

Immunologicals - Accessory reagents

Western/ImmunoBlotting technique

For Western / Immuno Blotting application, Interchim provides :

- ◆ **Blotter devices** for easy and high resolution blotting and probing
- ◆ sensitive ready to use **chromogenic and chemiluminescent detection kits**
- ◆ A re-stripping reagent to re-use probed blots
- ◆ A lot of **accessory tools**.

Please refer to other sections for polyvalent reagents (buffers and saturants, I and II antibodies, enzyme substrates,...), and the Assay kits section for blotting kits by applications.

Technical tip - Western Blotting technique

Western Blotting was popularized as a rapid and sensitive method for characterizing purified proteins or complex mixtures of proteins.

Western blotting consists in the separation of proteins by gel electrophoresis (usually by the high resolution of SDS-PAGE technique, eventually combined to IEF for so-called 2D electrophoresis) followed by the transfer of these proteins on a membrane to obtain a blot.

Immunoblotting combines this technique with the specificity and sensitivity of immunodetection. In fact, the blot can be easily probed to detect specific proteins (antigens) with monoclonal or polyclonal antibodies. The detection with a secondary antibody and chromogenic or chemiluminescent reagents allows visualizing usually <1 ng of antigens among 10ths of bands (WB), or 100ths of spots (2D). Immunoblotting is the major application for Western Blotting that's why it is usual to talk of Western Blotting as a general technique, including Immunoblotting.

Unlike ELISA techniques, Western blotting allows the researcher to specifically visualize and characterize (by MW, pI) the antigen(s) of interest. It replaces advantageously immunoprecipitation technique and it is often used subsequently to clarify results. Limitations include :

- ◆ Some proteins are not correctly detected because of SDS denaturation, membrane adsorption, low transfer to membrane (high MW), or un-sufficient membrane binding (low MW).
- ◆ The WB analysis is rather difficult to scale up, and multiple analysis of a same blot is possible only when performing chemiluminescence detection.
(please have a look at "Blotting reprobing" paragraph).

Several tips were proposed to address these limitations: i.e. addition of 0.1 % SDS as well reduction of the methanol concentration (to 10% or below) in the transfer buffer for high MW proteins, choice a smaller pore size membrane for low MW proteins, ...

Blotter system

Miniblotter™

The Miniblotter™ instruments enable you to perform multiple antibody or probe incubations on a single membrane, using microliter volumes of reagent. Typical applications include monoclonal antibody screening on western blots, animal and human serum screening, and DNA probe hybridizations. Sample processing is streamlined through use of the Washing Manifold, which enables washing of all sample lanes simultaneously. Reactions can be detected by all conventional methods, including enzyme conjugates, chemiluminescence and radiolabels.

- ◆ For membrane assays including Western, Northern, and Southern Blots and Checker-board Blots
- ◆ Requires microliter volumes, not milliliter volumes
- ◆ Multiple samples analyzed simultaneously
- ◆ No cross-contamination between channels
- ◆ Decrease processing time
- ◆ All results on a single membrane
- ◆ Compatible with all standard electrophoresis systems

Description	Cat.#
Miniblotter 45 Unit (for regular gel processing)	987900
	538370 (with washing manifold)

- 45 channels (samples) per blot.
- Holds up to 110µl of antibody or probe solution per channel.
- Dimensions compatible with most standard gel electrophoresis chambers :
 - Channel dimension : 2.0 mm x 13 cm
 - Blot width covered : 13 cm
- Washing Manifold (F2) available for simultaneously washing of all channels.
- Results on a single membrane are compared easily without switching or breaking of strips.
- Instruments function with all detection methods (i.e., enzymatic, radioactive, chemiluminescence).
- Combs available for gel electrophoresis to line up Miniblotter channels with electrophoresis lane

Miniblotter 25 Unit (for regular gel processing)	BN4550
	661060 (with washing manifold)

- 25 channels (samples) per blot.
- Holds up to 250 µl of antibody or probe solution per channel.
- Dimensions compatible with most standard gel electrophoresis chambers :
 - Channel dimension : 4.0 mm x 13 cm
 - Blot width covered : 13 cm
- Greater volume per channel for highest sensitivity detection of low affinity and low titer antibodies.
- Washing Manifold (F2) available for simultaneously washing of all channels.
- Results on a single membrane are compared easily without switching or breaking of strips.
- Instruments function with all detection methods (i.e., enzymatic, radioactive, chemiluminescence).

