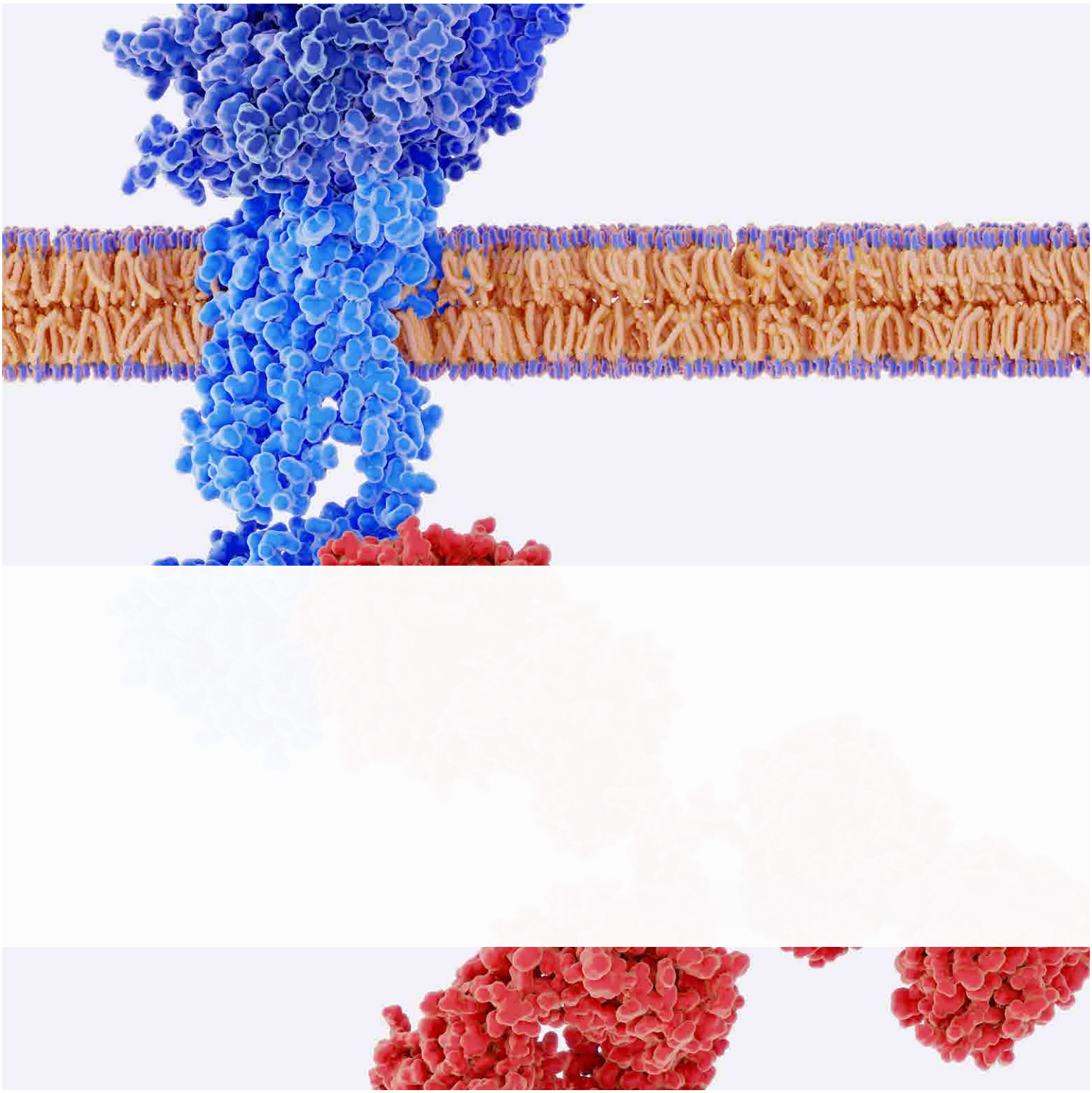
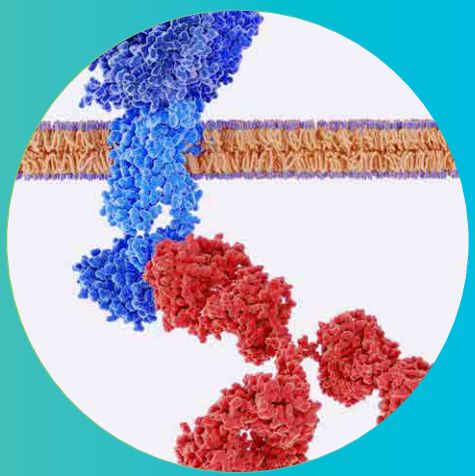


Focus: Western Blot

Western Blot detects specific proteins in a complex mixture of proteins extracted from cells or tissues. The three main steps in the workflow are separation by size, transfer to stable support, and visualization using appropriate primary and secondary antibodies.

For more information on products that fit each step in this workflow, visit **vwr.com/western-blot.**

2020



Western blot comprehensive comparison

Chemiluminescence or fluorescence?

By Justin Luh, Global Marketing Lead, Azure Biosystems

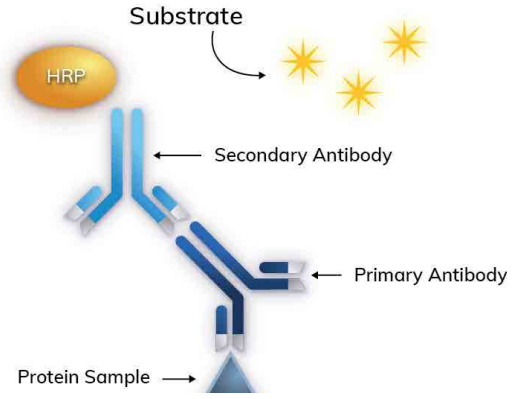
CHEMILUMINESCENCE OVERVIEW

Chemiluminescent Westerns are a popular indirect detection method for assessing protein expression, wherein an antibody that has conjugated to an enzyme reacts with chemiluminescent substrates to emit light that is then captured and archived on x-ray film or through digital imaging. Although only semi-quantitative, chemiluminescent Western blotting is a highly sensitive assay that can detect the presence or absence of a protein femtograms. Chemiluminescence is thus useful in detecting the induction of exogenous protein expression, confirming and following purification of a known protein, or for verification of antibodies during production, to list a few examples.

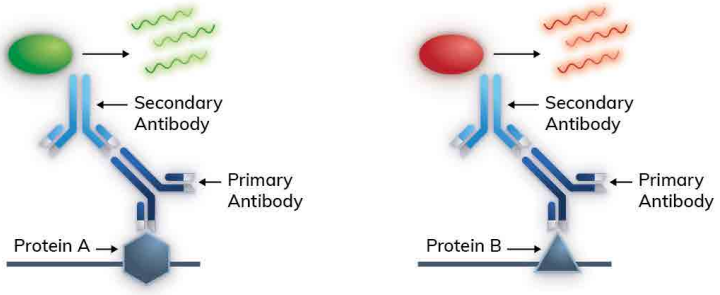
However, chemiluminescent Westerns can be difficult to perform. While the ultimate goal is to obtain a blot with a high signal-to-noise ratio, chemiluminescent Westerns can be plagued with high background, either in the form of an overall background that masks the signal from the protein of interest or as bright dots, speckles and/or splotches scattered randomly over the blot. Increased background noise can arise from a variety of factors, and it can also be difficult to obtain a strong signal from the protein of interest.

