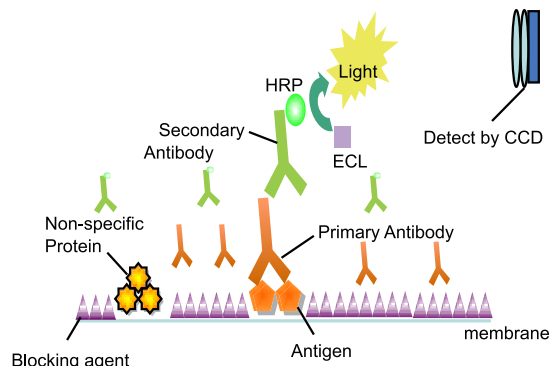


# Western blotting

Western blotting (西方墨點轉漬法) 是在 1977 年由 George Stark 所發表的一種技術。它是分子生物學、生物化學和免疫遺傳學中常被使用的一種實驗方法。其基本原理是通過特異性抗體與抗原的專一性結合，來偵測細胞、組織或體液中特定蛋白質的表現情形。這項技術結合了膠體電泳 (gel electrophoresis)、抗原抗體專一性結合、enzyme 受質的偵測。使得在一大群的樣品裡，找尋特定的某一個蛋白質，成為可能的事。

Western blotting 主要分成三個步驟：

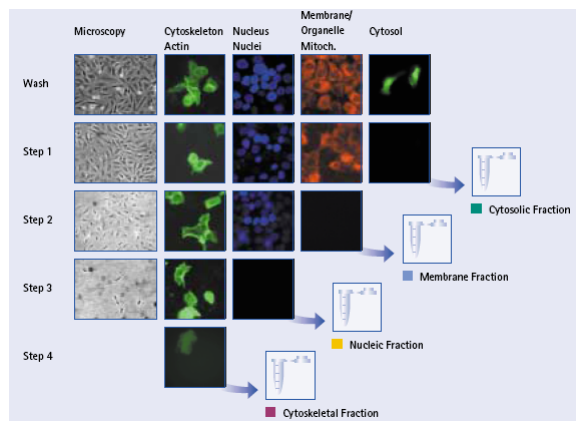
- 1. Gel electrophoresis & transfer**：將蛋白質混合物 loading 進 SDS-polyacrylamide 膠體上，蛋白質依分子量大小來分離。接著 transfer，將蛋白質從膠體轉移到 membrane 上。
- 2. Immunoblotting**：將轉漬好的 transfer membrane 浸於含有標地蛋白質的抗體 (primary antibody) 溶液中，形成抗原-抗體複合體。
- 3. Detection**：藉由對 primary antibody 具專一性的 enzyme-linked 抗體 (secondary antibody)，來漂洗此 membrane，再加入受質，產生有色物質或螢光，因而偵測感興趣的蛋白質表現。



## 樣品製備

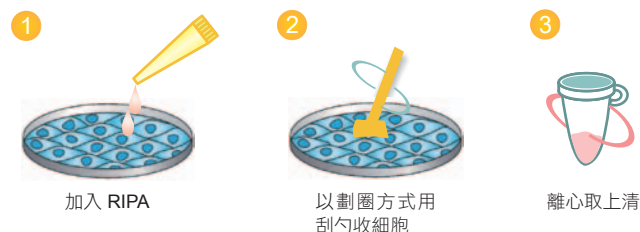
### Protein Buster 系列篇

- 包含實驗所需的所有 buffer
- 操作超簡單：不需超高速離心機
- 適用於各種細胞
- 能分離不同 subcellular localization 的蛋白
- 1D/ 2D 皆試用



### buffer 篇

- 經濟實惠
- 操作簡單：加入 dish 中，以劃圈方式刮取細胞即可
- 是你準備 Western blot sample 的最佳伴侶！
- 適用於各種哺乳類細胞



