

# Proteome Profiler™ Array

## Human Phospho-MAPK Array Kit

Catalog Number ARY002B

For the parallel determination of the relative levels of phosphorylation of Mitogen-Activated Protein Kinases (MAPKs) and other serine/threonine kinases.

This package insert must be read in its entirety before using this product.  
For research use only. Not for use in diagnostic procedures.

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## INTRODUCTION

Analyzing the phosphorylation status of all three major families of mitogen-activated protein kinases (MAPKs), the extracellular signal-regulated kinases (ERK1/2), c-Jun N-terminal kinases (JNK1-3), and different p38 isoforms ( $\alpha/\beta/\delta/\gamma$ ), is essential in understanding the roles these signaling molecules play in mechanisms underlying cell function and disease. Other intracellular proteins, such as Akt, GSK-3, p70 S6 Kinase, TOR, p53, and CREB, are additional important regulators of signal transduction, mediating development and cell proliferation. The Human Phospho-MAPK Array is a rapid, sensitive, and economical tool used to simultaneously detect the relative levels of phosphorylation of 26 kinases including nine MAPKs without performing numerous immunoprecipitations or Western blots. Each capture antibody was carefully selected using lysate samples prepared from cell lines known to express the target proteins.

## PRINCIPLE OF THE ASSAY

Capture and control antibodies have been spotted in duplicate on nitrocellulose membranes. Cell lysates are diluted, mixed with a cocktail of biotinylated detection antibodies, and incubated overnight with the Proteome Profiler Human Phospho-MAPK Array. The membrane is washed to remove unbound material. Streptavidin-HRP and chemiluminescent detection reagents are applied, and a signal is produced at each capture spot corresponding to the amount of phosphorylated protein bound. Refer to the Appendix for a list and coordinates of analytes and controls.

## TECHNICAL HINTS

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- This kit should not be used beyond the expiration date on the kit label.
- **Do not mix or substitute reagents with those from other lots or sources. Substitution of some high intensity chemiluminescent reagents for Chemi Reagents 1 and 2 may cause either increased background or diminished signal depending on the reagent.**
- Any variation in sample handling, buffers, operator, pipetting technique, washing technique, and incubation time or temperature can alter the performance of the kit.
- The Human Phospho-MAPK Array membranes are validated for single use only.
- Always use gloved hands and flat-tipped tweezers to handle the membranes.
- Pick up the membranes from the edge on the side with the identification number avoiding the area with the printed antibodies.
- A thorough and consistent wash technique is essential for proper assay performance. Individual membranes should be washed in separate containers to minimize background. Wash Buffer should be removed completely from the membrane before proceeding to the next step.
- Do not allow the membrane to dry out. This will cause high background.
- Avoid microbial contamination of reagents and buffers.

## PRECAUTION

Chemi Reagents 1 and 2 contain Boric Acid which is suspected of damaging fertility or the unborn child. Do not handle until all safety precautions in the MSDS have been read and understood. Wear protective gloves, clothing, eye, and face protection when using these reagents.

## MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	AMOUNT PROVIDED	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human Phospho-MAPK Array	893909	4 membranes	Return unused membranes to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 3 months at 2-8 °C.*
Array Buffer 1	895477	1 vial (21 mL)	May be stored for up to 3 months at 2-8 °C.*
Array Buffer 5	895876	1 vial (21 mL)	
Lysis Buffer 6	895561	1 vial (21 mL)	
Wash Buffer Concentrate, 25X	895003	2 vials (21 mL/vial)	
Detection Antibody Cocktail, Human Phospho-MAPK Array	893910	1 vial	
Streptavidin-HRP	890803	1 vial	
Chemi Reagent 1	894287	1 vial (2.5 mL)	
Chemi Reagent 2	894288	1 vial (2.5 mL)	
4-Well Rectangular Multi-dish	607544	1 dish	Store at room temperature.
Transparency Overlay Template	607725	1 template	

\* Provided this is within the expiration date of the kit.

