



Western Blotting

Some protein detection kits
make you wonder if your eyes
are playing tricks on you.



Where Better Science Begins

No matter which KPL Western Blotting system you use, you'll get a strong, clean signal every time.

When analyzing proteins immobilized on membrane, you'll get consistent, high quality results with KPL's Protein Detector™ Western Blot Kits. Choose from a broad line of kits and stand-alone reagents for either chemiluminescent or colorimetric methods of detecting and quantifying specific antigens in a complex protein mixture.

The cornerstone of Protein Detector systems is KPL's line of highly sensitive

liquid substrates. You have a choice among multiple chemiluminescent and chromogenic solutions for peroxidase and phosphatase that provide varied levels of sensitivity. The newest addition, LumiGLO Reserve™ Chemiluminescent Substrate, enables you to detect even lower amounts of protein than ever before. As a result of this option, you are now offered greater flexibility in selecting an assay that meets your changing research needs.

These substrates are combined with unique blocking and wash solutions and highly specific secondary antibodies to create comprehensive, fully optimized kits for Western blotting. KPL has designed each of these kits to deliver the maximum signal to noise ratio available. With Protein Detector, you will achieve clean, clear results blot after blot.

Protein Detector Western Blot Kits

Systems for peroxidase and phosphatase detection are available using high quality, reproducible chemiluminescent and chromogenic substrates. It's your choice!

	LumiGLO Reserve™ Western Blot Kit (54-13-50)	LumiGLO® Western Blot Kit (54-12-50)	TMB Western Blot Kit (54-11-50)	BCIP/NBT Western Blot Kit (55-11-50)
Substrate:				
Type	Chemiluminescent	Chemiluminescent	Chromogenic	Chromogenic
Format	2-Component: 1:2 Mix and use	2-Component: 1:1 Mix and use	1-Component: Ready-to-use	1-Component: Ready-to-use
Detection Limit	Sub-Picogram; Femtogram	6 picogram	50 picogram	500 picogram
Detection Method	Film or image analysis	Film or image analysis	Visual – dark blue ppt	Visual – purple ppt
Stability of Working Solution	8 hours	24 hours	1 year	1 year
Duration of Signal	4-8 hours	1-2 hours	NA	NA
Enzyme Catalyst	Peroxidase	Peroxidase	Peroxidase	Phosphatase
Recommended Membrane	Nitrocellulose or PVDF	Nitrocellulose or PVDF	Nitrocellulose or PVDF	Nitrocellulose or PVDF

All Protein Detector Western Blot Kits include Substrate, Detector Block, Wash Solution Concentrate and Enzyme-Labeled Goat Anti-Mouse and Anti-Rabbit Secondary Antibodies.



With KPL's Protein Detector™
Western Blotting Systems, the
results jump right out at you.

Western Blotting

Chemiluminescent Kits

Chemiluminescent detection involves the creation of light through the catalysis of an enzyme substrate. Use of this method for protein detection allows orders of magnitude increase in sensitivity compared to traditional colorimetric Western or dot blotting. KPL offers two unique Protein Detector Western Blot Kits containing proprietary luminol-based chemiluminescent substrates — traditional LumiGLO® and the new LumiGLO Reserve™ — for the rapid and sensitive detection of horseradish peroxidase (HRP)-labeled conjugates.

Protein Detector LumiGLO Reserve™ Western Blot Kit

With the recent development of LumiGLO Reserve Chemiluminescent Substrate, the Protein Detector product line has been expanded to include one of the most sensitive Western blotting systems available. You now have an option for those assays where enhanced sensitivity is critical to its success. For analysis of limited, hard-to-detect pro-

tein samples, see for yourself how Protein Detector LumiGLO Reserve Western Blot Kit can meet your needs.

Maximum Sensitivity

LumiGLO Reserve's proprietary two-component substrate formulation allows for greater than 20 times the sensitivity of standard LumiGLO. With detection levels as low as the femtogram range, LumiGLO Reserve is ideal for those situations where the protein of interest is expressed in low abundance (Figure 1).

LumiGLO Reserve emits light over the course of 4-8 hours with the most intense emission occurring within the first two hours. Because of its extreme light intensity, most images may be captured well under 10 minutes; multiple exposures are easy to obtain. This signal intensity makes this system ideal for use with chemiluminescent imagers.

Sample and Antibody Conservation

LumiGLO Reserve provides the added benefit of strong signal with the use of reduced amounts of precious target and

antibodies. Therefore, material of limited supply or higher expense can be conserved while maintaining your current level of sensitivity.

Superior Signal to Noise

No longer trade sensitivity for background. LumiGLO Reserve is specifically designed to deliver lower non-specific signal than competitor substrates in its class. For optimal signal to noise ratio, all Protein Detector Western Blot Kits contain KPL's unique Detector Block; very low background can be achieved without compromise to signal intensity (Figures 2 and 3).