FLUORESCENCE OVERVIEW

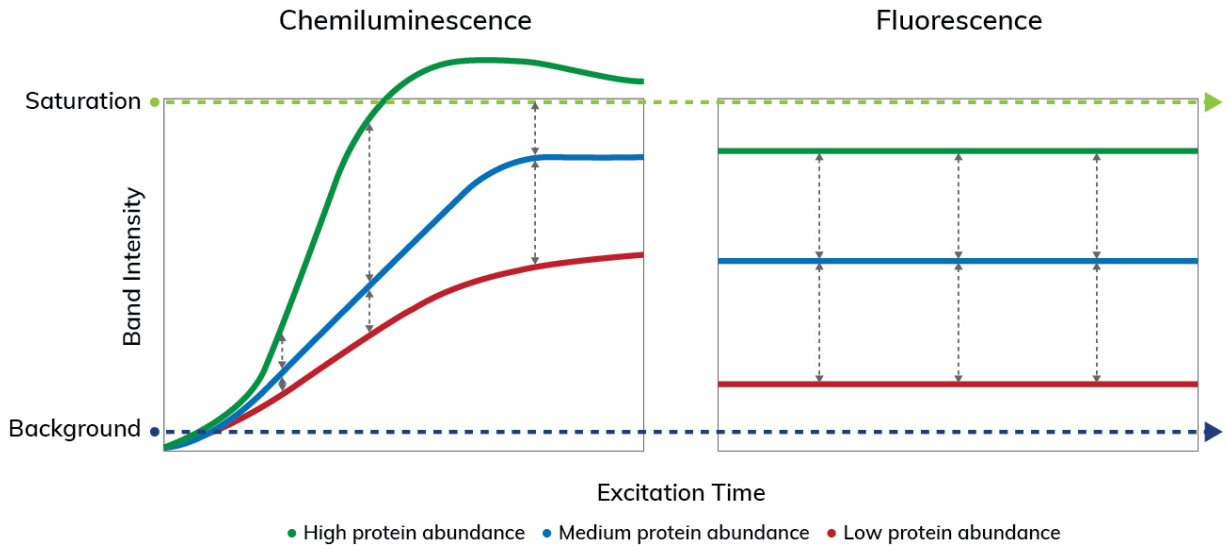
Fluorescent Western blots are the gold standard for quantitative Westerns, ideal for detecting multiple proteins simultaneously (multiplexing) and allowing in-lane normalization and detection of your protein of interest, as well as loading control. In addition, post-translational modifications can be studied and quantitated easily.



Primary antibodies probe for protein of interest. A secondary antibody is conjugated to horseradish peroxidase, an enzyme which enhances the light emitted from a chemiluminescent reaction.



Two primary antibodies can probe for two separate proteins of interest. Secondary antibodies are conjugated to fluorescent labels known as fluorophores. These fluorophores have distinct excitation and emission spectra.



Chemiluminescent vs. fluorescent signal. Due to the enzymatic nature of a chemiluminescent reaction, signal/band intensity increases greatly over time. This can be problematic for highly abundant proteins that saturate the detector. Since fluorophores are bound directly to the secondary fluorescent conjugate, the signal intensity is correlated directly to protein abundance.

In fluorescent Western blotting, the secondary antibody is directly conjugated to a dye that is excited by light, which is then detected by a digital imager and digitized for data analysis. Multiple proteins can be detected simultaneously by using secondary antibodies conjugated to different dyes with non-overlapping spectral emissions.

WHICH IS RIGHT FOR YOU?

Although the process of immunoblotting has been around for over 40 years, innovations in digital imaging have led to advances in both chemiluminescent and fluorescent western blots. Chemiluminescence has been established and integrated in many labs globally due to its traditional use with film and high sensitivity. However, the kinetic and enzymatic nature of this reaction promotes saturation in highly abundant samples, making a chemiluminescent blot non-quantitative as a signal will not accurately represent protein levels. Additionally, the reaction time of chemiluminescence is limited by the quality of substrate and only allows probing for a single protein of interest. There is increased cost associated with stripping a chemiluminescent blot and reprobing to perform semi-quantitative analysis.

Fluorescent western blotting has been rapidly adopted due to its capacity for multiplex detection and cost-efficiency. LED and laser-based excitation of fluorescent probes increases sensitivity and reduces cross-excitation between probes of adjacent wavelengths. The ability to identify up to four distinct proteins of interest reduces the time spent stripping and reprobing. The direct correlation between signal intensity and protein abundance makes fluorescent western blotting perfect for quantitative analysis of post-translational modifications.

No matter which Western blot method you prefer, Avantor offers a wide range of quality equipment and supplies to help you achieve the best results.

SOURCES

1. https://www.azurebiosystems.com/wp-content/uploads/2016/04/Improve-Chemi-Blots-app-note_3.pdf
2. https://www.azurebiosystems.com/wp-content/uploads/2016/04/Improve-Fluorescent-Blots-app-note_3.pdf

The ultimate all-in-one imager for gels and Western blots

AZURE IMAGING SYSTEMS, AZURE BIOSYSTEMS

INNOVATIVE WESTERN BLOT AND GEL DOCUMENTATION SYSTEMS DESIGNED TO BE EASY-TO-USE LAB WORKHORSES

- Designed for flexible Western blot applications
- A wide range of applications with multiple light sources and filters

The darkroom replacer, model 300, features fast chemiluminescent detection with no film or darkroom needed. It can be upgraded to any of the ascending Azure Imager models (400, 500 or 600) with a simple field upgrade.

The visible fluorescent western imaging system, model 400, features a three-channel RGB excitation.

Description	Cat. No.
Systems	
Chemi and NIR Imaging, Azure 500	**76353-886**
Chemi and RGB Imaging, Azure 400	**76353-888**
Chemiluminescet Western Imaging, Azure 300	**76353-890**
The Ultimate Western Blot Imaging System, Azure 600	**76353-884**

Everything you need

RADIANCE Q CHEMILUMINESCENT SUBSTRATE, AZURE BIOSYSTEMS

SETS THE BAR FOR SENSITIVITY AND QUANTITATIVE ABILITY

Specially developed for CCD imaging, Radiance Q produces a strong, long-lasting signal with extremely low background, perfect for detecting low abundance proteins.

Description	Volume_NA	Contents_NA	Cat. No.
HRP Substrate for CCD 150 mL Kit	150 mL	Substrate for 1500 cm^2 Membrane	**10147-296**

CHEMI BLOT BLOCKING BUFFER, AZURE

Size	Cat. No.
500 mL	**75818-198**

AZURESPECTRA FLUORESCENT WESTERN BLOTTING KITS, AZURE BIOSYSTEMS

PROVIDES EVERYTHING YOU NEED TO CONDUCT TWO-COLOR FLUORESCENT WESTERN BLOTS

Each kit includes pre-cut membranes, wash buffer, blocking buffer, quenching sheets, and secondary antibodies to detect two proteins per experiment.

Description	Sufficient For	Cat. No.
AzureSpectra rb700/ms800 Western Kit	10 Blots	**10147-374**
AzureSpectra ms700/rb800 Western Kit	10 Blots	**10147-376**
AzureSpectra rb660/ms578	10 Blots	**10147-378**

Run up to four gels

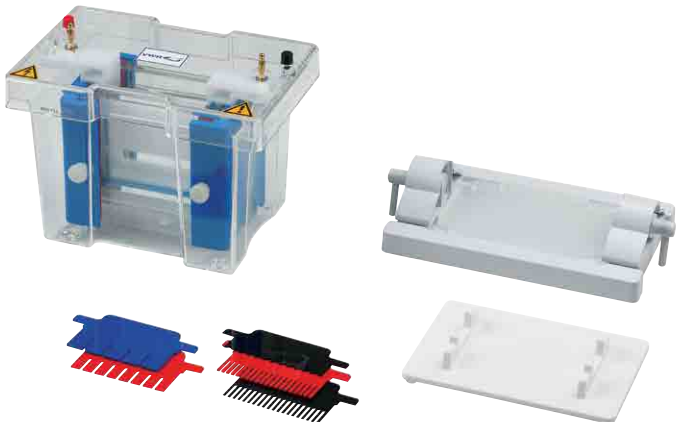
VWR® MINI VERTICAL PAGE SYSTEM

IDEAL FOR PROTEIN ELECTROPHORESIS, IEF, AND BLOTTING

- Can run up to four gels
- Inserts for each application
- Electrode assembly for each insert
- Common buffer tank

The system features inserts for each application and a common buffer tank.

