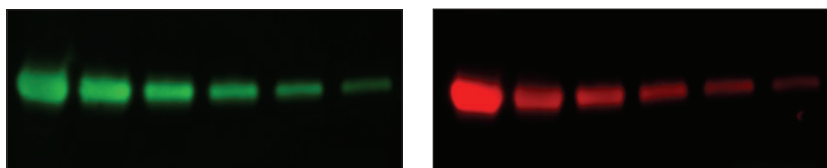
1. Run a gel of your sample and transfer to the provided PVDF membrane
2. Block using Fluorescent Blot Blocking Buffer
3. Probe using your chosen primary antibody
4. Wash with Fluorescent Blot Wash Buffer
5. Incubate with the provided secondary antibody
6. Wash again with Fluorescent Blot Wash Buffer
7. Rinse with PBS
8. Take an image of your fluorescent blot using the provided background quenching sheets



Each kit contains

Enough material for 1 Western blot

- 9x7cm Low Fluorescence PVDF membrane
- 10x Fluorescent Blot Washing Buffer, 30mL
- 1x Fluorescent Blot Blocking Buffer, 30mL
- Secondary Antibody, 4μL



Blots probed with AzureSpectra secondary antibodies and imaged using NIR with an Azure 600. Left: probed with AzureSpectra 800 secondary (green). Right: probed with AzureSpectra 700 (red).



ORDERING INFORMATION

Protein Gel Electrophoresis

AC2124 **AzureRed Fluorescent Protein Stain**—fluorescent total protein stain for 1D and 2D gels, sufficient for 20 minigels, 5 ml

Transfer

AC2105 **Low Fluorescence Western Membrane (PVDF)–0.45 µm**—pre-cut PVDF membrane, 9x7cm vertical gels, 10 sheets

AC2106 **Nitrocellulose Transfer Membrane–0.45 µm**—pre-cut membrane, 8x10 cm mini vertical gels, ideal for larger proteins, 10 sheets

AC2107 **Nitrocellulose Transfer Membrane–0.22 µm**—pre-cut membrane, 8x10 cm mini vertical gels, ideal for smaller proteins, 10 sheets

AC2108 **Low Fluorescence Western Membrane (PVDF)–0.45 µm**—pre-cut PVDF membrane, 10x15 cm vertical gels, 10 sheets

AC2109 **Low Fluorescence Western Membrane (PVDF)–0.45 µm**—pre-cut PVDF membrane, 13x18 cm vertical gels, 10 sheets

AC2110 **Blotting Paper for Western Blotting**—pre-cut blotting paper for Western blot transfer, 7x9 cm vertical gels, 60 sheets

AC2127 **Azure Transfer Buffer**—50x concentrated solution sufficient for 50–100 blots

AC2125 **Azure Ponceau Stain**—visible reversible total protein stain for proteins transferred to membrane, sufficient for staining 50 blots 9x7cm, 500 ml

Blocking

AC2112 **Azure Protein Free Blot Blocking Buffer**—protein-free blocking solution (5x concentrate) for immunoblots, 200 ml

AC2190 **Azure Fluorescent Blot Blocking Buffer**—blocking solution (1X) for fluorescent immunoblots, 500 ml

AC2148 **Azure Chemi Blot Blocking Buffer**—blocking solution (1X) for chemiluminescent immunoblots, 500 ml

Washing

AC2113 **Azure Blot Washing Buffer**—immunoblot washing solution 10x concentrate, 500ml

AC2145 **Azure Fluorescent Blot Washing Buffer**—immunoblot washing solution 10x concentrate, for use with fluorescent secondaries, 500 ml

Blot Accessories

AC2126 **Blot Development Folder**—transparent plastic sheets to hold blots for imaging (alternative to plastic wrap), 100 pcs

AC2144 **Quenching Sheets, small**—10 background quenching sheets, for use with fluorescent or chemiluminescent samples (9x12.5 cm)

AC2147 **Quenching Sheets, large**—10 background quenching sheets, for use with fluorescent or chemiluminescent samples (16x22 cm)

Detection Substrates

AC2101 **Radiance**—HRP Substrate for CCD 150 ml kit, contains substrate for 1500cm² membrane

AC2103 **Radiance Plus**—femtogram HRP Substrate 150 ml kit, contains substrate for 1500cm² membrane

AC2146 **Azure ChemiWriter ECL**—a chemiluminescent marker that enables you to write or draw on your transfer membranes, resulting in a chemiluminescent signal on your markings once substrate is added

Kits

AC2140 **AzureSpectra rb700/ms800 Western Kit, with Protein Free Block**—kit contains materials for 10 Western Blots: PVDF membranes, wash buffer, protein free blocking buffer, secondary antibodies AzureSpectra rb700/ms800

AC2141 **AzureSpectra ms700/rb800 Western Kit, with Protein Free Block**—kit contains materials for 10 Western Blots: PVDF membranes, wash buffer, protein free blocking buffer, secondary antibodies AzureSpectra ms700/rb800

