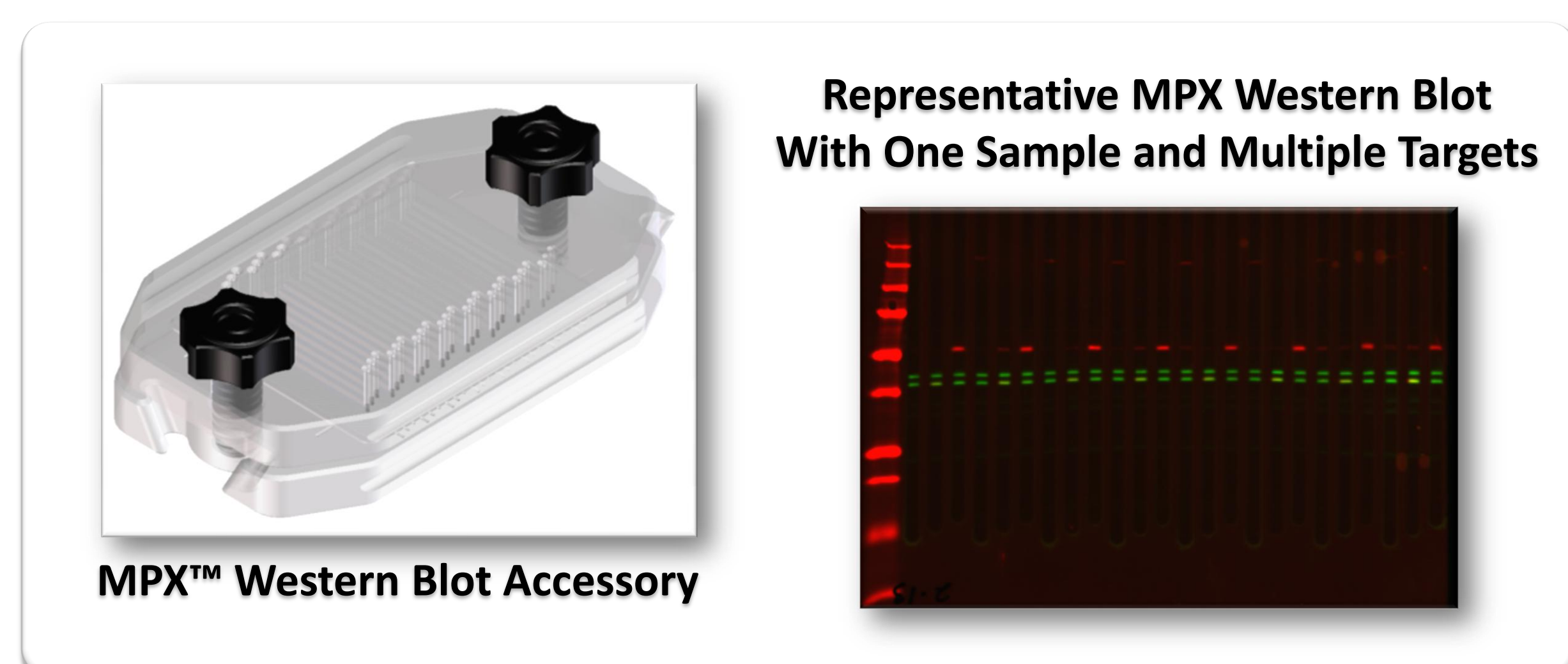


Multiplex (MPX™) Western Blot Detection for Antibody Validation

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INTRODUCTION

The MPX™ (multiplexer) can be used for multiple screening of one to four separate samples on a single Western blot. The MPX unit eliminates the limitations encountered with antibody cross reactivity in standard Western blots by effectively creating up to 24 independent channels on standard nitrocellulose or PVDF Western blot membrane. Up to 24 different targets can be detected per wavelength of the Odyssey® Infrared Imaging system for a maximum of 48 targets in a single sample on one blot. Channel ports are conveniently spaced and beveled to create a simple workflow using a standard 8-channel pipetter. The low volume channel ports accommodate a maximum volume of 160 µl conserving precious and costly antibodies to be used for detection. The nesting dovetail design allows multiple units to be locked together making it easier to handle additional blot processing. All data generated can be accurately quantified using the Odyssey software features. The MPX is compatible with all common Western blotting procedures that generate a blot of 7 X 8.5 cm.



