Western Blot Products Guide

Western Blotting Your Way

with PerkinElmer Products

PerkinElmer®
precisely.

Choose PerkinElmer Reagents

— For All Your Western Blotting Needs

Whether you're using chemiluminescent, chromogenic or radioisotopic methods, you can turn to PerkinElmer for economical products of the highest quality available, the convenience of one-stop shopping, and exceptional technical support.

PerkinElmer's full line of products for Western blotting includes:

- Chemiluminescence and chromogenic detection systems
- Chromogenic substrates
- ^{125}I-Protein A and Protein G
- Secondary antibodies
- Protein molecular weight markers
- Transfer membranes
- KODAK® films and intensifying screens

Three Detection Methods

Researchers may choose chemiluminescent, chromogenic, or radioisotopic detection methods for their Western blotting applications.

- **Chemiluminescence Detection:** Membrane-bound proteins are detected using specific protein-enzyme conjugates. The enzyme catalyzes chemiluminescence detection substrates to emit light, which produces an image on photosensitive film or appropriate instrumentation.
- **Chromogenic Detection:** Membrane-bound proteins are detected using specific protein-enzyme conjugates. The enzyme catalyzes chromogenic detection substrates to deposit dye directly on the membrane.
- **Radioisotopic Detection:** Membrane-bound proteins are detected using a specific radiolabeled antibody, which produces an image on autoradiography film or appropriate instrumentation, such as our Cyclone® Storage Phosphor System.

Detection Options for Western Blotting Applications

	Radiometric	Chemiluminescence		Chromogenic
Membrane	PVDF or Nitrocellulose	PVDF or Nitrocellulose	PVDF	PVDF or Nitrocellulose
Reporter	^{125}I Protein A ^{125}I Protein G	HRP conjugate	AP conjugate	HRP conjugate or AP conjugate
Detection Substrate	N/A	Enhanced Luminol	CDP-*Star*®	4CN Plus, DAB, or BCIP/NBT
Molecular Weight Markers	Multicolored or ^{14}C-Labeled	Multicolored or Biotinylated	Multicolored or Biotinylated	Multicolored or Biotinylated
Output Format	Film or Phorphor Imaging System	Film or Chemiluminescence Imaging System	Film or Chemiluminescence Imaging System	Membrane

Products for Chemiluminescent Detection

Western Lightning™ Chemiluminescence Reagent *Plus*

Our Western Lightning™ Chemiluminescence Reagent *Plus* is an enhanced luminol-based substrate for HRP-catalyzed detection. It is an economical choice that provides twice the sensitivity of standard reagents, and up to 8 times the sensitivity of some brands. Furthermore, it offers the best signal-to-noise ratio, and generates good results on either PVDF or nitrocellulose membranes.

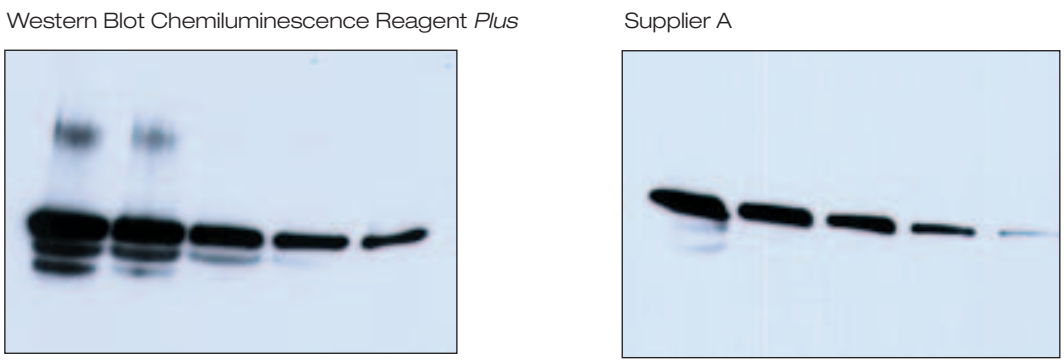
PerkinElmer guarantees the performance of Western Lightning Chemiluminescence Reagent *Plus* for 12 months from ship date. Unopened reagent can be stored for at least 8 weeks at room temperature. This extended shelf life means you can buy and use Western Lightning Chemiluminescence Reagent *Plus* with confidence.