All spacers and combs are color-coded or labeled to indicate thickness. The 8- and 16-tooth combs are designed for multi-channel pipettor loading.



Description	Buffer Volume	Cat. No.
Mini Vertical PAGE System	250 mL	**89032-300**

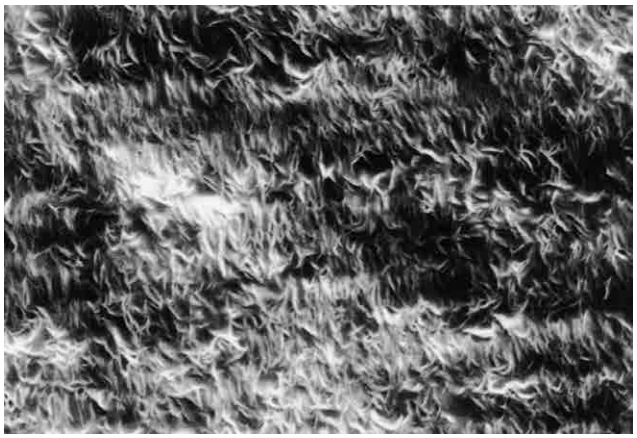
Sensitive protein detection with low background burnthrough

FLUOROTRANS® TRANSFER MEMBRANES, PALL LABORATORY

IDEAL FOR A WIDE VARIETY OF PROTEIN-ANALYSIS APPLICATIONS

- For protein analysis
- Naturally hydrophobic
- Resistant to chemicals

Filter membranes are resistant to a wide range of chemical solvents (i.e., acetone, DMSO, dimethyl formamide) and will not shrink during destaining with methanol. Resistance to trifluoroacetic acid and triethylamine allows direct insertion of FluoroTrans® membrane materials into amino acid analyzers and gas phase protein sequencing equipment.



Description	Size	Pore Size	Cat. No.
FluoroTrans® W PVDF Membrane	3.3 m Roll	0.2 µm	**29301-856**
FluoroTrans® W PVDF Membrane	7×9 cm	0.2 µm	**29301-852**
FluoroTrans® W PVDF Membrane	10×15 cm	0.2 µm	**29301-850**
FluoroTrans® W PVDF Membrane	20×20 cm	0.2 µm	**29301-854**

PromoCell

Designed to target a broad range of proteases

PROTEASE INHIBITOR COCKTAIL, PROMOCELL

CONTAINS A MIXTURE OF PROTEASE INHIBITORS WITH BROAD SPECIFICITY

- Optimized to generate the maximal protection to mammalian tissue and cell extracts
- Broad specificity for various proteases
- Water-soluble protease inhibitors

The protease inhibitor cocktail contains a mixture of water-soluble protease inhibitors with broad specificity for various proteases, and has been optimized to generate the maximal protection to mammalian tissue and cell extracts.

Description_NA	Size_NA	Cat. No.
Protease Inhibitor Cocktail	1 vial	**10183-804**

VWR® LIFE SCIENCE

Lightweight convenience

VWR® 200 HOMOGENIZER

HOMOGENIZE SAMPLES WITH VOLUMES AS SMALL AS 0.03 ML, OR AS LARGE AS 1L

- Lightweight handheld homogenizer unit
- For processing within various tube sizes to small beakers
- High-torque motor with variable speed adjustment of 5000 to 33,000 rpm
- Use handheld unit or post mount to a stand
- VWR® Generator probe sold separately

The VWR® 200 Homogenizer unit has a 144 watt, high-torque motor with variable speed adjustment, allowing for homogenization in mere seconds and a maximum noise level of only 68dB.

The unit is ideal for use with VWR® GenPack generator probes for low-cost, repeat homogenization of many samples without the risk of cross-contamination.

Description	Electrical	Volume Range	Speed Range	Cat. No.
Homogenizer				
VWR® 200 Homogenizer	120V	0.5 mL Tube to 1L (H_20)	Analog: 5000–35,000 rpm (with Separate ON/OFF Switch)	**10032-336**

Highly linear and reversible near-IR total protein labeling

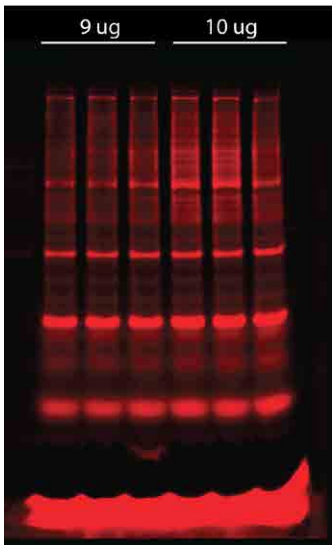
VERSABLOT™ TOTAL PROTEIN LABELING KITS, BIOTIUM

SENSITIVE, ACCURATE, AND REVERSIBLE NEAR-IR PROTEIN DETECTION FOR SDS-PAGE AND WESTERN BLOT NORMALIZATION

- Pre-label proteins or lysates with bright near-IR CF® dyes
- **Linear:** better western normalization than housekeeping proteins
- **Reversible:** allows the same channel to be used for western
- **Sensitive:** detect as little as 1 ng protein
- **Accurate:** detect 10% difference in protein content

VersaBlot™ Total Protein Normalization Kits are designed for rapid and sensitive in-gel protein detection and protein normalization on western blot (WB) membranes using near-infrared CF® Dyes that covalently label proteins. Available in 2 dye color options, CF®680T for the 700 channel on the LI-COR® Odyssey® or Cy®5 or Typhoon™ IR Short filter and CF®770T for the 800 channel on the LI-COR® Odyssey® or Typhoon™ IR Long filter.

Description	Size	Cat. No.
CF680T Protein Normalization Kit	1000 Assays	**76403-954**
CF770T Protein Normalization Kit	1000 Assays	**76403-958**



Quantitative visualization of HeLa lysates labeled with the VersaBlot™ CF®770T Total Protein Normalization Kits after transfer on PVDF membrane scanned on the Odyssey® Infrared Imaging System.

Ideal for SDS-PAGE and blotting applications

VWR® POWER SUPPLY

IDEAL FOR BASIC ELECTROPHORESIS APPLICATIONS SUCH AS NUCLEIC ACID, PROTEIN SEPARATION AND SMALL WET BLOT WESTERN BLOTTING APPLICATIONS

- Compact footprint, takes up minimal lab space
- 500 V/400 mA, 120 W maximum output
- 300 V/2000 mA, 300 W maximum output
- Ideally suited for horizontal DNA gels, vertical protein gels

The color touchscreen control panel is easy to use, allowing setting of voltage, amperage and run time. Four sets of output jacks allow connection of multiple electrophoresis tanks.

Description_NA	Voltage_NA	Cat. No.
115 VAC Unit		
VWR® Power Supply	10 - 500 V	**76196-454**
VWR® Power Supply	10 - 300 V	**76196-458**

High purity and protease-free

NONIDET® P 40 SUBSTITUTE (NP-40), PROTEOMICS GRADE

NON IONIC DETERGENT EFFICIENTLY SEPARATES HYDROPHILIC PROTEINS WITHOUT ALTERING BIOLOGICAL ACTIVITY

- High purity and protease-free
- Suitable for most biological applications

Non-ionic detergents have uncharged hydrophilic groups and are gentle, non-denaturing detergents ideal for studying the conformation and function of proteins because they keep secondary and tertiary structure intact.