分類	Cat No.	Description	適用	Qty/Pk
核蛋白	71183-3CN	NucBuster™ Protein Extraction Kit	Mammalian cells	100 rxns
膜蛋白	444810-1KITCN	ProteoExtract® Native Membrane Protein Extraction Kit	Mammalian tissues and cultured cells	20 Extractions
	71772-3CN	ProteoExtract® Transmembrane Protein Extraction Kit	Mammalian cells and tissue	20 Extractions
粒線體	QIA88-1KITCN	ProteoExtract® Cytosol/Mitochondria Fractionation Kit	Mammalian cells	100 Extractions
Subcellular Protein	539790-1KITCN	ProteoExtract® Subcellular Proteome Extraction Kit	Mammalian cells	20 Extractions
Total protein	71186-4CN	YeastBuster™ Protein Extraction Reagent	Yeast cells	500 ml
	539779-1KITCN	ProteoExtract® Complete Mammalian Proteome Extraction Kit	Mammalian cells and tissue	20 Extractions
	71456-3CN	BugBuster® Master Mix	Gram-positive & negative bacteria cells	100 ml
	71009-4CN	CytoBuster™ Protein Extraction Reagent	Mammalian and insect cells	250 ml
	20-188	RIPA Lysis Buffer, 10X	Mammalian cells	100 ml

## Protease inhibitor & Phosphatase inhibitor

- 純度高
- 超方便：使用 cocktail 的形式，方便使用
- 適用於各種細胞及組織
- 最多的發表文獻
- 最齊全的 Inhibitors
- 品質穩定，實驗再現性高

### Protease inhibitor 篇

Cat No.	Description	Target	Qty/Pk
539131-1VLCN	Inhibitor Cocktail Set I Dilute: 1:100	<ul style="list-style-type: none"> <li>■ AEBSF, Hydrochloride</li> <li>■ Aprotinin, Bovine Lung</li> <li>■ E-64, Protease Inhibitor</li> <li>■ Leupeptin, Hemisulfate</li> <li>■ EDTA, Disodium</li> </ul>	1 ml
539134-1MLCN	<b>Hot</b> Inhibitor Cocktail Set III ( EDTA-Free ) Dilute: 1:100	<ul style="list-style-type: none"> <li>■ AEBSF, Hydrochloride</li> <li>■ Aprotinin, Bovine Lung</li> <li>■ E-64, Protease Inhibitor</li> <li>■ Leupeptin, Hemisulfate</li> <li>■ Bestatin</li> <li>■ Pepstatin A</li> </ul>	1 ml
539137-1MLCN	Inhibitor Cocktail Set V ( EDTA-Free ) Dilute: 1:100	<ul style="list-style-type: none"> <li>■ AEBSF, Hydrochloride</li> <li>■ Aprotinin, Bovine Lung</li> <li>■ E-64, Protease Inhibitor</li> <li>■ Leupeptin, Hemisulfate</li> </ul>	1 ml

### Phosphatase inhibitor 篇

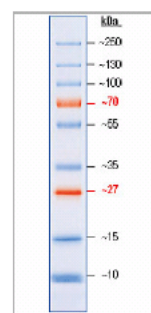
Cat No.	Description	Target	Qty/Pk
524625-1SETCN	Phosphatase Inhibitor Cocktail Set II Dilute: 1:100	<ul style="list-style-type: none"> <li>■ Acid Phosphatases</li> <li>■ Alkaline Phosphatase</li> <li>■ Protein Tyrosine Phosphatases ( PTPs )</li> </ul>	1 set ( 5x1 ml )
524628-1MLCN	Phosphatase Inhibitor Cocktail Set IV Dilute 1:50	<ul style="list-style-type: none"> <li>■ Alkaline Phosphatase</li> <li>■ Ser/ Thr phosphatases</li> </ul>	1 ml
524629-1MLCN	<b>Hot</b> Phosphatase Inhibitor Cocktail Set V	<ul style="list-style-type: none"> <li>■ Acid Phosphatases</li> <li>■ Alkaline Phosphatase</li> <li>■ Protein Tyrosine Phosphatases ( PTPs )</li> <li>■ Ser/ Thr phosphatases</li> </ul>	1 ml