Miniblotter 28 - Single Unit (for mini gel processing)	S31580
	590550 (with washing manifold)

- 28 channels (samples) per blot.
- Holds up to 50µl of antibody or probe solution per channel.
- Dimensions compatible with most standard gel electrophoresis chambers :
 - Channel dimension : 1.5 mm x 5.5 cm
 - Blot width covered : 8.3 cm
- Ideal for hybridoma screening from 96-well microplates. Hybridoma supernatants can be transferred directly into Miniblotter 28 channels using an 8-channel automatic pipette.
- Washing Manifold (F1) available for simultaneously washing of all channels.
- Results on a single membrane are compared easily without switching or breaking of strips.
- Instruments function with all detection methods (i.e., enzymatic, radioactive, chemiluminescence).

Miniblotter 28 - Dual Unit (for mini gel processing)	168830
	649400 (with washing manifold)

Same as Single Unit except there are two sets of 28 channels so that two membrane blots can be placed between the Top and Bottom Plates allowing for the processing of up to **56** samples (28 x 2).

Miniblotter 20 - Single Unit (for mini gel processing)	BN4580
	572090 (with washing manifold)

- 20 channels (samples) per blot.
- Holds up to 350 µl of antibody or probe solution per channel.
- Dimensions compatible with most minigel electrophoresis chambers :
 - Channel dimension : 3.0 mm x 5.5 cm
 - Blot width covered : 8.3 cm
- Greater volume per channel for highest sensitivity detection of low affinity and low titer antibodies.
- Washing Manifold (F1) available for simultaneously washing of all channels.
- Results on a single membrane are compared easily without switching or breaking of strips.
- Instruments function with all detection methods (i.e., enzymatic, radioactive, chemiluminescence).

Miniblotter 16 - Dual Unit	S31580
	590550 (with washing manifold)

Similar to Miniblotter 20 - Single Unit, except that there are two sets of 16 channels so that **two** membrane blots can be placed between the Top and Bottom Plates allowing for the processing of up to **32** samples (16x2).



Immunologicals - Accessory reagents

Western/ImmunoBlotting technique

Disposable Miniblot Systems for Minigel Processing

The Disposable Miniblot System operates exactly as the Miniblotter 28 unit, but its Top Plate frame (penned 'C-Shell') holds a disposable cassette. In addition to a test-specific blot, this disposable plastic cassette offers the same sample ports and 28 (enclosed) reaction channels as the Miniblotter 28 Unit. In the Disposable Miniblot System, however, once the assay is completed and the blot has been developed, the disposable cassette is thrown away - minimizing operator contact with potentially harmful reagents.

Cassette Miniblot - Single Unit

982720

- Disposable cassette has 28 enclosed reaction channels.
- Cassettes prevent risk of carry-over from one assay to another.
- Cassettes eliminate possibility of cross-contamination between samples
- Cassettes safely contain potentially hazardous serum during assay
- Holds up to 50 μ l of antibody or probe solution per channel.
- Dimensions compatible with most minigel electrophoresis chambers:
 - Channel dimension : 1.5 mm x 6.0 cm
 - Blot width covered : 8.3 cm
- Miniwash™ Manifold available for simultaneously washing of all channels - cutting assay time.
- Results on a single membrane are compared easily without switching or breaking of strips.
- Instruments function with all detection methods (i.e., enzymatic, radioactive, chemiluminescence).



Please inquire for other blotting accessories (rapid downward transfer system Turboblotter #BN4780, standard incubation trays #BN4801, sealable hybridization folders #BN4811,..).

Cassette Miniblot - Dual Unit

982721

- Same features as the Single Unit except the unit can hold two cassettes allowing for the processing of 56 samples (28 x 2).
- Each disposable cassette has 28 enclosed reaction channels.
- Disposable cassettes prevent risk of carry-over from one assay to another.
- The enclosed reaction channels eliminate the possibility of cross-contamination between samples and safely contain potentially hazardous serum during assay.
- Holds up to 50 μ l of antibody or probe solution per channel.
- Dimensions compatible with most minigel electrophoresis chambers :
 - Channel dimension: 1.5 mm x 6.0 cm
 - Blot width covered: 8.3 cm
- Miniwash™ Manifold available for simultaneously washing of all channels - cutting assay time.
- Results on a single membrane are compared easily without switching or breaking of strips.
- Instruments function with all detection methods (i.e., enzymatic, radioactive, chemiluminescence).