Size	Cat. No.
50 ml	**97064-922**
100 ml	**97064-918**
500 ml	**97064-920**

Ideal for monitoring your protein electrophoresis

VWR LIFE SCIENCE PROTEIN MOLECULAR WEIGHT MARKER, WIDE RANGE

- Biotechnology Grade
- 8 bands ranging from 14.0 to 212.0 kDa
- Ideal for high or low molecular weight applications

Description	Size	Application	Cat. No.
Protein MW Marker, Wide Range	500 µL	Sufficient for 150 lanes	**97063-556**

Consistent performance and quality

VWR LIFE SCIENCE TRIS-GLYCINE BUFFER

FOR USE IN IMMUNOBLOTTING OR SEQUENCING

- Convenience of liquid concentrates or powder blend
- Consistent performance and quality

Although there are different methods by which to achieve transfer, the one most routinely used is electroblotting. Electroblotting utilizes conductive buffers that do not interfere with proteins binding to the membrane and can be performed in a vertical buffer tank or in a semi-dry blotting apparatus.

Description	Size	Application	Cat. No.
READY-PACK™			
Tris-Glycine Buffer, 10x Ready-Pack™	2 pack	Enough powder to make 40 L of 1X buffer. Each pack makes 1L of 10X concentrate.	**97063-780**
Tris-Glycine Buffer, 10x Ready-Pack™	40 L	Enough powder to make 40 L of 1X buffer. Each pack makes 1L of 10X concentrate.	**97063-782**
SOLUTION			
Tris-Glycine Buffer, 10x Solution	1 L	A 1X working solution contains 25 mM Tris base, 0.192 M Glycine.	**97063-776**
Tris-Glycine Buffer, 10x Solution	4 L	A 1X working solution contains 25 mM Tris base, 0.192 M Glycine.	**97063-778**

Add methanol to make standard transfer buffer

Wells to Westerns:

Investigating the cellular heat shock response

In this application note we show how data for several related cellular parameters was collected using a single instrument, the SpectraMax® i3 Multi-Mode Detection Platform. Heat shock was used as a model system to show how different detection modes, including imaging and Western blot scanning, can be used to gain insights into a multi-faceted cellular response.

Exposure of cells to higher than normal temperatures is known to activate the apoptosis pathway, as well as increase expression of the heat shock protein HSP70. CHO-K1 cells under two different growth conditions – healthy or stressed – were exposed to heat shock then assayed for proliferation, viability and apoptosis using the imaging capability of the SpectraMax i3 system. Expression of HSP70, which is upregulated in response to heat shock, was analyzed using the ScanLater™ Western Blot Detection System.

One complete solution – with available options such as the SpectraMax® MiniMax™ 300 Imaging Cytometer, ScanLater™ Western Blot cartridge, optimized reagents, and the industry-leading data acquisition and analysis tool SoftMax Pro – the SpectraMax i3x* Detection Platform allows you to explore cellular pathways and protein activation and expression in one system.

MATERIALS

- CHO-K1 cells (ATCC P/N CCL-61)
- Cell culture medium (Ham's F12 with 10% fetal bovine serum and 1% penicillin/streptomycin)
- Black-wall, clear-bottom tissue culture microplates (29444-008)
- SpectraMax® i3 Microplate Reader (10014-924)
- SpectraMax® MiniMax™ 300 Cytometer (10192-218)
- EarlyTox™ Cell Integrity Kit (10127-640)
- CellEvent Caspase-3/7 Green Detection Reagent
- ScanLater™ Western Blot Detection System
- ScanLater™ Anti-Mouse Evaluation Kit with Europium-labeled goat anti-mouse antibody (10048-938)
- ScanLater™ Western Blot Detection Cartridge (10192-222)
- Anti-HSP70 mouse monoclonal antibody (R&D Systems P/N MAB1663)
- Immobilon-FL Membrane, 0.45 µm pore size (EMD Millipore P/N IPFL 000 10)

METHODS

Two populations of CHO-K1 cells were prepared: healthy and stressed. The healthy cells were given regular changes of culture medium and passaged at ≤ 80% confluence, while the stressed cells were allowed to become over-confluent and

Apoptosis
Cell Confluence
Cell Counting
Cell Cytotoxicity
Cell Proliferation
Cell Viability
Protein Expression
Western Blot Detection

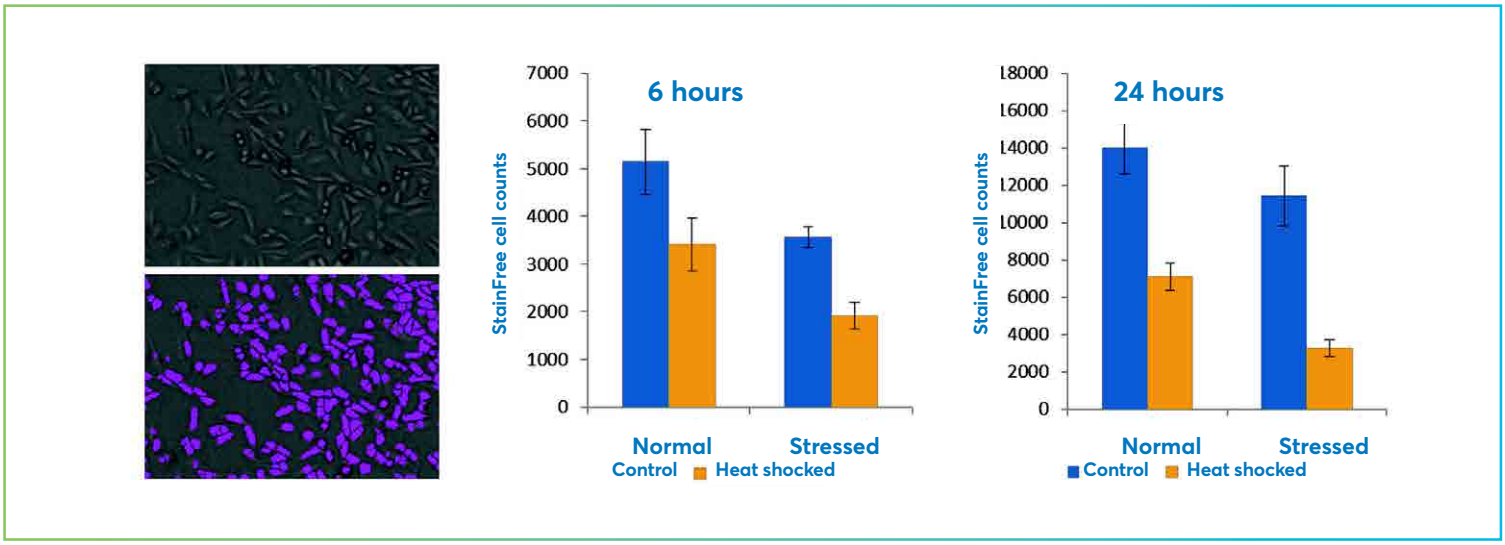


FIGURE 1. **StainFree measurement of cell proliferation after heat shock.** On the left are a transmitted light image of untreated CHO-K1 cells (top) and purple masks showing cells identified by StainFree analysis (bottom). Graphs show StainFree cell counts for normal and stressed populations of cells under control or heat shocked conditions. Cell counts were compared at 6 and 24 hours.

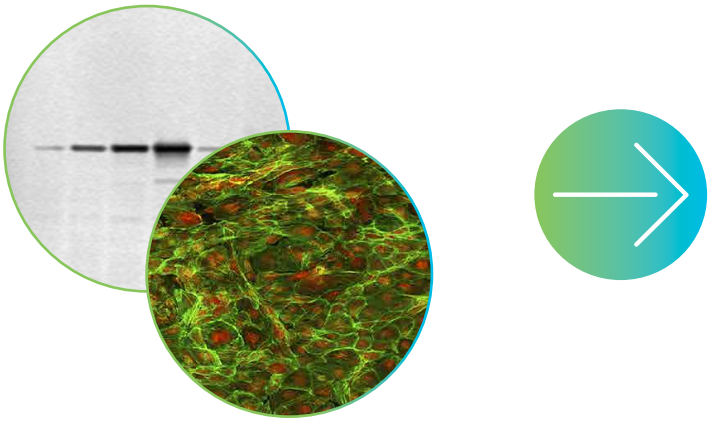
had less frequent replacement of their medium.