## Protein Marker

- 兩種顏色讓您好分辨大小
- 分子量位置穩定，變異性小
- Loading 5ul 即可清楚看見訊號

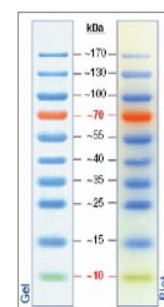
#### 訂購資訊

Cat No.	Description	Qty/Pk
IN1124	EZColor I™ Prestained Protein Marker	250 ul / vial
IN1125	EZColor II™ Prestained Protein Marker	250 ul / vial



5µl / lane  
4~15 % SDS-PAGE

Cat No. IN1124



5µl / lane  
4~15 % SDS-PAGE

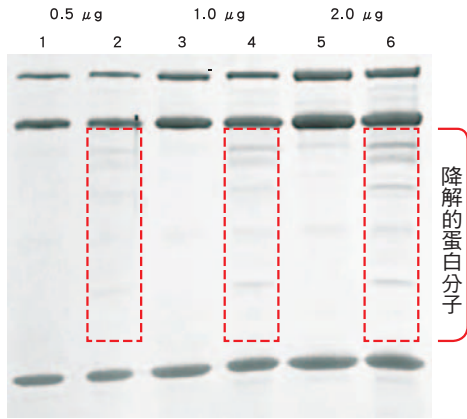
Cat No. IN1125

## Protein Loading Buffer

### Lonza

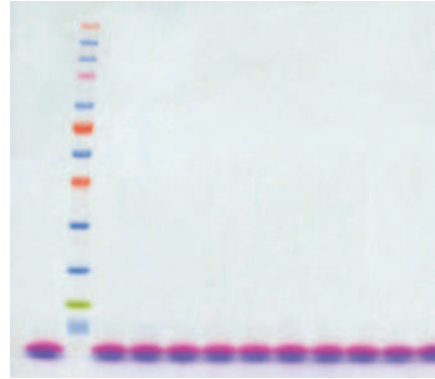
- 真放心 – 能保護樣品，避免電泳過程中，因溫度和 pH 變化所造成的 protein degradation。
- 真方便 – 雙色 loading buffer，藍色用來觀察電泳移動情形，粉紅色用來檢視 transfer 的效率。
- 真好用 – 4 倍濃縮，室溫保存。

成功的保護樣品，避免蛋白質 degradation



Dilutions of Phosphorylase B, BSA, and myoglobin proteins, separated on a 4 - 20% PAGE<sup>®</sup> Gel. Lanes 1, 3 and 5 are protected by ProSieve<sup>®</sup> ProTrack<sup>™</sup> Dual Color Protein Loading Buffer, Lanes 2, 4 and 6 are proteins prepared and run in a standard loading buffer.

在電泳的過程中，可觀察電泳移動的情形



Dilution series of tubulin run on a 4 - 20% PAGE<sup>®</sup> Gel. The ProSieve<sup>®</sup> QuadColor<sup>™</sup> Protein Marker in the left lane provided visual identification of run distance on the gel. ProSieve<sup>®</sup> ProTrack<sup>™</sup> Dual Color Protein Loading Buffer in the other lanes indicated the running front of the proteins (blue dye), and verification of the subsequent transfer efficiency of the proteins to the blot (pink dye).