Immunologicals - Accessory reagents

Western/ImmunoBlotting technique

E3® Transfer and Blotting System

E3® technology uses free solution pulsed electrophoresis to accelerate the rapid formation of stable and highly specific immune complexes in 6 minutes ! E3® blotting system dramatically reduces the incubation time required in standard Western and Dot Blotting procedures.

E3 Transfer

- Rapid (20-30 min procedure)

Standard Transfer

- 3-4 H

E3 Blotting

- Rapid (down 35 min procedure)
- Quantitative (readily standardized)
- Sensitivity comparable to standard WB
- Easy to use; no membrane shrinkage
- Reduction in reagent amount/cost (5x less Ab)
- For western and Dot blotting

Standard Blotting

- 3-4 H
- Poorly quantitative (variable incubation conditions temperature, timing,...)
- Sensitive
- Skilled / long operating
- Large and container dependant volumes of reagents



Description	Cat.#	Qty
E-3® Western Apparatus	FX6710	1 u
E-3® Power Switch (Power supply)	BP2721	1 u
E-3® Western Chemiluminescent Reagent Kit	FX6920	10 tests
Contains 10 PVDF Membranes, pre-cut filters, running/blocking/ transfer buffers, Chemiluminescent substrate		
E-3® Western Colorimetric Reagent Kit	FX6960	10 tests
Contains 10 PVDF Membranes, pre-cut filters, running/blocking/ transfer buffers, Chromogenic substrate		
E-3® Western Control Set	FX6910	1 u

Assembly to Transfer

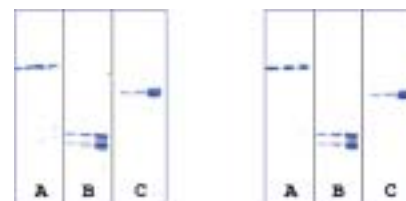


Assembly for Antibody Incubations



Comparison of Standard WB VS. E-3® WB (colorimetric)

Immunoblotting step	E3. Immuno blotting	Standard Immuno blotting
Blocking	10 min	20-30 min
I Ab incubation	6 min	1 H to OVN
Wash	3 min	20 min
II Ab incubation	6 min	1 H
Wash	10 min	20 min
Total blotting time :	35 min	4 H-OVN



Immunologicals - Accessory reagents

Western/ImmunoBlotting technique

Blotting membranes

Nitrocellulose membranes are preferred for protein application because they do not need pre-wetting in methanol. PVDF membranes are useful for applications that require higher binding capacity.

Protran® NitroCellulose membranes

Protran are superior nitrocellulose membranes :

- ◆ Compatible with all blotting methods
- ◆ Compatible with all standard probing and detection systems
- ◆ Excellent for proteins and carbohydrates (suits also nucleic acids)
- ◆ High binding, low background
- ◆ High retention of small proteins
- ◆ No methanol pre-wetting step (unlike PVDF membranes)
- ◆ Superior mechanical strength

Binding capacity :

0.45 μm : 75 -85 $\mu\text{g}/\text{cm}^2$

0.2 μm : 80 - 90 $\mu\text{g}/\text{cm}^2$

0.1 μm : 100 - 110 $\mu\text{g}/\text{cm}^2$

Protran are the most frequently specified media in the world for a wide range of applications. They are 100% pure nitrocellulose, unlike other membranes referred to as 'nitrocellulose' but contain actually cellulose acetate, and half tensile strength. As a result, they achieve higher protein binding capacity, lowest background without the need for stringent washing conditions, and excellent shelf life of proteins (molecular recognition for > five years, useful for archive and commercial products).

Protran excels for semi-dry and tank electroblotting, capillary and vacuum blotting, as well as dot blotting methods. For most protein transfers, the 0.2 μm is recommended, especially for proteins <20 kD. The 0.45 μm works fine for proteins >20 kD. The 0.1 μm is good for proteins down to about 1-2 kD, and 0.05 μm pore size is for smaller peptides. For nucleic acids , the standard pore size is 0.45 μm (or 0.2 μm for fragments smaller than 300 bp).

Immobilization of coated molecules is performed by drying (proteins, important for microarray and rapid tests), backing at 80°C (DNA/RNA) or UV-crosslinking (DNA/RNA). They accommodate all standard probing and detection (colorimetry, chemiluminescence, isotopes, Fluorescent/proteins) techniques.