Even users who don't require better images realize substantial benefits from enhanced sensitivity, by reducing film exposure times, or by decreasing concentration levels of primary antibodies, thus saving money.

Ordering Information

Cat. No.	Size
NEL103	For 1,000 cm^2 of membrane
NEL104	For 2,500 cm^2 of membrane
NEL105	For 5,000 cm^2 of membrane

Western Blots using Western Lightning Chemiluminescence Reagent *Plus*



Comparison of Enhanced Luminol Chemiluminescence Substrates: Two-fold serial dilutions of peanut lectin were electrophoresed and electroblotted onto nitrocellulose membranes. Western blot detection was carried out using anti-peanut lectin antibody and goat anti-rabbit HRP, followed by membrane incubation in the various enhanced luminol chemiluminescence reagents for 1 minute, and 1 minute film exposures. (Use of PVDF membranes produces comparably superior results.)

Western Lightning Chemiluminescence Reagent

Our original Western Lightning Chemiluminescence Reagent is an enhanced luminol chemiluminescence reagent for rapid HRP-catalyzed protein detection.

While not as sensitive as Western Lightning Chemiluminescence Reagent *Plus*, it combines excellent reliability with the safety of nonradiometric protocols.

Ordering Information

Cat. No.	Size
NEL100	For 1,000 cm^2 of membrane
NEL101	For 2,500 cm^2 of membrane
NEL102	For 5,000 cm^2 of membrane

CDP-*Star*® Western Blot Chemiluminescence Reagent

For alkaline phosphatase-catalyzed detection, CDP-*Star* Western Blot Chemiluminescence Reagent offers excellent value, and the fastest kinetics for short film exposures. A relatively strong signal develops in only 30 minutes, and maximum signal strength is attained in just two hours.

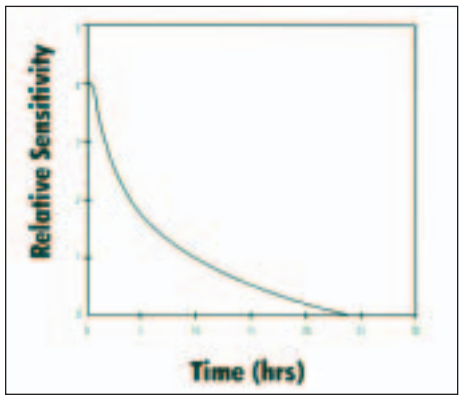
The signal remains strong continuously for 24 hours, and the substrate is well suited for re-exposure. CDP-*Star* Western Blot Chemiluminescence Reagent is provided ready to use, with no mixing required.

Ordering Information

Cat. No.	Size
NEL602	For 5,000 cm^2 of membrane

Schematic Representation of Light Emission Kinetics

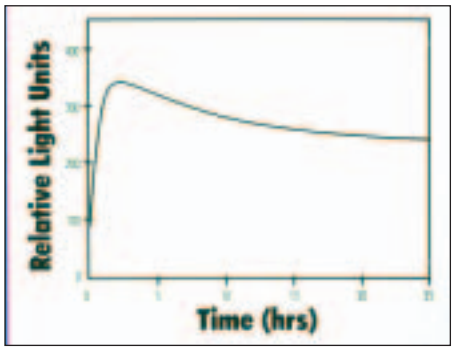
Light Emission of Enhanced Luminol: Relative Sensitivity vs. Time



Enhanced Luminol is characterized by:

- Immediate, intense signal.
- Maintenance of signal for approximately 2 hours.
- Rapid weakening of signal after 2 hours.
- Absence of remaining signal after 24 hours.
- Generally low background.