The MPX can be used for primary and secondary antibody screening, monoclonal antibody screening, signal transduction pathway analysis, or any Western blot application in which there are 1-4 samples needing to be evaluated for multiple targets. The use of the MPX for antibody screening is presented here.

MPX vs Standard Western Blot Cost Comparison Using Near-Infrared Detection

Reagents	IR Detection (15-well gel)		Chemiluminescence (15-well gel)		24-well MPX™ IR Detection
	2-targets	1 st -target	1 st -target	2 nd -target	
Blocker	7.80	7.80	7.80	7.80	5.20
Primary Antibody					
β-Tubulin (Upstate 05-661)	34.50	34.50	---	---	6.90
ERK 1 (Santa Cruz SC-94)	18.32	---	18.32	---	1.04
Secondary antibody*	0.82	0.09	0.09	0.09	0.08
(Recommended Dilutions: 1:15,000 for IR, 1:25,000 for Chemi, 1:5,000 for MPX)					
Chemiluminescent Substrate*	0.00	12.20	12.20	12.20	0.00
(0.1 ml/cm ²)					
Film (2 sheets/blot)	0.00	7.71	7.71	7.71	0.00
Protein Markers	0.22	2.26	2.26	2.26	0.22
(Recommended Amount: 1µl for IR; 10 µl for Chemi)					
Total (\$)	61.66	64.56	48.38	48.38	13.44
\$/per Data Point	2.06	4.30	3.23	3.23	0.28

*Based on typical prices of chemiluminescent reagents as of 17 October 2006

METHODS & MATERIALS

MPX Workflow



SmartGel™ (LI-COR Biosciences, PN 928-40040, 928-40042, 928-40044) gel electrophoresis chemistry was used to pour a denaturing gel matrix of 7.5%, 10 %, or 12.5% acrylamide. Using the MPX combs indicated in the table below, the sample number and desired targets can be optimized based on need. Additionally, NU-PAGE® Novex (Invitrogen) pre-cast 2D gels can be used to generate blots for use on the MPX (see Western blot above). Standard methods are used for gel electrophoresis and transfer to Odyssey® nitrocellulose membrane (LI-COR Biosciences, PN 926-31090). Standard 7 X 8.5 cm blots are blocked in Odyssey Blocking Buffer (LI-COR Biosciences, PN 927-40000) for 1 hour then placed wet into the MPX. The channel port plate is then clamped in place. Detection reagents are diluted in Odyssey blocking buffer as indicated in figure legends and 160 µl are added per channel and incubated appropriately. Channels are washed with 1 X PBST and imaged on the Odyssey infrared imaging system.

Comb Sample Number and Channel Guide for Optimal MPX Use

Single Marker Combs		Dual Marker Combs	
Combs	Samples	Combs	Samples
1 + Marker	24	1 + 2 Markers	22
2 + Marker	11	2 + 2 Markers	10
3 + Marker	7	3 + 2 Markers	6
4 + Marker	5	4 + 2 Markers	4