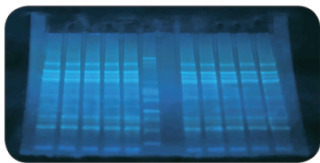
#### 訂購資訊

貨號	品名	包裝
193861	ProSieve ProTrack Dual Color 4X Protein Loading Buffer	1,000 loads of 20ul

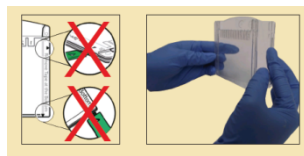
## Precast Gel



- 超長效期：一年半的長效期
- 方便觀察：不需染色，直接將 Gel 放置於 UV-box，即可看到所有蛋白的表現
- 方便拆除：不需任何工具，即可取得 Gel
- 方便 Loading：cassette 上標示數字，方便 Loading
- 避免污染：cassette 上有塑膠隔間，樣品 Loading 不會溢至隔壁 well
- 所需時間短：只要 30 分鐘即可跑完 SDS-PAGE
- 適用範圍廣：不同的 running buffer (MES, MOPS, Glycine)，皆適用
- 超貼心：不同廠牌的 Tanks 皆適用

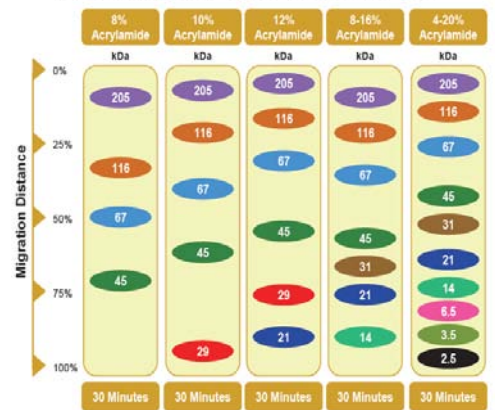


2 minute visualization under UV light



Requiring no key or knife to open

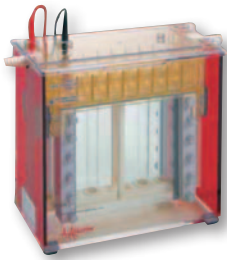
#### Broad Migration Range for nUView Gels



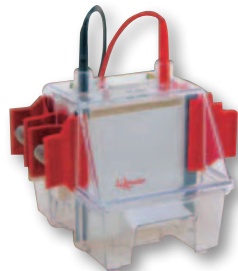
	% Acrylamide	Cat No. ( 10 well, 50ul )	Cat No. ( 12 well, 30ul )
NN cassette 10 cmx 10 cm Fits Xcell SureLock	4~20%	NN10-420	NN12-420
NB cassette 10 cmx 8.5 cm Fits Mini-Protean tanks	4~20%	NB10-420	NB12-420
NG cassette 10 cmx 8.0 cm Compatible with all other tanks	4~20%	NG21-420	NG11-420

# Electrophoresis & transfer

**Hofer** 您熟悉與信任的電泳相關設備



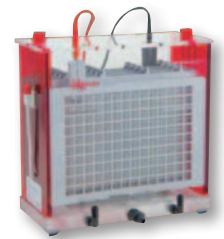
Vertical Electrophoresis  
Cat No. SE600



Mini Vertical Electrophoresis  
Cat No. SE260



Power Supply  
Cat No. SE600



Standard Transfer Tank  
Cat No. TE42

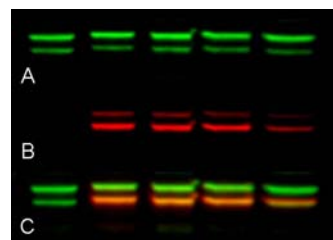
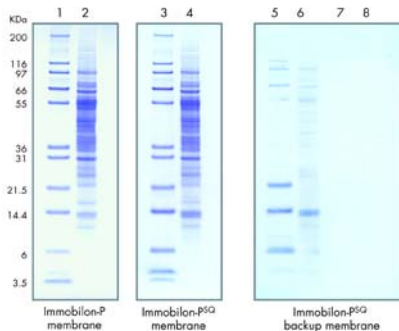
## Buffer : DNase, RNase, Protease Free