The day before heat shock, healthy or stressed cells were seeded at 7500 cells per well in 96-well black-wall, clear-bottom tissue culture microplates for imaging and cell-based assays. For Western blot samples, cells were seeded at 225,000 cells per well in 6-well tissue culture plates.

For Western blot, cells grown and treated in 6-well plates were trypsinized, pelleted, washed and lysed in PBS with 0.5% Tween and protease inhibitors. Lysates were centrifuged, and the supernatants were assayed for protein content. 2.1 µg of cell extracts were loaded on a 4-20% gel, transferred to a PVDF membrane, and probed with mouse anti-HSP70, followed by Europium-labeled anti-mouse secondary antibody. Protein bands were detected and quantified using the SpectraMax i3 reader with ScanLater Western Blot Detection Cartridge.

CELL PROLIFERATION

After heat shock, cells were allowed to recover for 6 or 24 hours prior to imaging and other assays. At the two timepoints, differences in cell number were quantified using StainFree cell counting on the SpectraMax MiniMax 300 imaging



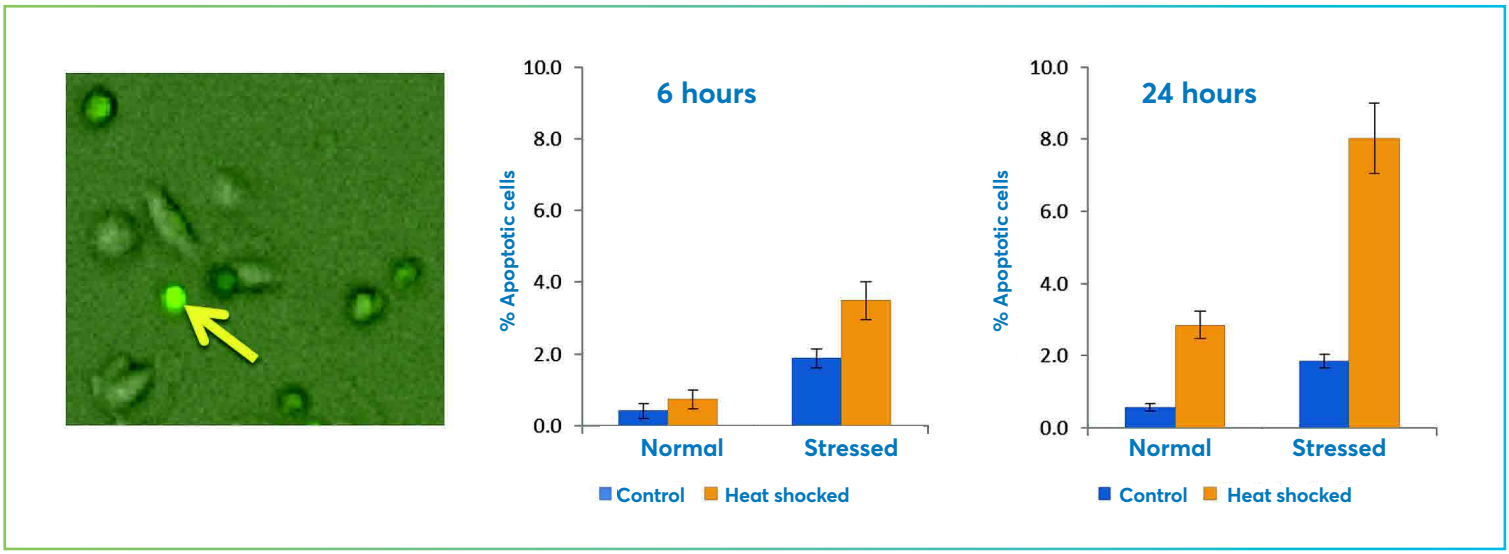


FIGURE 2. **Apoptosis in control and heat-shocked cells.** The left panel shows apoptotic (bright green) cells with rounded morphology, as well as several non-apoptotic cells with a more flattened, elongated shape. The yellow arrow points to an apoptotic cell with a high level of green fluorescence. The graphs show percent apoptosis in normal and stressed cell populations under control and heat shock conditions.

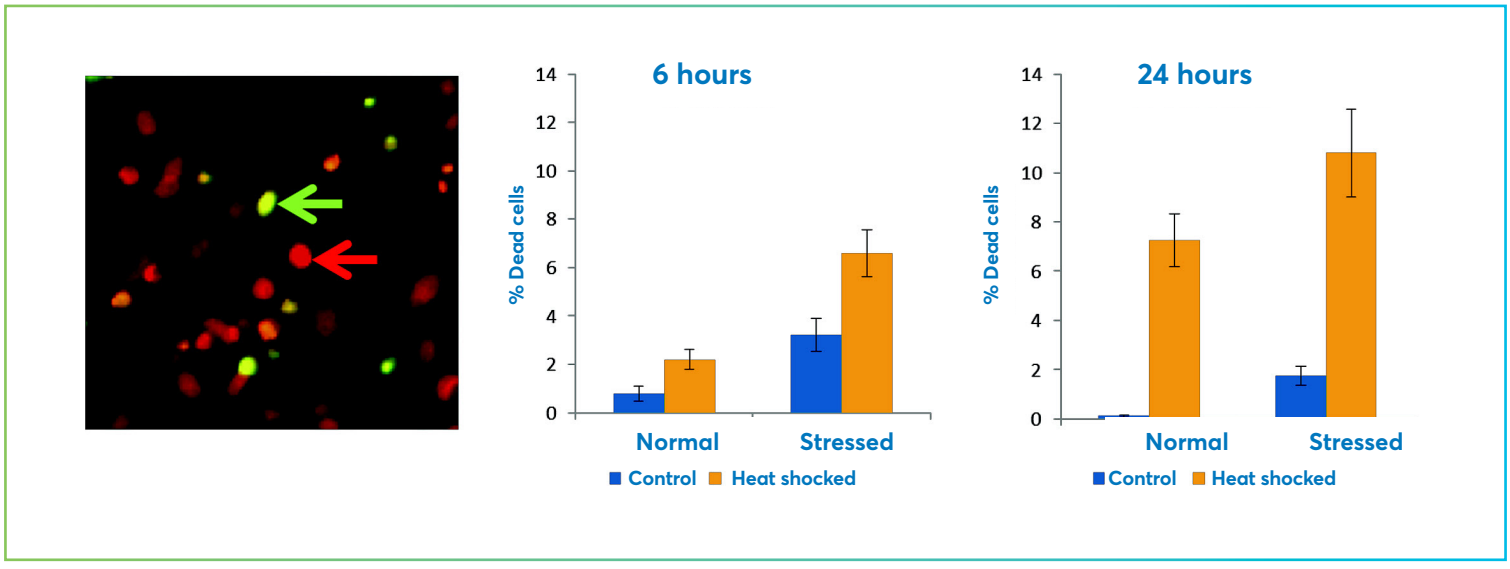


FIGURE 3. **Quantitation of cell viability after heat shock.** Heat shocked cells were imaged in the left panel (green arrow, dead cell; red arrow, live cell). Graphs show the increased percentage of dead cells upon heat shock, in both normal and stressed cell populations.

cytometer. No dye was required for the software to identify and count cells. A predefined setting in the SoftMax® Pro Software was used to identify cells. A decrease in proliferation in heat-shocked cells, particularly in the stressed population, was observed, and it was more pronounced after 24 hours (Figure 1, page 11).