AC2104 **AzureSpectra rb650/ms550 Western Kit, with Protein Free Block**—kit contains materials for 10 Western Blots: PVDF membranes, wash buffer, protein free blocking buffer, secondary antibodies rb650/ms550

AC2191 **AzureSpectra rb700/ms800 Western Kit, with Fluorescent Block**—kit contains materials for 10 Western Blots: PVDF membranes, wash buffer, fluorescent blocking buffer, secondary antibodies AzureSpectra rb700/ms800

AC2192 **AzureSpectra ms700/rb800 Western Kit, with Fluorescent Block**—kit contains materials for 10 Western Blots: PVDF membranes, wash buffer, fluorescent blocking buffer, secondary antibodies AzureSpectra ms700/rb800

AC2193 **AzureSpectra rb650/ms550 Western Kit, with Fluorescent Block**—kit contains materials for 10 Western Blots: PVDF membranes, wash buffer, fluorescent blocking buffer, secondary antibodies AzureSpectra rb650/ms550

Stripping Buffer

AC2154 **HRP Stripping Buffer**—uniformly removes antibodies from developed HRP Western blots so the blot can be reprobed with another set of antibodies



SCAN ME

Incubation Trays	
AC2120	7x5 cm, opaque black top and bottom —for staining and washing light sensitive gels and membranes, includes 5 trays
AC2121	9x6 cm, opaque black top and bottom —for staining and washing light sensitive gels and membranes, includes 4 trays
AC2122	11x9 cm, opaque black top and bottom —for staining and washing light sensitive gels and membranes, includes 3 trays
AC2123	15.5x10.5 cm, opaque black top and bottom —for staining and washing light sensitive gels and membranes, includes 2 trays
AC2150	7x5 cm, clear —for staining and washing gels and membranes, includes 5 trays
AC2151	9x6 cm, clear —for staining and washing gels and membranes, includes 4 trays
AC2152	11x9 cm, clear —for staining and washing gels and membranes, includes 3 trays
AC2153	15.5x10.5 cm, clear —for staining and washing gels and membranes, includes 2 trays

AzureSpectra 800 secondary antibodies

AC2134	Goat-anti-rabbit —AzureSpectra 800 conjugated secondary antibody for fluorescent Westerns, 250 µl
AC2135	Goat-anti-mouse —AzureSpectra 800 conjugated secondary antibody for fluorescent Westerns, 250 µl
AC2136	Goat-anti-human —AzureSpectra 800 conjugated secondary antibody for fluorescent Westerns, 250 µl
AC2137	Goat-anti-chicken —AzureSpectra 800 conjugated secondary antibody for fluorescent Westerns, 250 µl
AC2138	Goat-anti-rat —AzureSpectra 800 conjugated secondary antibody for fluorescent Westerns, 250 µl
AC2139	Goat-anti-guinea pig —AzureSpectra 800 conjugated secondary antibody for fluorescent Westerns, 250 µl
AC2157	Donkey-anti-goat —AzureSpectra 800 conjugated secondary antibody for fluorescent Westerns, 250 µl

AzureSpectra 700 secondary antibodies

AC2128	Goat-anti-rabbit —AzureSpectra 700 conjugated secondary antibody for fluorescent Westerns, 250 µl
AC2129	Goat-anti-mouse —AzureSpectra 700 conjugated secondary antibody for fluorescent Westerns, 250 µl
AC2130	Goat-anti-human —AzureSpectra 700 conjugated secondary antibody for fluorescent Westerns, 250 µl
AC2131	Goat-anti-chicken —AzureSpectra 700 conjugated secondary antibody for fluorescent Westerns, 250 µl
AC2132	Goat-anti-rat —AzureSpectra 700 conjugated secondary antibody for fluorescent Westerns, 250 µl

AC2132	Goat-anti-rat —AzureSpectra 700 conjugated secondary antibody for fluorescent Westerns, 250 µl
AC2133	Goat-anti-guinea pig —AzureSpectra 700 conjugated secondary antibody for fluorescent Westerns, 250 µl
AC2156	Donkey-anti-goat —AzureSpectra 700 conjugated secondary antibody for fluorescent Westerns, 250 µl

AzureSpectra 650 secondary antibodies

AC2165	Goat-anti-rabbit —AzureSpectra 650 conjugated secondary antibody for fluorescent Westerns, 250 µl
AC2166	Goat-anti-mouse —AzureSpectra 650 conjugated secondary antibody for fluorescent Westerns, 250 µl
AC2167	Goat-anti-human —AzureSpectra 650 conjugated secondary antibody for fluorescent Westerns, 250 µl
AC2168	Goat-anti-chicken —AzureSpectra 650 conjugated secondary antibody for fluorescent Westerns, 250 µl
AC2169	Goat-anti-rat —AzureSpectra 650 conjugated secondary antibody for fluorescent Westerns, 250 µl
AC2170	Goat-anti-guinea pig —AzureSpectra 650 conjugated secondary antibody for fluorescent Westerns, 250 µl
AC2171	Donkey-anti-goat —AzureSpectra 650 conjugated secondary antibody for fluorescent Westerns, 250 µl