### 訂購資訊

Cat No.	Description	Qty/Pk
2300CN	OmniPur® Ammonium Persulfate, APS	25 gm
3860CN	OmniPur® DTT	5 gm
4005CN	OmniPur® EDTA	500 gm
4010CN	OmniPur® EDTA, Disodium Salt, Dihydrate	250 gm
4750CN	OmniPur® Glycerin	1L
6505CN	OmniPur® 10X PBS Liquid Concentrate	4L
7710CN	OmniPur® Sodium Chloride	500 gm
8920CN	OmniPur® TEMED	25 ml
9410CN	OmniPur® Triton® X-100 Surfactant	1 L
9480CN	OmniPur® Tween®	1 L
4410CN	OmniPur® Ethidium Bromide, EtBr	10 ml
8820CN	OmniPur® 10X TBE Buffer	4L
9210CN	OmniPur® TRIS	500 gm
7910CN	OmniPur® Sodium Dodecyl Sulfate ( SDS )	500 gm
4810CN	OmniPur® Glycine	500 gm

## Transfer membrane

- 背景值低，實驗結果更具說服力
- Protein binding capacity 約 150-450 ug/cm<sup>2</sup>，比 NC membrane 高出兩倍
- 韌性強，操作過程中不易撕破
- 適用於各種呈色方式
- Immobilon-Psq 適用於 < 20kDa 蛋白質
- Immobilon-FL 低背景值干擾，適用螢光偵測



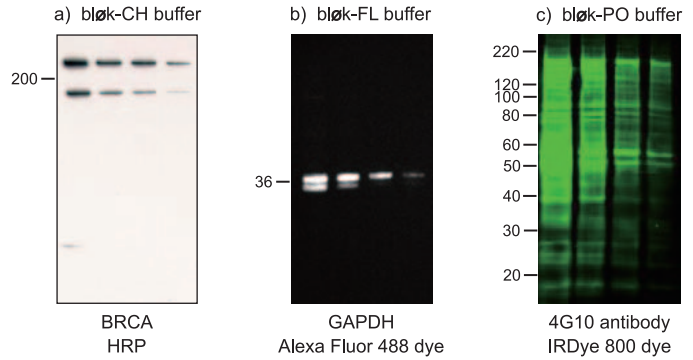
Immobilon-FL  
Cat No. IPFL00010

### 訂購資訊

Cat No.	Description	Applications	Qty/Pk
IPVH00010	Immobilon-P ; 0.45um	Western blotting ( General )	26.5 cm x 3.75 m / roll
ISEQ00010	Immobilon-Psq ; 0.22um	Low molecular weight ( MW < 20kDa )	26.5 cm x 3.75 m / roll
IPFL00010	Immobilon-FL ; 0.45 μm	Fluorescence immunodetection	26.5 cm x 3.75 m / roll

## Blocking Solution

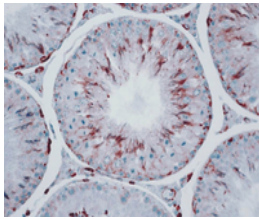
- 背景值低，實驗結果更具說服力
- Protein-free
- Ready to use
- 適用於各種呈色方式



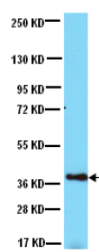
### 訂購資訊

Cat No.	Description	Applications	Qty/Pk
2170	ChemiBLOCKER	ELISA Western Blotting Immunohistochemistry	2 × 500 mL
WBAVDCH01	Blok - CH Noise Cancelling Reagents	Chemiluminescence Detection	500 mL
WBAVDFL01	Blok - FL Noise Cancelling Reagents	Fluorescent Detection	500 mL
WBAVDP001	Blok - PO Noise Cancelling Reagents	Phosphoprotein Detection	500 mL

## Internal control Anti-body ( 1st Ab )



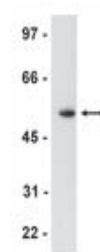
Cat No. MAB1501



Cat No. MAB374



Cat No. 05-661



Cat No. 05-829

### 訂購資訊

Cat No.	Description	Applications	Qty/Pk
MAB1501	Anti-Actin, clone C4	ELISA, IC, IF, IH, IH(P), WB	100 μL
MAB374	Anti-Glyceraldehyde-3-Phosphate Dehydrogenase, clone 6C5	ELISA, IAP, IC, IF, IH, WB	200 μg
05-661	Anti-β-Tubulin, clone AA2	WB	200 μg
05-829	Anti-α-Tubulin, clone DM1A	IC, IF, WB	200 μg