Compared to competitors, Protran suits better the requirements of most demanding proteomics studies, as well of glycomics. For example, Protran offers highest sensitivity for protein-protein, protein-DNA interaction studies (i.e. down 150 amol of CRP). Also 10 fold higher signal intensity for oligosaccharides has been showed (i.e. down 5pmol 16 mer Chondroitin Sulfate).

Immunologicals - Accessory reagents

Western/ImmunoBlotting technique

Nitrocellulose Blotting membranes

Size	BA79 NC membranes 0.1 µm		BA83 NC membranes 0.2 µm		BA85 NC membranes 0.45 µm	
	Cat.#	Qty/pack	Cat.#	Qty/pack	Cat.#	Qty/pack
Sheets 20 x 20 cm	BN3651	5	S31441	5	BN3801	5
	BN3652	25	S31442	25	BN3802	25
Sheets 30 x 60 cm	BN3671	5	875900	5	324621	5
Rolls NC membrane						
20 cm x 3 m			BN3851	1	U60630	1
30 cm x 3 m	BN3831	1	BN3841	1	U60640	1

Blotting paper

Size	3MM sheets (0.34 mm thick)		17Chr (0.92 mm thick)		GB004 (1 mm thick)		GB005 (1.5 mm thick)	
	Cat.#	Qty/pack	Cat.#	Qty/pack	Cat.#	Qty/pack	Cat.#	Qty/pack
20 x 20 cm	BP2761	100						
460 x 570	BP2771	100	BP2781	25 u	BP2791	100		
580 x 580 cm							BP2801	25 u

PVDF and Nylon membranes

See description of TotalBlot™ membranes in section D "Genomics".

Chromogenic Blotting detection kits

Interchim provides Western Max Kits for rapid and sensitive chromogenic detection of either rabbit or mouse primary antibodies complexed with antigen immobilized on PVDF or nitrocellulose membranes. The Western Max HRP Kits use horseradish peroxidase conjugated goat anti-IgG antibody and the substrate 3,3'-diaminobenzidine (DAB) to produce a brown precipitate. The Western Max AP Kit uses alkaline phosphatase conjugated goat anti-IgG antibody and includes the substrate 5-bromo-4-chloro-indolyl phosphate/nitro blue tetrazolium chloride (BCIP/NBT) for dark blue-gray color development.

The highly purified secondary antibody conjugates and matching reagents provide superior sensitivity, minimize species cross-reactivity, and reduce lot-to-lot variation. Components in each kit are sufficient to develop up to 40 immunoblots (10 cm x 10 cm each). The amount of antibody supplied when diluted as recommended will yield 200 ml of working solution. These kits are stable for at least one year when stored at 4°C.

Description	Cat.#	Qty
Western MAX™ AP Kit, Chromogenic Detection, Anti-Mouse	N15130	1 Kit
Western MAX™ AP Kit, Chromogenic Detection, Anti-Rabbit	N15140	1 Kit
Western MAX™ HRP Kit, Chromogenic Detection, Anti-Mouse	GS3190	1 Kit
Western MAX™ HRP Kit, Chromogenic Detection, Anti-Rabbit	GS3200	1 Kit

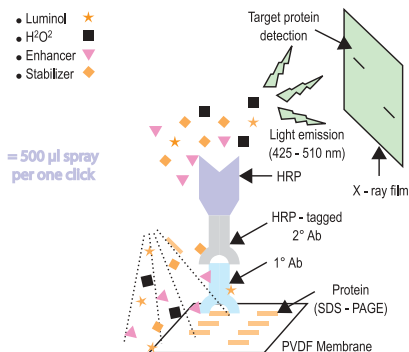
Related products

- ◆ Blotting Membranes
- ◆ Electrophoresis reagents (Acrylamides, SDS,...)
- ◆ Buffers and saturating agents (TBS, BioBlock...)

See also other AP and HRP substrates pages A357-A360.

Immunologicals - Accessory reagents

Western/ImmunoBlotting technique



Chemiluminescent detection - UptiLight

UptiLight WB HRP chemiluminescent substrate

Uptima is pleased to introduce UptiLight, an exceptional formulation of luminol, the best chemiluminescent substrate of peroxidase. UptiLight produces in presence of peroxidase a particularly intense luminous emission thanks to different proprietary agents increasing the photonic emission. UptiLight blotting substrate is optimized for the detection of the immobilized peroxidase, especially in Dot, Western, Southern and Northern- blotting techniques on nitrocellulose, nylon and PVDF. The signal is registered on radiographic films or by scanners.