AzureSpectra 550 secondary antibodies

AC2158	Goat-anti-rabbit —AzureSpectra 550 conjugated secondary antibody for fluorescent Westerns, 250 µl
AC2159	Goat-anti-mouse —AzureSpectra 550 conjugated secondary antibody for fluorescent Westerns, 250 µl
AC2160	Goat-anti-human —AzureSpectra 550 conjugated secondary antibody for fluorescent Westerns, 250 µl
AC2161	Goat-anti-chicken —AzureSpectra 550 conjugated secondary antibody for fluorescent Westerns, 250 µl
AC2162	Goat-anti-rat —AzureSpectra 550 conjugated secondary antibody for fluorescent Westerns, 250 µl
AC2163	Goat-anti-guinea pig —AzureSpectra 550 conjugated secondary antibody for fluorescent Westerns, 250 µl
AC2164	Donkey-anti-goat —AzureSpectra 550 conjugated secondary antibody for fluorescent Westerns, 250 µl

HRP secondary antibodies

AC2114	Goat-anti-rabbit —HRP conjugated secondary antibody for chemi Westerns, 500 µl
AC2115	Goat-anti-mouse —HRP conjugated secondary antibody for chemi Westerns, 500 µl
AC2116	Goat-anti-human —HRP conjugated secondary antibody for chemi Westerns, 500 µl
AC2117	Goat-anti-chicken —HRP conjugated secondary antibody for chemi Westerns, 500 µl

AC2118	Goat-anti-rat —HRP conjugated secondary antibody for chemi Westerns, 500 µl
AC2119	Goat-anti-guinea pig —HRP conjugated secondary antibody for chemi Westerns, 500 µl
AC2149	Donkey-anti-goat —HRP conjugated secondary antibody for chemi Westerns, 500 µl

AzureSpectra Demo Kits

AC2172	Goat-anti-mouse 700 —kit contains 1 PVDF membrane, blocking buffer, wash buffer, goat anti-mouse 700 secondary antibody
AC2173	Goat-anti-rabbit 700 —kit contains 1 PVDF membrane, blocking buffer, wash buffer, goat anti-rabbit 700 secondary antibody
AC2174	Goat-anti-mouse 800 —kit contains 1 PVDF membrane, blocking buffer, wash buffer, goat anti-mouse 800 secondary antibody
AC2175	Goat-anti-rabbit 800 —kit contains 1 PVDF membrane, blocking buffer, wash buffer, goat anti-rabbit 800 secondary antibody
AC2176	Goat-anti-mouse 650 —kit contains 1 PVDF membrane, blocking buffer, wash buffer, goat anti-mouse 650 secondary antibody
AC2177	Goat-anti-rabbit 650 —kit contains 1 PVDF membrane, blocking buffer, wash buffer, goat anti-rabbit 650 secondary antibody
AC2178	Goat-anti-mouse 550 —kit contains 1 PVDF membrane, blocking buffer, wash buffer, goat anti-mouse 550 secondary antibody
AC2179	Goat-anti-rabbit 550 —kit contains 1 PVDF membrane, blocking buffer, wash buffer, goat anti-rabbit 550 secondary antibody

AzureSpectra Labeling Kits

AC2185	For Westerns – 490 —for covalently labeling antibodies with a fluorescent dye. Includes labeling buffer, dye solution, quenching solution, neutralization buffer and two G-25 columns. Each kit includes sufficient reagents to perform two labellings of 50 micrograms of antibody.
AC2186	For Westerns – 550 —for covalently labeling antibodies with a fluorescent dye. Includes labeling buffer, dye solution, quenching solution, neutralization buffer and two G-25 columns. Each kit includes sufficient reagents to perform two labellings of 50 micrograms of antibody.

AC2187	For Westerns – 650 —for covalently labeling antibodies with a fluorescent dye. Includes labeling buffer, dye solution, quenching solution, neutralization buffer and two G-25 columns. Each kit includes sufficient reagents to perform two labellings of 50 micrograms of antibody.
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AC2188	For Westerns – 700 —for covalently labeling antibodies with a fluorescent dye. Includes labeling buffer, dye solution, quenching solution, neutralization buffer and two G-25 columns. Each kit includes sufficient reagents to perform two labellings of 50 micrograms of antibody.
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AC2189	For Westerns – 800 —for covalently labeling antibodies with a fluorescent dye. Includes labeling buffer, dye solution, quenching solution, neutralization buffer and two G-25 columns. Each kit includes sufficient reagents to perform two labellings of 50 micrograms of antibody.
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Azure Imaging Systems

AZI600-01	Azure Imager 600
AZI500-01	Azure Imager 500
AZI400-01	Azure Imager 400
AZI300-01	Azure Imager 300
AZI280-01	Azure Imager 280
AZI200-01	Azure Imager 200
CS1000	chemiSOLO Imager



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MM-0102 R1