APOPTOSIS ASSAY

At the 6- and 24-hour time points, cells were assayed for apoptosis. By 6 hours post-heat shock, stressed cells exhibited a higher percentage of apoptotic cells, but by 24 hours there were many more apoptotic cells in the stressed, heat-shocked population (Figure 2). By comparison, the non-heat-shocked

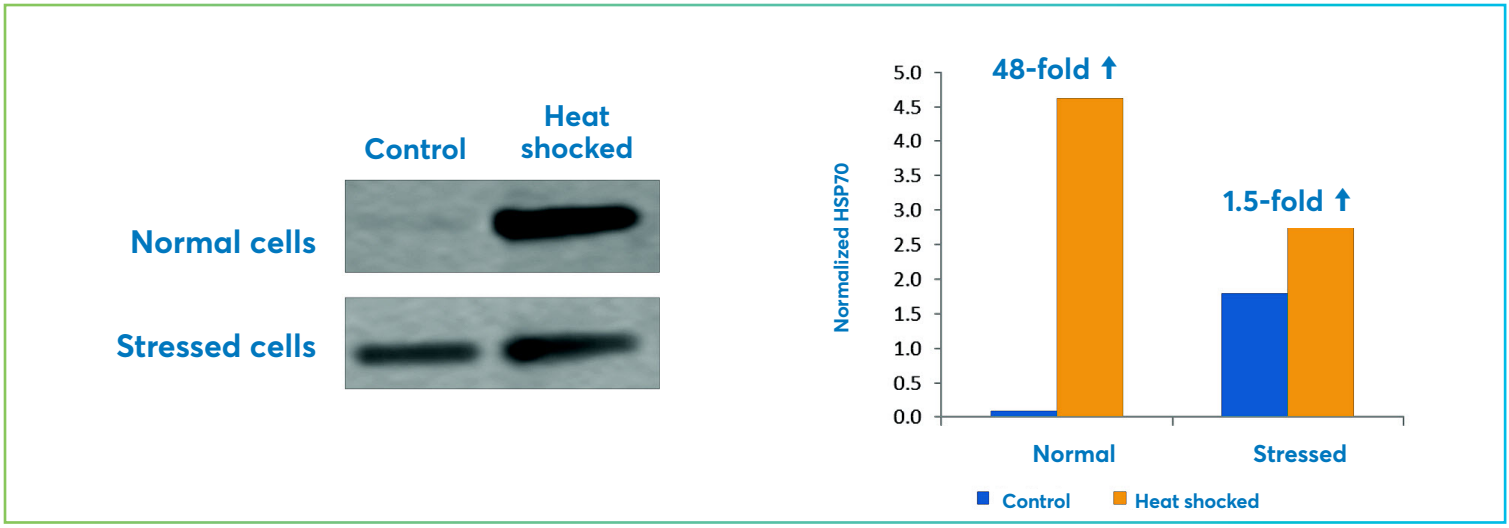


FIGURE 4. **Western blot analysis of HSP70 expression.** A Western blot for HSP70 expression in control and heat shocked cells, 6 hours after treatment, is shown in the left panel. Quantitation performed with ScanLater analysis in SoftMax Pro Software is shown in the graph. 'Normalized HSP70' indicates integrated density minus background, expressed in millions.

cells maintained a steady and low percentage of apoptotic cells.

CELL VIABILITY ASSAY

The EarlyTox Cell Integrity Kit was used to investigate whether cells were dying by other means than apoptosis. Increased cell death was observed with heat shock, particularly after 24 hours. Stressed cells had a higher overall percentage of dead cells than did the normal population. These results match reasonably well with the apoptosis result, suggesting that all or most of the cell death observed is due to apoptosis.

HSP70 INDUCTION

ScanLater Western blot analysis revealed that HSP70 was induced in response to heat shock as expected, but this response was much greater in the normal cell population than in stressed cells. With ScanLater analysis in SoftMax Pro Software, the intensity of bands on the blot was quantitated. A 48-fold increase in HSP70 was seen in the healthy cells 6 hours after heat shock, but only a 1.5-fold increase was seen in stressed cells (Figure 4). The amount of HSP70 expressed in non-heat-shocked control cells was about 18-fold higher for the stressed cell population than for normal cells.

CONCLUSIONS

Using the SpectraMax i3 Multi-Mode Detection Platform, cell-based imaging assays and western blots were performed to examine the cellular response to heat shock. StainFree cell counting identified changes in proliferation with heat shock in normal and stressed cells. Apoptosis and cell viability assays provided insights into the mechanism of heat shock-induced cell death. Finally, Western blot analysis and enabled quantitation of the induction of HSP70 in control and heat-shocked cells.

From the results of apoptosis and cell viability assays, we hypothesize that all or most of the cell death occurring in response to heat shock was due to apoptosis. Induction of HSP70 was highest in the normal (non-stressed) cells under heat shock conditions. HSP70 was induced in stressed cells even prior to heat shock, and then induced further under heat shock.

However, HSP70 in stressed cells never reached the high levels induced in healthy cells. As a result the stressed cells may have had less protection from apoptosis, leading to the higher percentage of apoptotic cells observed.

With the SpectraMax i3 system, and analysis enabled by SoftMax Pro Software, a multi-faceted investigation of cellular function that used to require multiple detection instruments and several different data analysis tools can now be performed on a single platform.

BENEFITS

- Monitor cell proliferation without dyes
- Investigate mechanisms of cell death with fluorescent cell imaging
- Image cells, run cell-based assays, and detect western blots, all on one platform

* Technical specifications for the SpectraMax i3x are similar to those of the SpectraMax i3. For luminescence, the LLD specification for the SpectraMax i3x is 5x lower (better) than for the SpectraMax i3.

Unravel the mysteries of science with one system

SPECTRAMAX® I3X MULTI-MODE DETECTION PLATFORM, MOLECULAR DEVICES

MEASURES SPECTRAL-BASED ABSORBANCE, FLUORESCENCE, AND LUMINESCENCE

- Modular upgrades
- Expanded dynamic range
- Spectral Fusion™ illumination with a power consumption <200 watts

The SpectraMax® i3x offers an expanded dynamic range. It is engineered for performance with Spectral Fusion™ illumination for increased sensitivity across the entire excitation range. The SpectraMax® i3x also features a cooled photomultiplier tube (PMT) for improved detection in extremely low light. These features enable users to generate more data points without the need to dilute.

Description	Power	Weight	Cat. No.
SpectraMax® i3x Multi-mode detection platform	100 - 240 VAC, 2 A, 50/60 Hz	68.3 lbs. (31.0 kg)	**10014-924**

Description	Details	Cat. No.
Accessories		
ScanLater™ Western blot detection cartridge	Includes Membrane sample holder, SoftMax Pro software, Image Plug-in for data analysis	**10192-222**

Time-resolved fluorescence detection

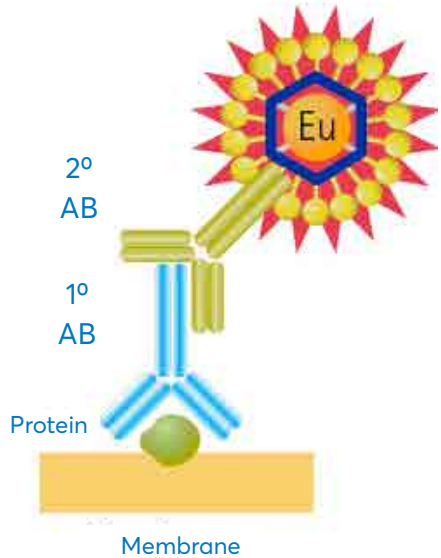
SCANLATER™ WESTERN BLOT ASSAY KIT, MOLECULAR DEVICES

OPTIMAL FOR QUANTITATING AS LITTLE AS FEMTOGRAM PROTEIN SAMPLES

- Time-resolved fluorescence detection
- Supports nitrocellulose and PVDF membranes
- 340/80 nm excitation with 616/10 nm emission range
- Optimized for use with ScanLater™

Using this novel western blot method, researchers can eliminate time-dependent substrate addition steps and sustain blot signal stability for at least one month.