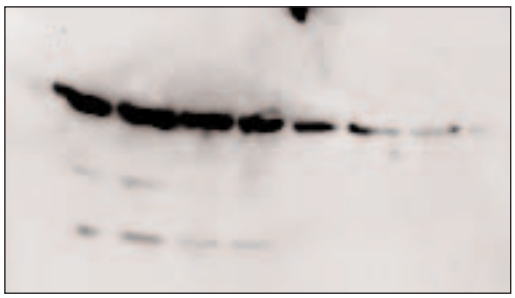
Light Emission of Western Lightning CDP-*Star*: Relative Light Units vs. Time



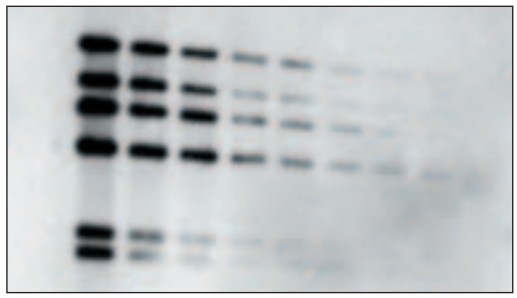
Western Lightning CDP-*Star* is characterized by:

- Relatively strong signal after only 30 minutes.
- Maximum signal in 2 hours.
- Maintenance of strong signal for 24 hours.
- Well-suited for re-exposure.

Western Blots using Western Lightning CDP-*Star*



Western Lightning CDP-*Star*® Western blot chemiluminescence detection on commercial imaging system. Dilutions of bovine α-tubulin (starting at 400 ng) were electrophoresed and electroblotted onto PolyScreen® PVDF Transfer Membrane. Western blot detection was carried out using anti-tubulin antibody and goat anti-mouse AP, followed by membrane incubation in Western Lightning CDP-*Star*. Chemiluminescence signal detection by 5 minute exposures on a commercial imaging system.



Western Lightning CDP-*Star* Southern blot chemiluminescence detection on commercial imaging system. Two-fold serial dilutions of Lambda Hind III (starting at 1 ng) were electrophoresed and capillary blotted onto GeneScreen™ transfer membrane. After UV crosslinking, the blot was hybridized with fluorescein-labeled Lambda Hind III digest using PerkinElmer's Random Primer Fluorescein Labeling Kit. Southern blot detection was carried out using Antifluorescein-AP followed by membrane incubation in Western Lightning CDP-*Star*. Chemiluminescence signal detection by 5 minute exposures on a commercial imaging system.

Products for Chromogenic Detection

Chromogenic Substrates

These substrates are for colorimetric visualization of membrane-bound proteins. Choice of substrates is largely a matter of personal preference.

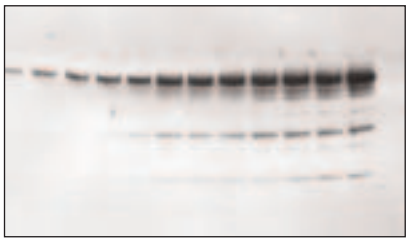
Ordering Information

Cat. No.	Substrate	Chemical Composition	Volume	Color
NEL300	4CN Plus	Enhanced 4-chloro-nephthol reagent	15 mL of substrate, 75 mL of diluent for up to 3,000 cm^2 of membrane	Dark purple precipitate in presence of HRP
NEL937	BCIP/NBT	5-bromo-4-chloro-3-indolyl-phosphate and nitroblue tetrazolium	2 x 250 mL of ready-to-use solution for up to 2,000 cm^2 of membrane	Permanent dark purple stain on membrane sites retaining phosphatase.
NEL938	DAB	3,3'-diamino-benzidine	10 mL liquid concentrate	Brown oxidation product in the presence of HRP