## HRP conjugate ( 2nd Ab )

### 訂購資訊

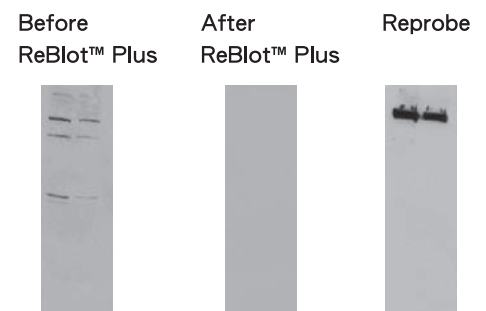
Cat No.	Description	Applications	Qty/Pk
AP124P	Goat anti-Mouse IgG, Peroxidase Conjugated	ELISA, IH, WB	2 mL
AP132P	Goat anti-Rabbit IgG, Peroxidase Conjugated	ELISA, IH, WB	2 mL
AP112P	Goat anti-Human IgG, HRP conjugate	ELISA, WB	2 mL
AP136P	Goat anti-Rat IgG, HRP conjugate	ELISA, IH, WB	2 mL

## Stripping buffer

- 不需加熱，室溫即可進行
- 不含 Mercaptoethanol，沒有刺鼻的臭味
- 節省時間，不需再進行費時的 Gel electrophoresis 及 transfer 過程
- 同一片 membrane，可以觀察不同的蛋白，節省您寶貴的樣品

### 訂購資訊

Cat No.	Description	Qty/Pk
2502	ReBlot Plus Mild Antibody Stripping Solution, 10x	50 mL
2504	ReBlot Plus Strong Antibody Stripping Solution, 10x	50 mL
2060	ReBlot Western Blot Recycling Kit ( 包含 blocking reagent )	kit



## Substrate

### Premix 設計

- premix 形式：減少污染及試劑的浪費
- 靈敏度高
- 再現性高：減少 pipetting error，增加實驗再現性
- 適用性高：PVDF 及 NC 皆適用
- 選擇性廣：針對不同的實驗需求，提供不同靈敏度等級的呈色劑



	Luminata Classico	Luminata Crescendo	Luminata Forte
Unique Feature	Premixed	Premixed	Premixed
Best used for	Blots where the primary antibody is incubated for ~1 hr	Blot where the primary antibody is incubated > 2 hrs	Blots with overnight primary antibody incubation, or detection of PTM** proteins
Detection Range	~6 pg	~1-3 pg	~400 fg
Signal Duration	1 hr	3 hr	3 hr
Stock Solution Stability	1 yr at 4 °C	1 yr at 4 °C	1 yr at room temperature

\*\*PTM – Post-translationally modified



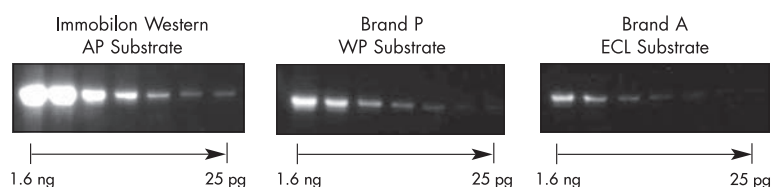
### 訂購資訊

分類	Cat No.	Description	Qty/Pk
低階	WBLUC0100	Luminata Classico Western HRP substrate	100 mL
	WBLUC0500	Luminata Classico Western HRP substrate	500 mL
中階	WBLUR0100	Luminata Crescendo Western HRP substrate	100 mL
	WBLUR0500	Luminata Crescendo Western HRP substrate	500 mL
高階	WBLUF0100	Luminata Forte Western HRP substrate	100 mL
	WBLUF0500	Luminata Forte Western HRP substrate	500 mL