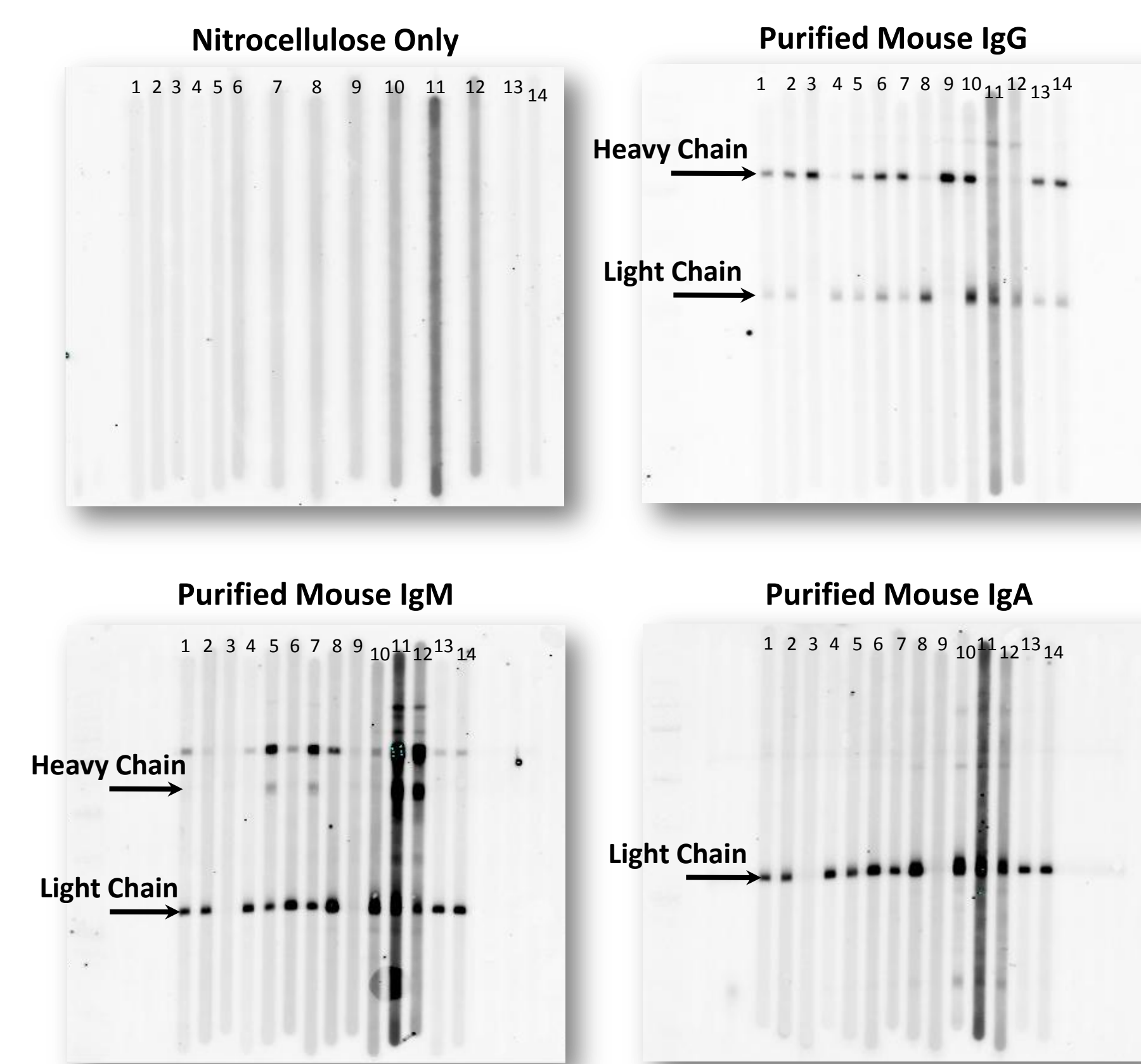
RESULTS & DISCUSSION

Secondary Antibody Screening

The MPX blotting system was used to screen a panel of anti-mouse antibodies. The objective was to identify a secondary antibody that performed the best for detection of IgG, IgA, and IgM primary antibody. Whole mouse IgG, IgA, or IgM was electrophoresed and then transferred to Odyssey nitrocellulose membrane. Twelve IRDye® 800CW conjugated anti-mouse antibodies were evaluated. LI-COR antibodies performed as expected. IgA, IgG, IgM antibody did not perform better than LI-COR donkey anti-mouse or goat anti-mouse. No advantage in detection was seen when using F(ab)₂ antibodies. Fc specific antibodies performed as expected and offer no benefit over LI-COR donkey anti-mouse or goat anti-mouse for IgG, IgM, or IgA detection. IgM specific antibody is recommended for IgM antibody detection.