Excellent results are obtained with shorter exposure duration than competitor products. Optimal sensitivity/background/time of operating is usually achieved in only 1-15 min. The good stability of signal allows the multiple re-exposure of the radiographic film in order to refine the intensity of revelation, or in order to obtain several copies.

UptiLight comes in following convenient packagings :

- ◆ The classic kit UP99619A is the most cost effective ECL product in the market with improved features.
- ◆ The spray format BM4961 is a breakthrough put in the labs : just spray the reagent ! This format is dedicated to radiography.

UptiLight one Spray WB chemiluminescent substrate

Benefits :

- ◆ Spray type - Un-rivalled ease of use : just spray, and expose blot to X-ray film
- ◆ Rapid reaction time - Specific detection achieved in less than 1-10minute
- ◆ High Sensitivity - High contrast signal, detect as little as 1-2pg of antigen
- ◆ Long duration time - More stable reagent, more reproducible data

Description	Cat.#	Qty
UptiLight One Spray WB chemiluminescent substrate	BM4961	1 u (for 2500 cm ²)
contains 100 ml reagent, sufficient for 100 miniblots		

UptiLight WB chemiluminescent Substrate kit

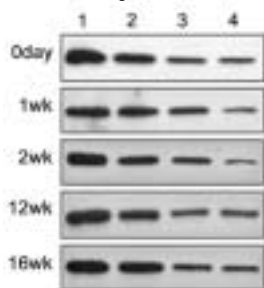
Benefits :

- ◆ Top Detection Sensitivity (high signal, low background)
- ◆ Excellent light emission stability
- ◆ No need to measure exact volumes !

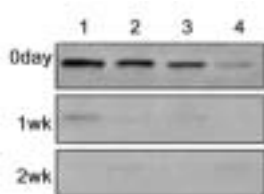
Description	Cat.#	Qty
UptiLight WB chemiluminescent substrate kit	UP99619A	1 kit (for 4000 cm ²)
contains 500 ml total reagent, sufficient for 80 miniblots (8 x 8 cm ²)		
UptiLight WB chemiluminescent substrate kit, 1 bottle/use	UP99619D	1 kit (for 1200 cm ²)

Superior reagent stability

a-tubulin (20, 10, 5, 2µg in lanes 1. 2. 3. 4) detected by WB using UptiLight One Spray (A) shows substrate activity is not affected for weeks compared with a mix of A and B reagents from a competitor Enzyme Chemi-Luminescent reagent (B)

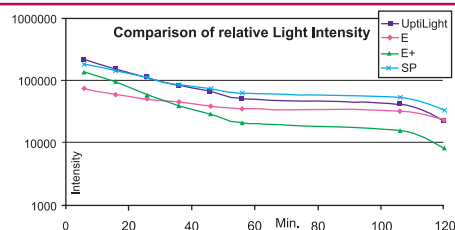


UptiLight Onespray



Competitor ECL (A+B mix)

UptiLight Comparative Study



The different Substrates (UptiLight, ECL (E), ECL +(E+), SS West pico (SP) were compared on a blot of IgGs revealed with an HRP labeled anti IgG. Blots were scanned simultaneously during 1minute with a phosphorImager™ after a 16min to 2Hours incubation of the blot with substrate.

Blotting reprobing

Because of the nature of chemiluminescence detection, it is possible to reprobe blotted membranes. Conventionally, Western blots have been stripped using extremely harsh conditions that may alter the antigen for subsequent immunoprobings. Our Stripping buffer provides a gentle method of removing primary and secondary antibodies from the membranes, which allows several reprobings on the same membrane.


Recycling protein blots offers many advantages :

1. When protein samples are available in limited quantities, are difficult to obtain or expensive.
2. When samples are to be analyzed with different antibodies under identical conditions, e.g. subtype or isoform specific antibodies.
3. When a blot gives unexpected results and needs confirmation with the same or different antibody.
4. When a blot is mistakenly incubated with wrong antibody.
5. It is simply more economical and less time consuming to reuse the same blot.

Description	Cat.#	Qty
Antibody Stripping Buffer	L7710A	500 ml

- ◆ Mild stripping conditions
- ◆ Do not affect immobilized proteins
- ◆ Rapid procedure (15 min)
- ◆ No pungent smelling, not harsh (acid) conditions
- ◆ No heating required

Antibody stripping buffer do not contains acids, mercaptoethanol or other reducer as used in home-made solutions. It allows rapid stripping.