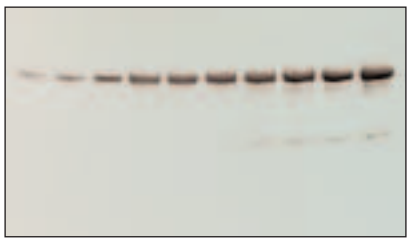
Comparison of Chromogenic Substrates

4CN Plus

Imaged

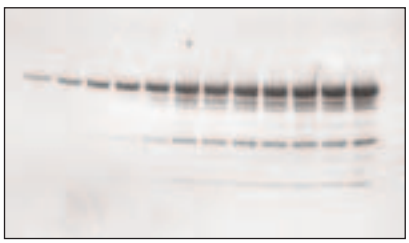


Photograph of membrane

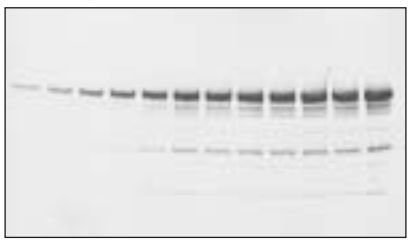


DAB

Imaged

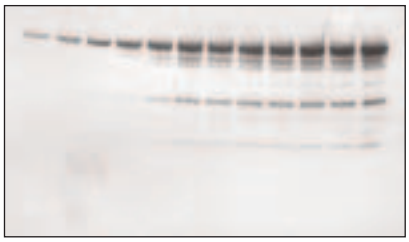


Photograph of membrane

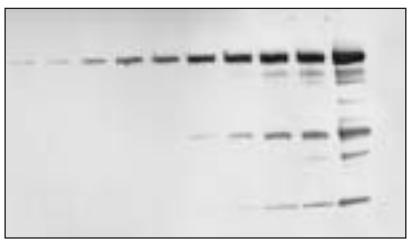


BCIP/NBT

Imaged



Photograph of membrane



Comparison of chromogenic detection methods for Western blotting with visualization on commercial imaging system. Dilutions of bovine α-tubulin (starting at 800 ng) were electrophoresed and electroblotted onto PolyScreen® PVDF Transfer Membrane. Western blot detection was carried out using anti-tubulin antibody, either goat anti-mouse HRP or goat anti-mouse AP followed by detection with either 4CN Plus, DAB, or BCIP/NBT. Stained blots were visualized on a commercially available imaging system.

BLAST® BLotting Amplification SysTem

Our proprietary BLAST® Blotting Amplification System increases signal strength by 8- to 10-fold using standard chromogenic detection methods, permitting vivid visualization of otherwise weak or invisible signals. Alternately, it may be used to reduce usage of precious antibodies, thus saving costs while achieving equivalent detection levels.

The BLAST system uses horseradish peroxidase to catalyze the deposition of biotin-labeled tyramide onto membranes that have been previously blocked with proteins. The reaction takes less than 15 minutes, and results in the deposition of numerous biotin labels that can be detected using standard chromogenic techniques. Resolution is excellent, because the biotin labels are deposited in close proximity to the bound enzyme.

BLAST Kit Components

- Streptavidin-HRP
- Blocking Reagent
- Amplification Diluent
- Control Protein
- BLAST Reagent
- 4CN Plus Chromogenic Substrate*
- 4CN Plus Substrate Diluent*
- Comprehensive Manual with Troubleshooting Guide

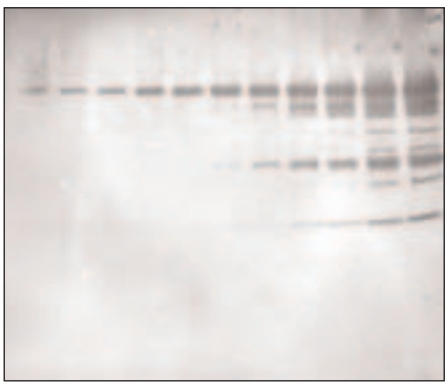
*4CN Plus Chromogenic Substrate is a sensitive, nontoxic detection substrate. Other substrates may be substituted in the protocol if desired.

Ordering Information

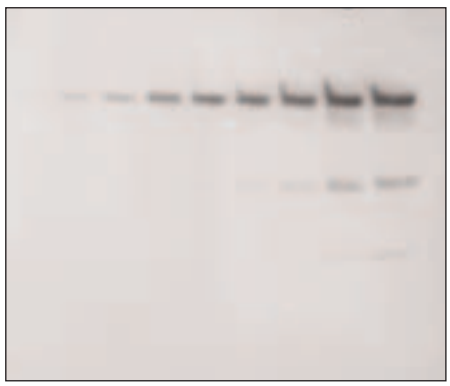
Cat. No.	Size	Cat. No.	Size
NEL761A	For 500 cm^2 of membrane	NEL761	For 2,500 cm^2 of membrane

Increase signals 8- to 10-fold by applying BLAST to your chromogenic detection protocol.