Convenience

LumiGLO Reserve is supplied in several kit formats to offer the most flexibility. The comprehensive Protein Detector LumiGLO Reserve Western Blot Kit is available for a fully optimized approach to chemiluminescent Western blot detection. Alternatively, LumiGLO Reserve Chemiluminescent Substrate Kits can be used in existing assays where greater sensitivity is desired.

LumiGLO Reserve Signal to Noise Comparison



Figure 1: Relative expression of transcription factor, c-myc, using different chemiluminescent substrates. Five two-fold serial dilutions of purified c-myc (25 ng–1.56 ng, lanes 1–5) were compared to a 64 µg total protein HeLa nuclear lysate (lane 6). Following separation on a 4–20% PAGE gel and transfer to PVDF, protein was detected using a rabbit anti-c-myc antibody (1:200) and anti-rabbit HRP conjugate (1:10,000). Detection conditions were identical with the exception of substrate. While the c-myc lysate sample was not detectable with A) LumiGLO or C) ECL Plus™ after 10 minutes, the sample was easily detected with B) LumiGLO Reserve after just a 2-minute film exposure.



Figure 2: Comparison of low-end sensitivity using LumiGLO Reserve and ECL Detection Kits. Two-fold serial dilutions of Mouse IgG (1 ng – 31 µg) were separated by SDS-PAGE and transferred to PVDF. Under manufacturer's recommended conditions, protein was detected using HRP-labeled anti-mouse antibody (varied dilutions according to recommended optimization) and each respective substrate: A) LumiGLO Reserve, B) ECL Plus™, C) ECL Advance™. Film was exposed for 10 minutes and analyzed for sensitivity and signal to noise.

LumiGLO vs. Leading Traditional Chemiluminescent Substrates

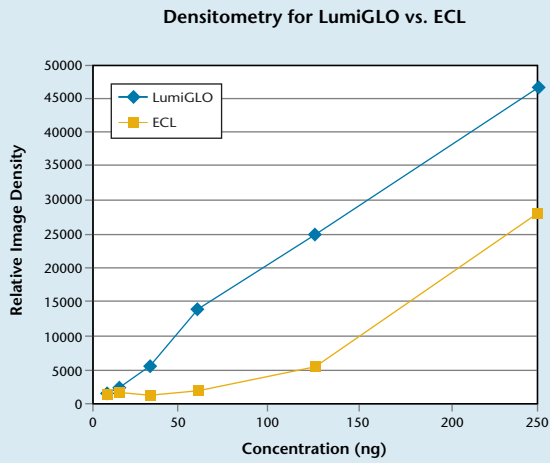


Figure 4: Signal response comparison of LumiGLO vs. ECL™ chemiluminescent substrates in Western blotting. β -galactosidase was electrophoresed, transferred to PVDF membrane and subsequently detected using rabbit anti- β -gal followed by HRP conjugated goat anti-rabbit IgG (H+L). Each blot was treated with 5 mL of LumiGLO or ECL substrate and exposed to film for 10 minutes. The density of each band was analyzed on a Syngene GeneGenius™ image analyzer, using automatic background subtract.

Superior Signal to Noise with Detector™ Block

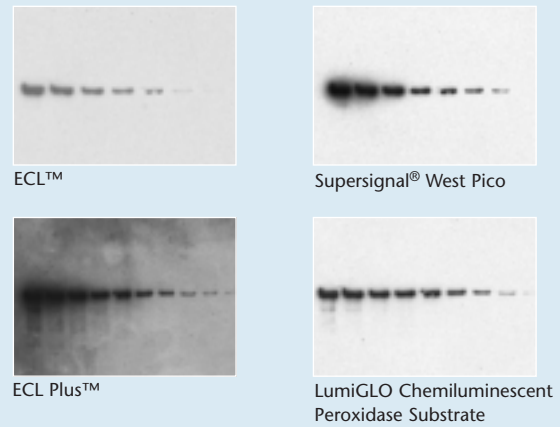


Figure 5: Detection of β -galactosidase in Western blots using alternative chemiluminescent substrates. A two-fold dilution series of purified β -galactosidase (from 25 μ g) was electrophoresed on a polyacrylamide gel and transferred to KODAK BIOMAX Multi-Blot Kit for Proteins. Each blot was detected under the same conditions using Protein Detector LumiGLO Western Blotting Kit, substituting 5 mL LumiGLO on 3 of the 4 blots with 5 mL of leading competitive chemiluminescent substrates, respectively. Following a 10 minute film exposure, results were evaluated for sensitivity and signal:noise ratio.