IRDye 800CW Conjugated Secondary Antibodies Used to Screen IgG, IgA and IgM

ID	Secondary Antibody	Dilution
1	Goat anti-mouse IgA, IgG, IgM	1:5000
2	Rabbit anti-mouse IgG	1:5000
3	Goat anti-mouse IgG Fcy	1:5000
4	Goat anti-mouse IgG F(ab) ₂	1:5000
5	Goat anti-mouse IgG, IgM	1:5000
6	F(ab) ₂ Goat anti-mouse IgG	1:5000
7	F(ab) ₂ Goat anti-mouse IgG, IgM	1:5000
8	F(ab) ₂ Goat anti-mouse IgG F(ab) ₂	1:5000
9	F(ab) ₂ Goat anti-mouse Fcy	1:5000
10	Donkey anti-mouse IgG (LI-COR, PN 926-32212)	1:5000
11	Goat anti-mouse IgM	1:5000
12	Goat anti-mouse IgM	1:7500
13	Goat anti-mouse IgG (LI-COR, PN 926-32210)	1:2500
14	Goat anti-mouse IgG (LI-COR, PN 926-32210)	1:5000

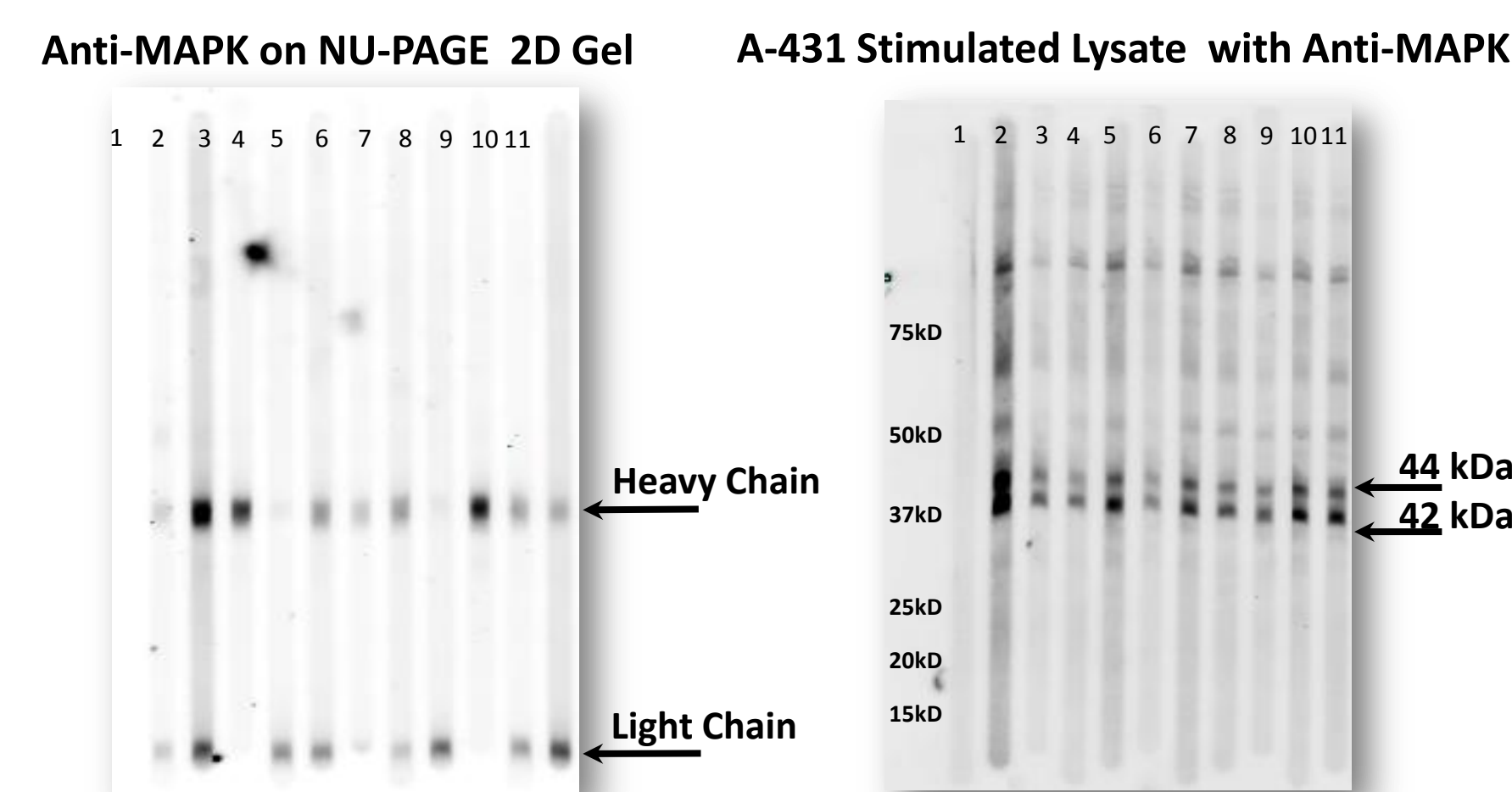


Primary Antibody Optimization of MAP Kinase

MAP Kinase, activated (diphosphorylated ERK 1 and 2) (Sigma, PN M8159) primary antibody was run on NU-PAGE Novex (Invitrogen) pre-cast 2D gels, transferred to Odyssey nitrocellulose membrane, and detected using IRDye 800CW conjugated anti-mouse antibodies. The same antibodies were also used to detect ERK 1 and 2 on A431 EGF stimulated lysate. The donkey