BLAST Amplified Detection on Commercial Imaging System



Unamplified Detection on Commercial Imaging System



Comparison of amplified and unamplified chromogenic detection for Western blotting, with visualization on a commercially available imaging system. Dilutions of bovine α-tubulin (starting at 400 ng) were electrophoresed and electroblotted onto PolyScreen PVDF Transfer Membrane. Western blot detection was carried out using anti-tubulin antibody and goat anti-mouse HRP for the standard detection method. For the amplified method, detection was performed as above, followed by BLAST®. Signal detection was by 4CN Plus on a commercially available imaging system.

Reduce the use of precious antibodies.

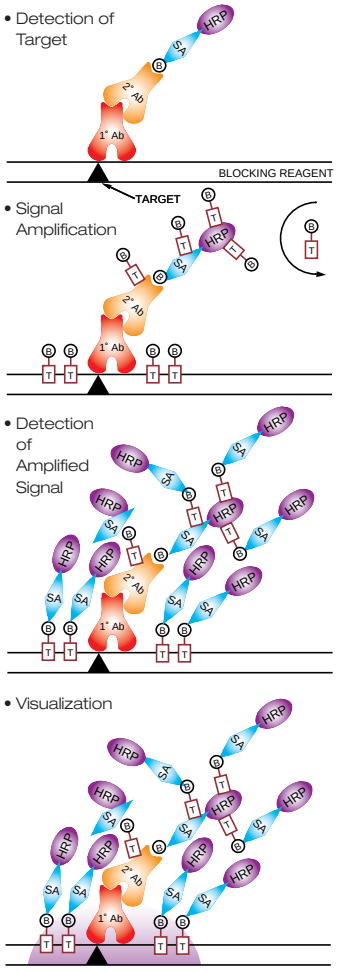
Column 1
Primary antibody
dilution 1:1K
Chromogenic
Detection System

Column 2
Primary antibody
dilution 1:1K
Chromogenic
Detection System
BLAST Enhanced

Column 3
Primary antibody
dilution 1:10K
Chromogenic
Detection System
BLAST Enhanced

Slot blots of 2-fold serial dilutions
of rabbit IgG (starting at 10 ng)
on nitrocellulose.

Diagram of BLAST Signal Amplification Procedure



BLAST Blocking Buffer	60 minutes
Incubation with Primary Antibody	60 minutes
BLAST Wash Buffer	4 x 5 minutes
Incubate in HRP-labeled Reagent	30-60 minutes
BLAST Wash Buffer	4 x 5 minutes
Incubation with BLAST Biotin Reagent	10-15 minutes
DMSO Wash Buffer	4 x 5 minutes
BLAST Wash Buffer	1 x 5 minutes
Incubation with Streptavidin-HRP	30 minutes
BLAST Wash Buffer	4 x 5 minutes
Incubation with 4CN Plus	Up to 30 minutes

Recombinant ^{125}I-Protein A and ^{125}I-Protein G

Protein A and Protein G are both suitable for direct autoradiographic detection of membrane-bound antibody in Western blotting applications.

Protein A reacts with most common IgG subclasses. Protein G reacts more broadly with all IgG subclasses.

Ordering Information

Cat. No.	Product	Size
NEX146L	^{125}I- Protein A (recombinant) 2-10 µCi (74-370 kBq)/µg in phosphate buffer (pH 4.0) containing 35% ethanol. Store at 4°C.	100 µCi (3.70 MBq) 250 µCi (9.25 MBq)
NEX237	^{125}I- Protein G (recombinant), Bolton-Hunter labeled 15-25 µCi (555-925 MBq)/µg in sodium phosphate buffered saline (pH 5.2) containing glycine and BSA. Store at -20°C.	10 µCi (370 kBq) 50 µCi (1.85 MBq) 100 µCi (3.70 MBq)