Protein Detector™ LumiGLO® Western Blot Kit

KPL's original LumiGLO Chemiluminescent Substrate has consistently provided high quality results in a variety of immunoassays for years. Researchers continue to select LumiGLO kits for their reliable performance in Western blot detection. Take a look at the difference.

Ideal for Routine Western Blotting

LumiGLO detects low picogram quantities of target protein on blots.

After reaction with membrane-bound HRP conjugates, light emission begins immediately and is sustained for approximately 1–2 hours. Detection is possible within minutes of blot exposure.

Greater Signal Linearity

LumiGLO produces a quantitatively linear signal on film that ensures a broader dynamic range of detection. While the light output from other chemiluminescent substrates tends to reduce sharply as the concentration of protein is titrated, a more proportional

reduction in sensitivity is achieved with LumiGLO (Figure 4).

Low Background

Like other KPL substrates, LumiGLO delivers superior signal to noise by design. In addition to the substrate, efficient detection of immunoblots is dependent on the reduction of non-specific binding through the use of blocks. The newly improved Detector™ Block is a critical component of the Detector blotting systems. Its use ensures low background without loss of signal intensity. Figure 5 illustrates the superior signal to noise from blots blocked with Detector Block and subsequently detected with LumiGLO as compared to leading competitor substrates.

Cost Effective

Protein Detector LumiGLO Western Blot Kit and LumiGLO Chemiluminescent Substrate are more economical blot for blot compared to competitive products. As a result, you have a product that is dependable and efficient — a great value!

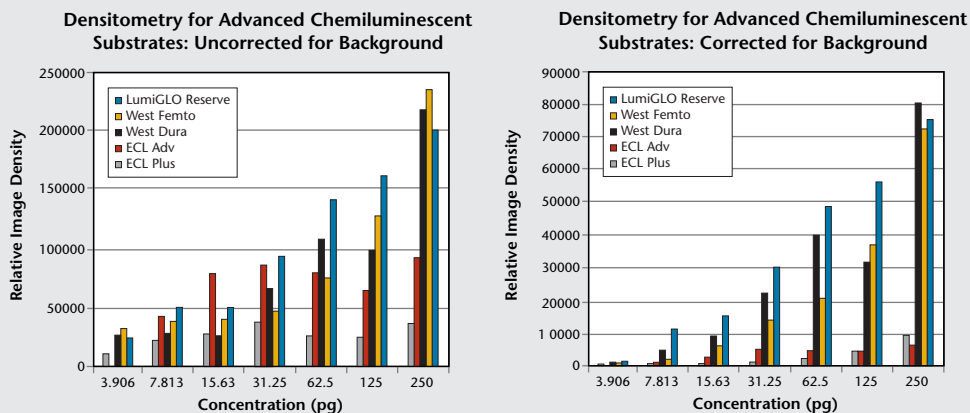


Figure 3: Signal linearity and signal-to-noise ratio comparison of advanced chemiluminescent substrates. Mouse IgG (250 pg to 3.91 pg) was transferred to nitrocellulose. Blots were detected under identical conditions with a 1:10,000 dilution of HRP-Goat-anti-Mouse IgG (except ECL Advance, 1:100,000) and exposed to film for 10 minutes. Densitometry was then performed using the Syngene GeneGenius. LumiGLO Reserve demonstrates superior signal linearity over a larger dynamic range and the greatest comparative signal-to-noise.

Western Blotting

Chromogenic Kits

KPL also offers chromogenic systems for those labs where chemiluminescent detection is not preferred. When reacted with the appropriate enzyme, these substrates produce precipitating color on the membrane at the site of the protein. Results can be interpreted by direct visualization of the blot. As with all Protein Detector kits, all of the critical reagents are supplied for convenience and optimal performance. Everything you need to detect your antigen and primary antibody is included.

Protein Detector TMB Western Blot Kit

Protein Detector TMB Western Blot Kits utilize TMB Peroxidase Membrane Substrate (3,3',5,5'-tetramethylbenzidine), the most sensitive chromogenic peroxidase substrate for Western and dot blotting applications. Detection limits are significantly increased as compared to other chromogenic membrane substrates. TMB produces a dark blue precipitate upon reaction with HRP.

Protein Detector BCIP/NBT Western Blot Kit

For detection of phosphatase-labeled conjugates, Protein Detector BCIP/NBT Western Blot Kit is available. When reacted with alkaline phosphatase, BCIP/NBT produces clean, intense bands of purple precipitate. It also provides stable, more permanent results than other chromogenic substrates.

Western Blot Reagents

The reagents in Protein Detector Kits are also available individually for flexibility in assay design. You can select your preferred combination of reagents and set up your own system. KPL offers a broad line of secondary antibodies and conjugates, substrates, and support reagents in various packaging formats to suit your needs.