anti-mouse or goat anti-mouse antibodies gave very clear positive signals of the light and heavy chains of the anti-MAPK antibody. All of the antibodies evaluated were able to detect this antibody under standard Western blot conditions except the goat anti-mouse IgG, IgM, IgA antibody. The anti-MAPK antibody was then used in combination with the various secondaries to detect ERK 1 and 2 in A-431 lysate. All but the goat anti-mouse IgA, IgG, IgM antibody detected ERK 1 and 2 under standard western blot conditions.

IRDye 800CW Conjugated Secondary Antibodies Used to Screen MAP Kinase

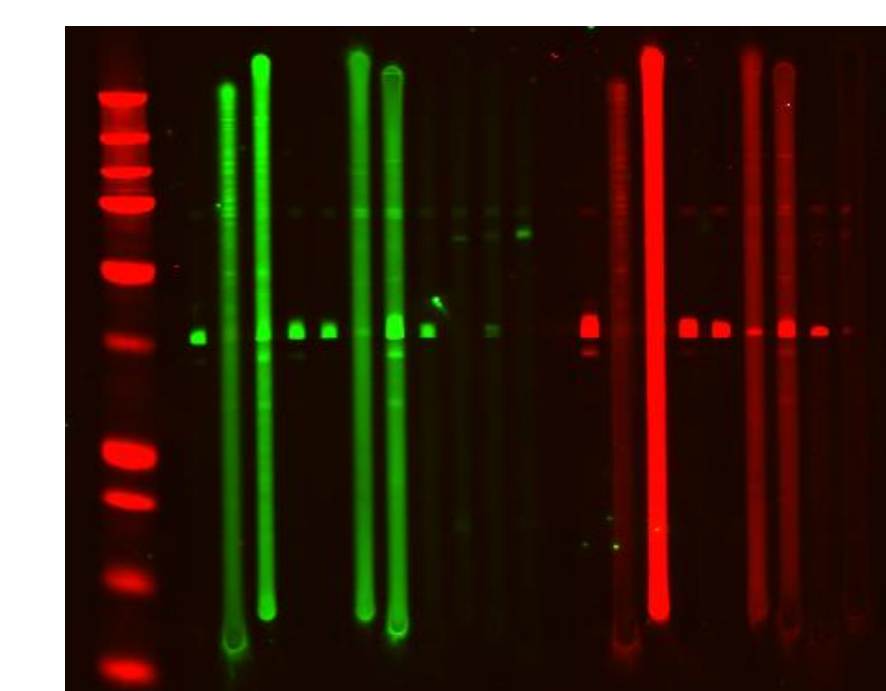
ID	Secondary Antibody	Dilution
1	Goat anti-mouse IgA, IgG, IgM	1:5000
2	Rabbit anti-mouse IgG	1:5000
3	Goat anti-mouse IgG Fcy	1:5000
4	Goat anti-mouse IgG F(ab) ₂	1:5000
5	Goat anti-mouse IgG, IgM	1:5000
6	F(ab) ₂ Goat anti-mouse IgG	1:5000
7	F(ab) ₂ Goat anti-mouse IgG, IgM	1:5000
8	F(ab) ₂ Goat anti-mouse IgG F(ab) ₂	1:5000
9	F(ab) ₂ Goat anti-mouse IgG Fcy	1:5000
10	Donkey anti-mouse IgG (LI-COR, PN 926-32212)	1:7000
11	Goat anti-mouse IgG (LI-COR, PN 926-32210)	1:5000