Europium-labeled goat, anti-rabbit, anti-mouse or streptavidin labeled antibodies. Supports nitrocellulose and PVDF membranes. 340/80 nm excitation with 616/10 nm emission range. Optimized for use with ScanLater™ Western Blot Detection Cartridge for the SpectraMax® i3 and Paradigm® Multi-Mode Platforms.



Description_NA	Cat. No.
Assay Kits	
ScanLater™ Assay Kit, Goat Anti-Mouse	**10048-868**
ScanLater™ Assay Kit, Goat Anti-Rabbit	**10048-858**
ScanLater™ Assay Kit, Streptavidin	**10048-856**

Pure, unsupported nitrocellulose membrane

BIOTRACE™ NT NITROCELLULOSE TRANSFER MEMBRANES, PALL LABORATORY

PURE UNSUPPORTED NITROCELLULOSE MEMBRANES HAVE HIGH BINDING AFFINITY

- Strong and durable, less likely to tear or crack than competitor nitrocellulose
- High binding capacity for proteins and nucleic acids
- Lower protein burn-through than competitors in electrophoretic transfers

BioTrace™ NT provides a good binding matrix for macromolecules. The unique structure and lack of growth-inhibiting ingredients facilitate bacterial growth even when the bacteria are stressed by transformation. Typical thickness: 140 µm.

Applications include protein transfers, western blots, southern blots, northern blots, and nucleic acid and protein dot blots.

Description	Size	Format	Thickness	Pore Size	Sterility	Cat. No.
Transfer membrane BioTrace NT	30 cm × 3 m	Roll	101.6 - 190.5 µm	0.2 µm	Nonsterile	**27376-991**
Transfer membrane BioTrace NT	20×20 cm	Sheet	101.6 - 190.5 µm	0.2 µm	Nonsterile	**27377-000**
Transfer membrane BioTrace NT	7×8.5 cm	Sheet	101.6 - 190.5 µm	0.2 µm	Nonsterile	**28139-306**

VWR LIFE SCIENCE

Quick and efficient transfer of proteins

VWR LIFE SCIENCE RAPID TRANSFER BUFFER, 10X

A SIMPLE, SINGLE COMPONENT SYSTEM FOR QUICK AND EFFICIENT TRANSFER OF PROTEINS

- Fast, efficient transfer in 10–20 minutes
- Utilizes standard transfer equipment
- Non-hazardous formulation
- Transfers to PVDF and nitrocellulose from multiple types of gels

Rapid Transfer Buffer is a methanol-free, non-hazardous formulation that is compatible with PVDF and nitrocellulose membranes. It is used for transfer of proteins from standard Laemmli gels, commercial pre-cast gels, and VWR's NEXT GEL™. The transfer efficiency is equivalent to that observed when using a Tris-Glycine-Methanol transfer buffer.

Description	Size	Cat. No.
Rapid Transfer Buffer, 10X	1 L	**97064-312**
Rapid Transfer Buffer, 10X	4 L	**97064-314**

VWR LIFE SCIENCE

Allows sensitive immuno-detection

VWR LIFE SCIENCE TOTALBLOT+™ PVDF MEMBRANES

NATURALLY HYDROPHOBIC MEMBRANES WHICH EXHIBIT STRONG INTERACTIONS WITH A WIDE RANGE OF PROTEINS

- Chemically resistant and does not shrink or disfigure during destaining in methanol
- Pure white microporous solid phase support
- Allows sensitive immuno-detection down to nanogram or picogram levels
- Direct visualization of immobilized proteins with all common staining reagents, including Coomassie Blue, Amido Black, and Ponceau S
- Pore size: 0,2 µm

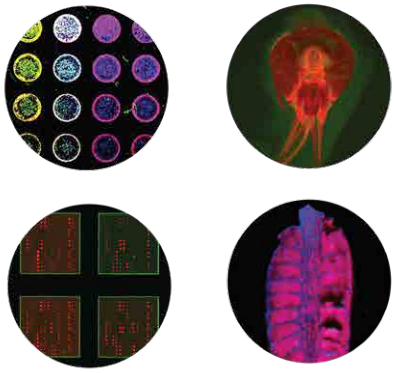
Description	Size	Pore Size	Cat. No.
TotalBLOT+™ PVDF Membranes	10 x 10 cm2	0.2 µm	**97062-898**
TotalBLOT+™ PVDF Membranes	1 Roll (30 cm x 3 m)	0.2 µm	**97062-900**
TotalBLOT+™ PVDF Membranes	5 x 15 cm2	0.2 µm	**97062-902**

The next generation lab imaging platform

SAPPHIRE BIOMOLECULAR IMAGER, AZURE BIOSYSTEMS

- Next generation laser scanning system which offers the best of both worlds
- Unparalleled flexibility with excellent sensitivity and image quality
- Capture software will deliver high quality quantifiable data

Up to four solid state lasers (488, 520, 658 and 784 nm) offering ultimate excitation sensitivity. Photon multiplier tube (PMT) for fluorescence and phosphor imaging, avalanche photodiodies (APD) for near-infrared imaging and a CCD sensor for chemiluminescent and visible imaging. Ultra-wide dynamic range for imaging and quantifying low and high abundance samples simultaneously. Image resolution down to 10 microns for high-quality image analysis.



Description	Cat. No.
Imagers	
Sapphire Biomolecular Imager - NIR	**76317-604**
Sapphire Biomolecular Imager - RGB	**76317-606**
Sapphire Biomolecular Imager - RGBNIR	**76317-608**
Sapphire Biomolecular Imager - PI	**76317-610**

Femtomole sensitivity ECL substrate

RADIANCE PLUS CHEMILUMINESCENT SUBSTRATE, AZURE BIOSYSTEMS

THE MOST SENSITIVE HRP SUBSTRATE FOR CHEMILUMINESCENT WESTERN BLOTTING AVAILABLE FROM AZURE

Radiance Plus has attomole sensitivity and a long-lasting signal, allowing you to detect bands not visualized with other substrates.

High signal-to-noise and a large dynamic range make Radiance Plus ideal for quantifying low-intensity bands.

Description	Volume	Contents	Cat. No.
Femtogram HRP Substrate 150 mL Kit	150 mL	Substrate for 1500 cm^2 Membrane	**10147-298**

IgGy Antibody Selector

Search. Select. Simple.