### 傳統設計

提供您高敏感度的呈色劑

將樣品以兩倍序列稀釋，Millipore 可以偵測到其它產牌皆無法偵測到的樣品量。

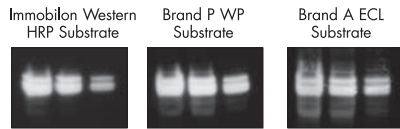


**節省您寶貴又昂貴的抗體**

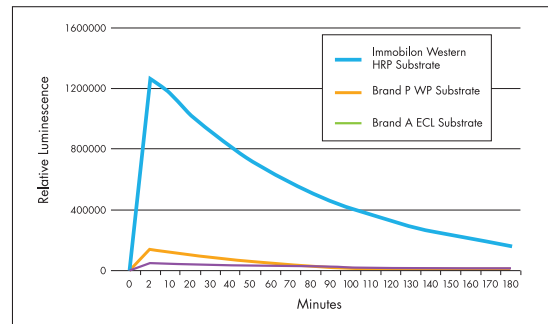
即使抗體稀釋至兩萬倍，Millipore 依然可以偵測到與其它產牌相同的訊號。

**維持較長時間的訊號及強度**

Millipore 可以比其它產牌維持較長及較強的訊號反應。



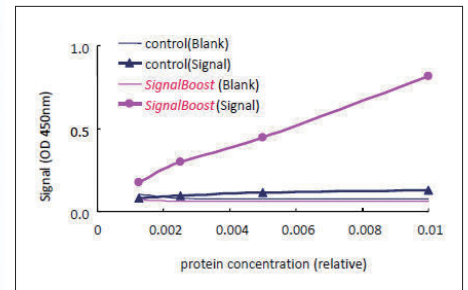
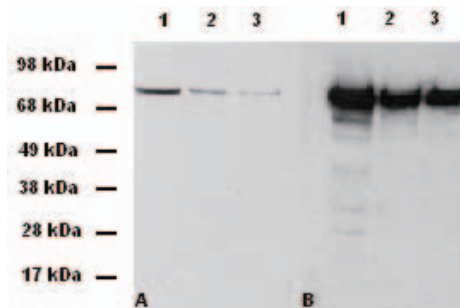
Primary Ab (400 µg/ml)	1:20,000	1:1,000	1:1,000
Secondary Ab (200 µg/ml)	1:20,000	1:5,000	1: 5,000
Exposure	1 min	1 min	5 min*



Cat No.	Description	Membrane Coverage, cm <sup>2</sup>	Qty/Pk
WBKLS0500	Immobilon Western HRP Substrate	5000	500 mL
WBKDS0100	Immobilon Western AP Substrate	2000	100 mL
17-373	Visualizer™ Spray & Glow™ ECL Western Blotting Detection System	-	100 mL

**Signal Enhancer**

- 省錢：節省抗體量
- 訊號加強效果好
- 操作簡單：和抗體一起添加使用
- 背景值低
- 適用於 WB 及 ELISA



**訂購資訊**

Cat No.	Description	Qty/Pk
407207-1KITCN	SignalBoost™ Immunoreaction Enhancer Kit	kit ( 20 Miniblots or 1600 cm <sup>2</sup> )

**Detection**



- 適用於 Blotting 及 Sequence autoradiography
- 可用於偵測 <sup>14</sup>C、<sup>35</sup>S、<sup>32</sup>P、<sup>33</sup>P、<sup>125</sup>I 等放射性實驗及冷光偵測實驗
- 高靈敏度與高對比解析能力
- 適用於自動化洗片系統

**訂購資訊**

Cat No.	Description	Qty/Pk
E3018	Denville HyBlot CL film ; 8 x 10"	100 片
E3012	Denville HyBlot CL film ; 5 x 7"	100 片