Primary Antibody Screening of Various GAPDH Antibodies

A battery of GAPDH primary antibodies were screened against HeLa cell lysate for Fluorophore-Linked Immunosorbent Assay (FLISA) development. Lysate was electrophoresed and transferred to Odyssey nitrocellulose membrane. Eight different sources of anti-GAPDH from mouse, rabbit and goat species were diluted in 160 µl of Odyssey blocking buffer at manufacturers recommended dilutions. Detection was performed using a 1:5000 dilution of IRDye 800CW & IRDye 680 goat anti-mouse, goat anti-rabbit, or donkey anti-goat (LI-COR Biosciences, PNs 926-32210, 926-32211, 926-32214, 926-32220, 926-32221, 926-32224). This screening method successfully displays the use of different primary antibodies as well as different secondary antibodies applied to each channel of the MPX.

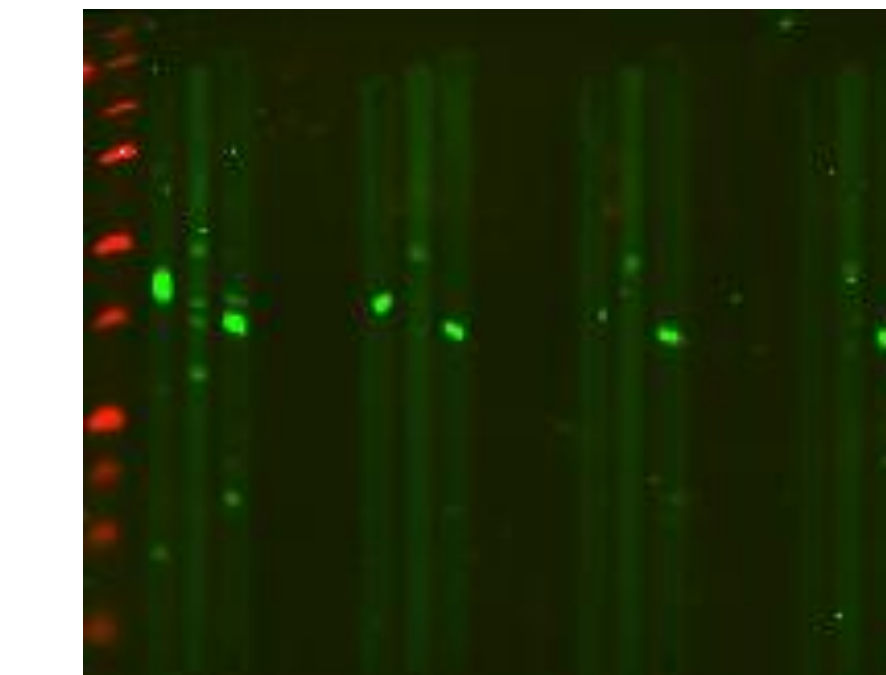
Anti-GAPDH screening using IRDye 800CW and IRDye 680 detection



General Western Blot Analysis of Multiple Samples

A-431, HeLa, Jurkat, and 3T3-L1 cell lysates were electrophoresed using the SmartGel chemistry and MPX 4-well, single marker comb and transferred to nitrocellulose. Primary antibodies anti-ERK2 (Santa Cruz, SC-1647), anti-β Tubulin (Santa Cruz, SC-9104), and anti-Actin (Chemicon, MAB1501) were diluted as per manufacturer recommendation and detected using IRDye 800CW Goat anti-mouse/rabbit secondary antibodies at 1:5000.

Sample Screen with 4 + Marker Blot



SUMMARY

- The MPX blotting system can be effectively used for many types of antibody screening and evaluation.
- Smaller volumes of valuable antibody are used for MPX detection versus traditional Western blot methods.
- One to four samples can be evaluated simultaneously on one Western blot, saving time and money per data point collected.
- This system is ideal for increasing throughput of multiple target evaluation on a small sample number.
- Most standard Western blot applications can be converted for use on the MPX system.