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Using the IgGy Antibody Selector makes searching for antibodies easier. VWR, part of Avantor, has brought together a multitude of antibody suppliers and manufacturers with hundreds of thousands of antibodies to meet your specific application needs. With VWR, IgGy offers:

- More than 350,000 antibodies
- Brands you know and trust
- Wide range of conjugations
- Choices from multiple suppliers
- Resource for all application areas

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High signal intensity and sensitivity

AMERSHAM ECL WESTERN BLOTTING PRIME BLOTTING AND BLOCKING REAGENTS, CYTIVA (FORMERLY GE HEALTHCARE LIFE SCIENCES)

A HIGHLY SENSITIVE CHEMILUMINESCENT DETECTION REAGENT

- High signal intensity and sensitivity allows for use with low abundance proteins and highly diluted primary and secondary antibodies
- Long lasting, stable signal >3 hours
- Compatible with Amersham ECL Rainbow molecular weight markers and Amersham ECL DualVue Western blotting markers
- Recommended for membrane blocking in chemiluminescent and fluorescent applications to improve specificity and sensitivity
- Each pack of blocking agent contains 40g; sufficient for at least 20 mini blots

Description	For Membrane Area	Cat. No.
Amersham ECL Prime Western Blotting Detection Reagent	1000 cm^2	**89168-782**
Amersham ECL Prime Western Blotting Detection Reagent	3000 cm^2	**89238-012**
Amersham ECL Prime Blocking Reagent	40 g	**95040-068**

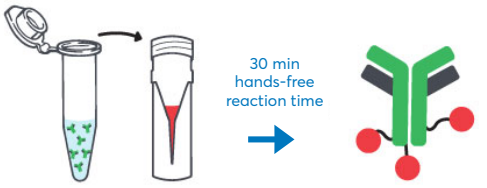
Simplify the process of anitbody labeling

MIX-N-STAIN™ ANTIBODY LABELING KITS, BIOTIUM

DRAMATICALLY SIMPLIFY THE PROCESS OF LABELING PRIMARY ANTIBODIES WITH FLUORESCENT DYES OR BIOTIN

Mix-n-Stain™ Antibody Labeling Kits feature Biotium's line of CF® dyes, which offer advantages in brighness and/or photostability compared to Alexa Fluor® dyes and other similar commercial dyes. A large selection of CF® dye colors spanning the visible and near-infrared spectra offers flexibility for labeling primary antibodies with dye colors that are optimal for your instrument detection settings for multi-color detection. See the CF® Dye Reference Table under the Datasheets tab on **vwr.com** for more information.

Mix-n-Stain CF® Dye Antibody Labeling Kits



Description	Size	Ex/Em	Cat. No.
Mix-n-Stain™ CF® Dye Antibody Labeling Kits			
CF® 647 Mix-n-Stain™ Antibody Labeling Kit	50 - 100 µg	650/655 nm	**89171-530**
CF® 594 Mix-n-Stain™ Antibody Labeling Kit	50 - 100 µg	593/614 nm	**89171-524**
CF® 405S Mix-n-Stain™ Antibody Labeling Kit	50 - 100 µg	404/431 nm	**89171-514**
CF® 790 Mix-n-Stain™ Antibody Labeling Kit	50 - 100 µg	784/806 nm	**89411-608**
CF® 568 Mix-n-Stain™ Antibody Labeling Kit	5 - 20 µg	562/583 nm	**89171-586**
CF® 488A Mix-n-Stain™ Antibody Labeling Kit	20 - 50 µg	490/515 nm	**89171-550**
CF® 750 Mix-n-Stain™ Antibody Labeling Kit	50 - 100 µg	755/777 nm	**89171-540**
Mix-n-Stain™ Biotin Antibody Labeling Kits			
Biotin Mix-n-Stain™ Antibody Labeling Kit	50 - 100 µg	N/A	**89411-604**

For additional kits or sizes, visit **vwr.com**

Variety of immunochemicals

BLOCKING BUFFERS, ROCKLAND IMMUNOCHEMICALS

ELISA microwell blocking buffer with stabilizer is designed to block microwells coated with antigens, antibodies, or other ligands and stabilize the plates for drying. Once plates are stabilized, they can be stored for up to one year without a significant loss of signal. The near infra-red blocking buffer is designed to be used with IRDye® 700Dx, IRDye 800, Alexa Fluor® 680, and Cy™5.5 antibody and reagent conjugates.

BLOTTO A is a ready-to-use general purpose blocking agent. It is 5% non-fat dry milk in TBS. BLOTTO B is recommended for use when phospho-specific antibodies are used. It is 1% non-fat dry milk in TBS.

Description	Size	Cat. No.
Near Infra-Red Blocking Buffer	3 x 500 mL	**RLMB-070-003**
Near Infra-Red Blocking Buffer	10 x 500 mL	**RLMB-070-010**
Near Infra-Red Blocking Buffer	500 mL	**RLMB-070**

Exceptional sharpness and resolution

GENEMATE BLUE AUTORADIOGRAPHY FILM

OUTSTANDING FILM FOR DIVERSE APPLICATIONS INCLUDING CHEMILUMINESCENCE AND AUTORADIOGRAPHY

Exceptional sharpness and resolution and extremely low backgrounds lead to publishing-grade results. Manual or automatic development consistently superior results for exposure of blotting experiments. Sharp and clear results, box after box.

Three double emulsion blue autorad films available including Blue Basic for ideal for autoradiography and chemiluminescence, Blue Ultra for the highest level of sensitivity for all application and Blue Lite, which features the lowest background and low graininess, ideal for chemiluminescence. The shelf life lasts 2 years from the manufacturing date.

Description	Size	Cat. No.
Blue Basic Autorad Film	35.6 x 43.2 cm (14 x 17")	**490001-928**
Blue Basic Autorad Film	20.3 x 25.4 cm (8 x 10")	**490001-952**
Blue Lite Autorad Film	35.6 x 43.2 cm (14 x 17")	**490001-934**
Blue Ultra Autorad Film	35.6 x 43.2 cm (14 x 17")	**490001-946**
Blue Extra Autorad Film	35.6 x 43.2 cm (14 x 17")	**490014-238**

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With a studio in your lab, your research is unlimited

UVP CHEMSTUDIO PLUS IMAGING SYSTEMS, ANALYTIK JENA

THE EXTENSIVE ANALYTICAL SOFTWARE AND RANGE OF EPI LEDS ENSURE EVERY APPLICATION NEED IS MET

US ONLY

- Take images of the highest quality with deep cooling, efficient photon-to-signal conversion and high performance cameras
- Camera options include either the highest resolution 8.1MP camera or the exceptionally sensitive 3.2MP camera
- Wide aperture lens optics, capture more light in low-light applications (signals come faster and are stronger; this guarantees extremely fast image capture)
- Available as either a PC-operated unit or as a stand-alone instrument with an integrated color touchscreen
- Overhead white, green, red and blue LEDs come as standard in the series
- Includes Ethidium Bromide emission filter in an easy-to-access filter wheel with up to five positions
- Includes unlimited copies of VisionWorks® Software, with comprehensive features, optimizes image acquisition and analysis
- Unmodified raw data is collected, giving users the choice of applying image enhancement tools

The UVP ChemStudio systems were built with flexibility in mind, providing RGB detection as standard. These Chemiluminescent imaging systems offer high-resolution and sensitive imaging of gels, western blots, colorimetry, fluorescent westerns, NIR, colony plates, plants, IR dyes, and a wide range of fluorescent dyes. No matter your preferred research method, the UVP ChemStudio product family was built to streamline your protocol from detection to analysis, providing the most accurate quantitation of data for an unlimited range of applications.

Multiple illumination sources–Each UVP ChemStudio system comes with a 302 nm UV transilluminator and overhead RGBW LEDs as standard enabling a wide scope of fluorescent and nonfluorescent imaging applications. Optional NIR laser modules with excitation wavelengths of 660 nm and 787 nm are available to allow multiplex lighting for IR1 and IR2. The included Ethidium Bromide emission filter allows for imaging of the most common applications; additional filters are available for extended applications.

Description	ElectricaA	Camera	Cat. No.
115 V Units			
UVP ChemStudio PLUS 615	115 V	3.2 MP 615 Camera, Automated 25 mm f/0.95 lens	**76307-478**
UVP ChemStudio PLUS touch 615	115 V	3.2 MP 615 Camera, Automated 25 mm f/0.95 lens	**76307-474**
UVP ChemStudio PLUS 815	115 V	8.1 MP 815 Camera, Automated 42.5 mm f/0.95 lens	**76208-584**
UVP ChemStudio PLUS touch 815	115 V	8.1 MP 815 Camera, Automated 42.5 mm f/0.95 lens	**76208-582**

These products are coming soon to Canada. Please contact your VWR sales rep for availability or similar options currently available in your region.

27262-KK-WH

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