## OTHER SUPPLIES REQUIRED

- Pipettes and pipette tips
- Gloves
- Phosphate-Buffered Saline (PBS)
- Deionized or distilled water
- Flat-tipped tweezers
- Rocking platform shaker
- Microcentrifuge
- Plastic containers with the capacity to hold 50 mL (for washing the arrays)
- Plastic transparent sheet protector (trimmed to 10 cm x 12 cm and open on three sides)
- Plastic wrap
- Absorbent lab wipes (KimWipes® or equivalent)
- Paper towels
- Autoradiography cassette
- Film developer
- X-ray film (Kodak® BioMax™ Light-1, Catalog # 1788207) or equivalent
- Flatbed scanner with transparency adapter capable of transmission mode
- Computer capable of running image analysis software and Microsoft® Excel

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## SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Since the Human Phospho-MAPK Array detects relative phosphorylation levels of individual analytes, it is important to include appropriate control samples.

**Note:** *Sample amount may be empirically adjusted to attain optimal sensitivity with minimal background. The suggested starting range for cell lysates is 100-300 µg.*

**Cell Lysates** - Rinse cells with PBS, making sure to remove any remaining PBS before adding lysis buffer. Solubilize the cells at  $1 \times 10^7$  cells/mL in Lysis Buffer 6. Pipette up and down to resuspend and rock the lysates gently at 2-8 °C for 30 minutes. Microcentrifuge at 14,000 x g for 5 minutes, and transfer the supernate into a clean test tube. Quantitation of sample protein concentrations using a total protein assay is recommended. Assay immediately or aliquot and store at  $\leq -70$  °C. Avoid repeated freeze-thaw cycles.

## REAGENT PREPARATION

**Bring all reagents to room temperature before use.**

**Human Phospho-MAPK Array** - Four nitrocellulose membranes each containing 26 different capture antibodies printed in duplicate. **Handle the membranes only with gloved hands and flat-tipped tweezers.**

**Detection Antibody Cocktail** - One vial of lyophilized biotinylated phospho-specific antibodies. Before use, reconstitute the Detection Antibody Cocktail in 100 µL of deionized or distilled water.

**1X Wash Buffer** - If crystals have formed in the concentrate, warm the bottles to room temperature and mix gently until the crystals have completely dissolved. Dilute 40 mL of 25X Wash Buffer Concentrate into 960 mL of deionized or distilled water.

**Chemi Reagent Mix** - Chemi Reagent 1 and 2 should be mixed in equal volumes within 15 minutes of use. **Protect from light. 1 mL of the resultant mixture is required for each membrane.**

## ARRAY PROCEDURE

**Bring all reagents to room temperature before use. Keep samples on ice. To avoid contamination, wear gloves while performing the procedures.**

1. Prepare all reagents and samples as directed in the previous sections.
2. Pipette 2.0 mL of Array Buffer 5 into each well of the 4-Well Multi-dish to be used. Array Buffer 5 serves as a block buffer.
3. Using flat-tip tweezers, remove each membrane to be used from between the protective sheets and place in a well of the 4-Well Multi-dish. The number on the membrane should be facing upward.

**Note:** *Upon contact with Array Buffer 5, the blue dye from the spots will disappear, but the capture antibodies are retained in their specific locations.*

4. Incubate for one hour on a rocking platform shaker. Orient the tray so that each membrane rocks end to end in its well.
5. While the membranes are blocking, prepare samples by adding up to 400  $\mu$ L of each sample to separate tubes. Adjust to a final volume of 1.5 mL with Array Buffer 1.
6. Add 20  $\mu$ L of reconstituted Detection Antibody Cocktail to each prepared sample. Mix and incubate at room temperature for one hour.
7. Aspirate Array Buffer 5 from the wells of the 4-Well Multi-dish and add the prepared sample/antibody mixtures. Place the lid on the 4-Well Multi-dish.
8. Incubate overnight at 2-8 °C on a rocking platform shaker.

**Note:** *A shorter incubation time may be used if optimal sensitivity is not required.*

9. Carefully remove each membrane and place into individual plastic containers with 20 mL of 1X Wash Buffer. Rinse the 4-Well Multi-dish with deionized or distilled water and dry thoroughly.
10. Wash each membrane with 1X Wash Buffer for 10 minutes on a rocking platform shaker. Repeat two times for a total of three washes.
11. Dilute the Streptavidin-HRP in Array Buffer 5 using the dilution factor on the vial label. Pipette 2.0 mL of diluted Streptavidin-HRP into each well of the 4-Well Multi-dish.
12. Carefully remove each membrane from its wash container. Allow excess Wash Buffer to drain from the membrane. Return the membrane to the 4-Well Multi-dish containing the diluted Streptavidin-HRP. Cover the wells with the lid.
13. Incubate for 30 minutes at room temperature on a rocking platform shaker.