Immunoglobulin Binding Specificities of Protein G and Protein A Conjugates

Immunoglobulin	Protein G		Protein A		Immunoglobulin	Protein G		Protein A	
	STRONG	WEAK	STRONG	WEAK		STRONG	WEAK	STRONG	WEAK
Human IgG_1	•		•		Rat IgG_1		•		•
Human IgG_2	•		•		Rat IgG_{2a}	•		–	–
Human IgG_3	•		–	–	Rat IgG_{2b}		•	–	–
Human IgG_4	•		•		Rat IgG_{2c}	•		•	
Pig IgG	•			•	Sheep IgG_1	•		–	–
Rabbit IgG	•		•		Sheep IgG_2	•		•	
Bovine IgG_1	•		–	–	Goat IgG_1	•			•
Bovine IgG_2	•		•		Goat IgG_2	•		•	
Mouse IgG_1		•		•/–	Horse IgG (ab)	•			•
Mouse IgG_{2a}	•		•		Horse IgG (c)	•			•
Mouse IgG_{2b}	•		•		Horse IgG (T)		•	–	–
Mouse IgG_3	•		•		Dog IgG		•	•	

KEY:

– No Binding

• Binding

Protein Molecular Weight Markers

These markers provide standards for molecular weight determinations in Western blotting applications.

Multicolor Protein Molecular Weight Markers

Multicolor markers with high, low, and wide molecular weight ranges are designed for use as standards, and provide a visual monitor of migration during electrophoresis and transfer efficiency for isotopic and nonisotopic applications.

Ordering Information

Cat. No.	Molecular Weight Range	Size
NEL311	Wide range	500 mL
NEL312	High range	500 mL

Cat. No.	Molecular Weight Range	Size
NEL313	Low range	500 mL

Protein Mix of Multicolor Molecular Weight Markers

Protein	Molecular Weight (Daltons)	Color	Wide Range	High Range	Low Range
Myosin, rabbit muscle	205,000	Blue	X	X	
B-Galactosidase, *E. coli*	116,000	Turquoise	X	X	
Albumin, bovine serum	66,000	Pink	X	X	
Ovalbumin, chicken egg	45,000	Yellow	X	X	X
Bovine erythrocytes carbonic anhydrase	29,000	Orange	X	X	X
Trypsin inhibitor, soybean	20,100	Green	X		X
α-Lactalbumin, bovine milk	14,200	Purple	X		X
Aprotinin, bovine lung	6,500	Blue	X		X

1. Wide Range
2. High Range
3. Low Range

Biotinylated Molecular Weight Markers

This mix of biotin-labeled proteins results in a ladder of six equal intensity bands ranging from 12,300 to 97,400 Daltons, for use in nonisotopic Western blotting applications.

Ordering Information

Cat. No.	Concentration	Size
NEL310	1 µg/mL	50 µL

Protein Mix of Biotinylated Molecular Weight Markers

Protein	Molecular Weight (Daltons)	Protein	Molecular Weight (Daltons)
Phosphorylase B	97,400	Carbonase anhydrase	30,000
Albumin, bovine serum	69,000	Trypsin inhibitor	20,100
Ovalbumin	46,000	Cytochrome C	12,300

^{14}C Methylated Molecular Weight Markers

^{14}C-labeled markers are for use in isotopic applications. The electrophoretic mobility of methylated molecular weight markers is identical to the unmodified individual protein.

Ordering Information

Cat. No.	Size
NEC811	5 µCi (185 kBq)

Protein Mix of ^{14}C Methylated Molecular Weight Markers

Protein	Molecular Weight (Daltons)	Protein	Molecular Weight (Daltons)
Phosphorylase B	97,400	Ovalbumin	46,000
Albumin, bovine serum	69,000	Gamma globulin (subunit 2)	22,500
Gamma globulin (subunit 1)	53,000	Cytochrome C	12,300

Secondary Antibodies

PerkinElmer offers secondary antibodies, affinity purified from goat serum, immunized with purified IgG, then labeled with alkaline phosphatase, horseradish peroxidase, or biotin. They provide high sensitivity and specificity when used in either chromogenic or chemiluminescent applications.