Secondary Antibodies

- Highly purified and specific.
- One of the largest offerings of species-specific polyclonal antibodies available.
- Accommodates multiple detection methods with enzyme, biotin and fluorochrome-labeled antibodies.

Substrates

- Packaged for convenience in ready-to-use and easy-to-prepare liquid formats.
- Choice of chemiluminescent and chromogenic products for both phosphatase and peroxidase systems.
- Highly stable with a long active shelf life.

Support Reagents

- One source for all necessary block solutions, wash buffers, diluents, and stabilizers.
- Consistently produced for reliable results.

Try KPL's Protein Detector Kits and See What You've Been Missing.

KPL has extensive experience in antibody purification and the development of stable liquid substrates. This long-standing expertise is the foundation of our line of Protein Detector systems, and ensures consistent and reliable product performance from lot-to-lot. Try our kits and get the results you're looking for: a strong, clean signal without background, every time.

To take a closer look at our product line, call or visit us online for more information.

Your eyes aren't playing tricks on you. You've found the finest line of Western Blotting Systems at KPL.

LumiGLO is a registered trademark and LumiGLO Reserve, Protein Detector, Detector are trademarks of KPL, Inc.

ECL Plus, ECL, and ECL Advance are trademarks of Amersham Biosciences UK Limited.

SuperSignal West is a registered trademark of Pierce Biotechnology, Inc.

BIOMAX is a trademark of KODAK.

GeneGenius is a trademark of Syngene.

Catalog #	Description	Size
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Protein Detector™ Western Blotting Kits

Each kit includes anti-mouse and anti-rabbit conjugates, Detector Block, Wash Solution Concentrate and Substrate.

Phosphatase

55-11-50	BCIP/NBT Western Blot Kit	2500 cm ²
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Peroxidase

54-11-50	TMB Western Blot Kit	2500 cm ²
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Peroxidase Chemiluminescent

54-12-50	LumiGLO® Western Blot Kit	2500 cm ²
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54-13-50	LumiGLO Reserve™ Western Blot Kit	2400 cm ²
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Related Reagents and Kits

Antibody Conjugates

All antibodies listed below are produced in goat. For a complete antibody listing, refer to KPL's Product Catalog.

Phosphatase-labeled

475-1006	Anti-Human IgG (H+L)	1.0 mL, liquid
475-1806	Anti-Mouse IgG (H+L), HSA	1.0 mL, liquid
475-1506	Anti-Rabbit IgG (H+L)	1.0 mL, liquid

Peroxidase-labeled

474-1006	Anti-Human IgG (H+L)	1.0 mL, liquid
474-1806	Anti-Mouse IgG (H+L) HSA	1.0 mL, liquid
474-1506	Anti-Rabbit IgG (H+L)	1.0 mL, liquid

Biotin-labeled

16-10-06	Anti-Human IgG (H+L)	0.5 mg
176-1006	Anti-Human IgG (H+L)	2.0 mg

Labeled Streptavidin

474-3000	HRP-labeled	1.0 mL, liquid
475-3000	AP-labeled	1.0 mL, liquid

Catalog #	Description	Size
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Substrates for Western Blotting

Phosphatase Chromogenic Substrates

50-81-18	BCIP/NBT Substrate	100 mL
50-81-07	BCIP/NBT Substrate	600 mL

Peroxidase Chromogenic Substrates

50-77-18	TMB Membrane Substrate	100 mL
50-77-03	TMB Membrane Substrate	200 mL
50-73-00	4 CN Substrate	600 mL
50-73-04	4 CN Substrate	2700 mL

Peroxidase Chemiluminescent Substrates

54-61-00	LumiGLO Chemiluminescent Substrate	240 mL
54-61-01	LumiGLO Chemiluminescent Substrate	720 mL
54-71-00	LumiGLO Reserve Substrate Kit	2400 cm ²
54-71-01	LumiGLO Reserve Substrate Kit	600 cm ²

Assay Support Reagents

50-84-00	Coating Solution Concentrate	50 mL
54-15-01	HRPStabilizer	200 mL
55-15-00	APStabilizer	200 mL
50-61-00	10% BSA Diluent/Blocking Solution Concentrate	200 mL
50-82-01	Milk Diluent/Blocking Solution Concentrate	200 mL
71-83-00	Detector Block (5X)	240 mL
50-63-00	Wash Solution Concentrate (20X)	800 mL
50-63-06	Biotin Wash Solution Concentrate (10X)	200 mL
60-00-50	Biodyne B Nylon Membrane	1 roll

HSA=human serum adsorbed.

To order or for more information, call KPL at 800-638-3167, 301-948-7755, Fax: 301-948-0169. www.kpl.com or contact your local distributor.



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