Daily Updated

Prices of + 500 000 products, Including BioScience Innovations cat. number, are available from our web site :

www.interchim.com

Other tools for Blotting (biochemicals, accessories)

Buffers

Conventional buffers (PBS, TBS) and saturants are presented from page A352.

Antigen-Antibody pens™

Antigen-Antibody pens™ solves several problems of conventional Western using Chemiluminescence. (see figure 2A, B, C below)

Antigen-Antibody pens™ allow you to put marks on blots and visualize the MW weight scale or every different lanes after a chemiluminescent detection. You see right away, what samples produced results and what the sizes are even if the whole blot image size were to change. There is no need to compare with the original.

- ◆ Pen should be chosen from the same specie as primary antibody : annotations will be visible only if the right secondary was used. That's why the pen can also be used as a positive control.
- ◆ Antigen Pens are formulated with Color-coded dyes : **Red** dye for **Rabbit**, **Green** dye for **Goat**, and **Black** dye for **Mouse** primary antibodies.
- ◆ Pens are independent of the label of the secondary antibody.
- ◆ They are stable for 6-12 months and sufficient to mark 100-1000s of blots.

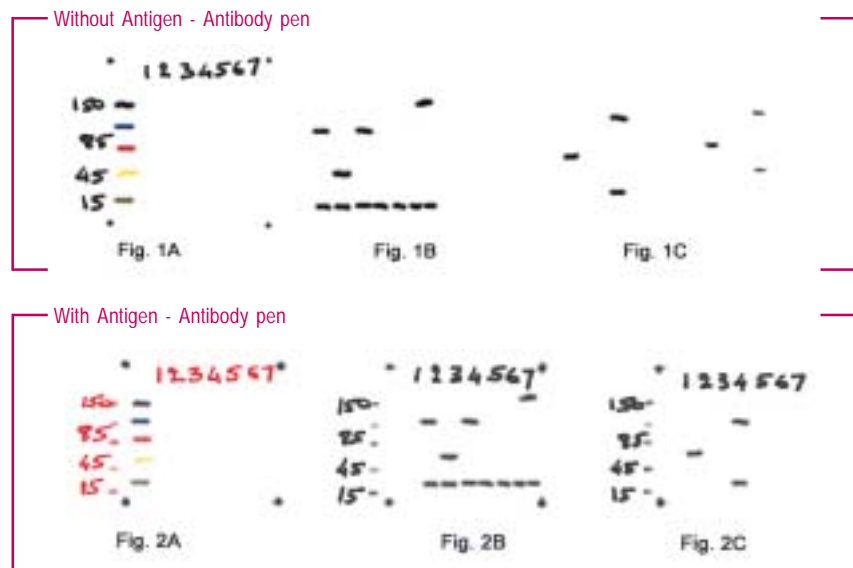
Description	Cat.#	Qty
Antigen-Antibody Pen for Rabbit primary antibodies	BN4360	1 u
Antigen-Antibody Pen for Mouse primary antibodies	BN4370	1 u
Antigen-Antibody Pen for Goat primary antibodies	BN4380	1 u
Antigen-Antibody Pen for Biotin-tagged primary antibodies (applicable for all species)	BN4390	1 u

Other pens specificities can be provided on inquire.

Antigen - Antibody pens

Difficulties of blot tracking in WB/ECL

In recent years, highly sensitive enzyme chemiluminescent substrate (Uptilight, ECL...) have been developed that emit light, instead of producing color and that can be recorded on x-ray films or more convenient film-less digital imager. However, it has become extremely problematic to keep track of the sample location and the size of the protein bands. As can be seen in Fig.1A, the marking of the protein size (15-150 kDa) or the lanes (1-7) by ordinary pen or pencil is lost when results are recorded on X-ray films or digital imager (Fig. 1B). The identity/location of the sample producing the band becomes more problematic when only a few lanes produce the band (Fig. 1C) or when there is a change in the size of the image (Fig. 1D) shows the image smaller; often the image is larger than the original blot) even if one tries to superimpose the image over the original.



Membrane Permanent Marking pen

This pen permanently writes on nitrocellulose and nylon membranes used in standard transfer procedures, even if the membrane is damp. The pen is compatible with hybridization and incubation solutions.

Description	Cat.#	Qty
Membrane Permanent Marking pen	BN4761	10 u