Ordering Information

Cat. No.	Antibody	Label	Size	Concentration
NEF801	Anti-Human IgG (goat)*	AP	1 mL	1 mg/mL
NEF802	Anti-Human IgG (goat)*	HRP	1 mL	1 mg/mL
NEF803	Anti-Human IgG (goat)	Biotin	0.5 mg	Lyophilized
NEF811	Anti-Rabbit IgG (goat)	AP	1 mL	1 mg/mL
NEF812	Anti-Rabbit IgG (goat)	HRP	1 mL	1 mg/mL
NEF813	Anti-Rabbit IgG (goat)	Biotin	0.5 mg	Lyophilized
NEF821	Anti-Mouse IgG (goat)	AP	1 mL	1 mg/mL
NEF822	Anti-Mouse IgG (goat)	HRP	1 mL	1 mg/mL
NEF823	Anti-Mouse IgG (goat)	Biotin	0.5 mg	Lyophilized

* Bovine serum albumin added as a stabilizer.

PolyScreen® PVDF Transfer Membrane

Our PolyScreen® polyvinylidene difluoride (PVDF) membrane exhibits excellent sensitivity for Western blots, generating superb results with direct specific staining, indirect immunolabels, and all common chromogenic and chemiluminescent substrates. PolyScreen membranes exhibit high signal and low background noise, and can be used in most nitrocellulose protocols with the addition of a simple prewetting step. Blocking is easily done with a variety of blocking agents, such as nonfat dry milk, casein, non-ionic surfactants, BSA, and others.

Due to its hydrophobic surface, PolyScreen produces strong protein binding, and so retains more sample than nitrocellulose membranes, even during the most stringent blocking and washing steps. Unlike nitrocellulose, it does not crack, shatter, or discolor over time. Additionally, it is nonflammable, and requires no special handling, storage, or shipping precautions.

Ordering Information

Cat. No.	Quantity/Package	Size
NEF1000	10 sheets	20 cm x 20 cm sheets
NEF1002	1 sheet	26.5 cm x 3.75 m roll

Cat. No.	Quantity/Package	Size
NEF1003	50 sheets	7 cm x 8.4 cm sheets

Schleicher & Schuell BioScience Nitrocellulose Transfer Membranes

PerkinElmer is an authorized, worldwide dealer of Schleicher & Schuell BioScience membranes. Choose from a full selection of superior S&S Protran nitrocellulose membranes to suit your needs.

Protran Nitrocellulose Transfer Membranes feature:

- **100% pure nitrocellulose**—no cellulose acetate added, ensuring the highest binding capacity possible
- **Low Background**—surface properties guarantee superior signal-to-noise ratios, without the need for stringent washing conditions
- **Choice of two pore sizes**—0.2 µ size ensures high retention of small proteins below 20 kD by reducing "blowthrough", while 0.45 µ pore size membrane is ideal for larger molecular weight samples.)
- **Easy to use**—no methanol pre-wetting step. Simply wet in water then in transfer buffer prior to transfer. No other pre-treatment steps are necessary.

Ordering Information

Product	Quantity & Size	Cat. No.
Protran Nitrocellulose Rolls and Sheets (0.2 µ pore size)	1 (15 x 15 cm) sample sheet	NBA 083S
	1 (15 cm x 3 m) roll	NBA 083A
	1 (20 cm x 3 m) roll	NBA 083B
	1 (30 cm x 3.5 m) roll	NBA 083C
	5 (15 x 15 cm) sheets	NBA 083D
	5 (20 x 20 cm) sheets	NBA 083E
	5 (25 x 25 cm) sheets	NBA 083F
	5 (33 x 56 cm) sheets	NBA 083G
Protran Nitrocellulose Rolls and Sheets (0.45 µ pore size)	1 (15 x 15 cm) sample sheet	NBA 085S
	1 (15 cm x 3 m) roll	NBA 085A
	1 (20 cm x 3 m) roll	NBA 085B
	1 (30 cm x 3.5 m) roll	NBA 085C
	5 (15 x 15 cm) sheets	NBA 085D
	5 (20 x 20 cm) sheets	NBA 085E
	5 (25 x 25 cm) sheets	NBA 085F
	5 (33 x 56 cm) sheets	NBA 085G
Protran Unsupported Nitrocellulose Circles (0.45 µ pore size)	100 circles, 25 mm	NBA 085H
	50 circles, 82 mm	NBA 085I
	50 circles, 132 mm	NBA 085J
	50 circles, 137 mm	NBA 085K

Storage and Handling Precautions: Highly flammable. Store in a cool location, away from chemical vapors and direct sunlight.

KODAK® Films and Screens for Use with Western Blots

As a full-line supplier of KODAK scientific films, intensifying screens, and accessories, PerkinElmer offers the convenience of "one-stop shopping," and products of the highest quality for detection of isotopic and chemiluminescent Western blots.

KODAK BioMax Light Film

BioMax Light is KODAK's best film for chemiluminescence, offering maximum clarity and sensitivity, and the highest signal-to-noise ratio. Available in boxes of 50 sheets, either as ReadyPack packaging, with each sheet individually wrapped, or in environmentally friendly, non-interleaved packaging.

Ordering Information

Cat. No.	Quantity/Package	Size
178 8207	50 sheets, non-interleaved packaging	20.3 cm x 25.4 cm (8" x 10")
868 9358	50 sheets, non-interleaved packaging	13 cm x 18 cm (5" x 7")
819 4540	50 sheets, non-interleaved packaging	18 cm x 24 cm (7" x 9.5")
876 1520	50 sheet ReadyPack: each sheet individually wrapped	20.3 cm x 25.3 cm (8" x 10")
191 7012	50 sheet ReadyPack: each sheet individually wrapped	13 cm x 18 cm (5" x 7")

KODAK X-OMAT Blue Film

Available exclusively from PerkinElmer, KODAK X-OMAT Blue film is the economical choice, that provides excellent sensitivity, sharp resolution, and high contrast images. It can be used for both chemiluminescence detection and with all isotopes. Available in environmentally sensitive packaging, X-OMAT Blue film is compatible with all standard darkroom chemistries and autoprocessors.

Ordering Information

Cat. No.	Quantity/Package	Size
NEF586	100 sheets	13 cm x 18 cm (5" x 7")
NEF585	100 sheets	18 cm x 24 cm (7" x 9.5")

Cat. No.	Quantity/Package	Size
NEF596	100 sheets	20 cm x 25 cm (8" x 10")
NEF595	100 sheets	35 cm x 43 cm (14" x 17")

KODAK BioMax XAR Film

A general purpose film for use with all commonly used isotopes and chemiluminescence. Coated with emulsion on both sides of a clear base, it provides high sensitivity for both direct autoradiography and exposures with the BioMax MS or TranScreen intensifying screens.

Ordering Information

Cat. No.	Quantity/Package	Size
165 1454	50 sheets	20.3 x 25.4 cm (8" x 10")
165 1512	50 sheets	35 x 43 cm (14" x 17")
165 1496	50 sheets	13 x 18 cm (5" x 7")
853 2665	50 sheets	18 x 24 cm (7" x 9.5")

Cat. No.	Quantity/Package	Size
150 1451	50 sheets	24 x 30 cm (9.5" x 11.8")
150 6955	50 sheets (11.8" x 15.8")	30 x 40 cm
165 1579	50 sheet ReadyPack; each sheet individually wrapped	20.3 x 25.4 cm (8" x 10")
165 1678	50 sheet ReadyPack; each sheet individually wrapped	35 x 43 cm (14" x 17")

KODAK TranScreen HE Intensifying Screen

This innovative intensifying technology for high-energy emitting radiolabeled samples (e.g., ^{125}I) produces publication-quality images.

Ordering Information

Cat. No.	Quantity/Package	Size
856 3959	1 screen	8" x 10"

Choose PerkinElmer Reagents—For All Your Western Blotting Needs

Why go anywhere else? PerkinElmer has everything you need for Western blotting—products, performance, experience, and support! To order, call 1-800-762-4000 or visit our website at www.perkinelmer.com/las.

PerkinElmer Life and Analytical Sciences
710 Bridgeport Avenue
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