14. Wash each array as described in steps 9 and 10.

**Note:** *Complete the remaining steps without interruption.*

15. Carefully remove each membrane from its wash container. Allow excess Wash Buffer to drain from the membrane by blotting the lower edge onto paper towels. Place each membrane on the bottom sheet of the plastic sheet protector with the identification number facing up.

16. Pipette 1 mL of the prepared Chemi Reagent Mix evenly onto each membrane.

**Note:** *Using less than 1 mL of Chemi Reagent Mix per membrane may result in incomplete membrane coverage.*

17. Carefully cover with the top sheet of the plastic sheet protector. Gently smooth out any air bubbles and ensure Chemi Reagent Mix is spread evenly to all corners of each membrane. Incubate for 1 minute.

18. Position paper towels on top and sides of plastic sheet protector containing the membranes and carefully squeeze out excess Chemi Reagent Mix.

19. Remove the top plastic sheet protector and carefully lay an absorbent lab wipe on top of the membranes to blot off any remaining Chemi Reagent Mix.

20. Leaving membranes on the bottom plastic sheet protector, cover the membranes with plastic wrap taking care to gently smooth out any air bubbles. Wrap the excess plastic wrap around the back of the sheet protector so that the membranes and sheet protector are completely wrapped.

21. Place the membranes with the identification numbers facing up in an autoradiography film cassette.

**Note:** *Use an autoradiography cassette that is not used with radioactive isotope detection.*

22. Expose membranes to X-ray film for 1-10 minutes. Multiple exposure times are recommended.

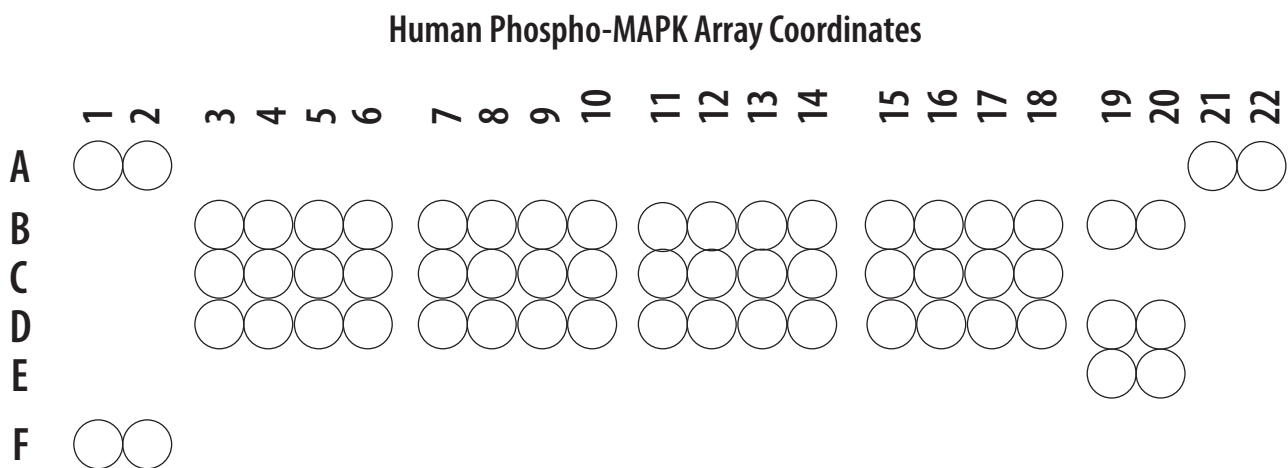
## DATA ANALYSIS

The positive signals seen on developed film can be quickly identified by placing the transparency overlay on the array image and aligning it with the pairs of reference spots in three corners of each array. The stamped identification number on the membrane should be placed on the left hand side. The location of controls and capture antibodies is listed in the Appendix.

**Note:** Reference spots are included to align the transparency overlay template and to demonstrate that the array has been incubated with Streptavidin-HRP during the assay procedure.

Pixel densities on developed X-ray film can be collected and analyzed using a transmission-mode scanner and image analysis software.

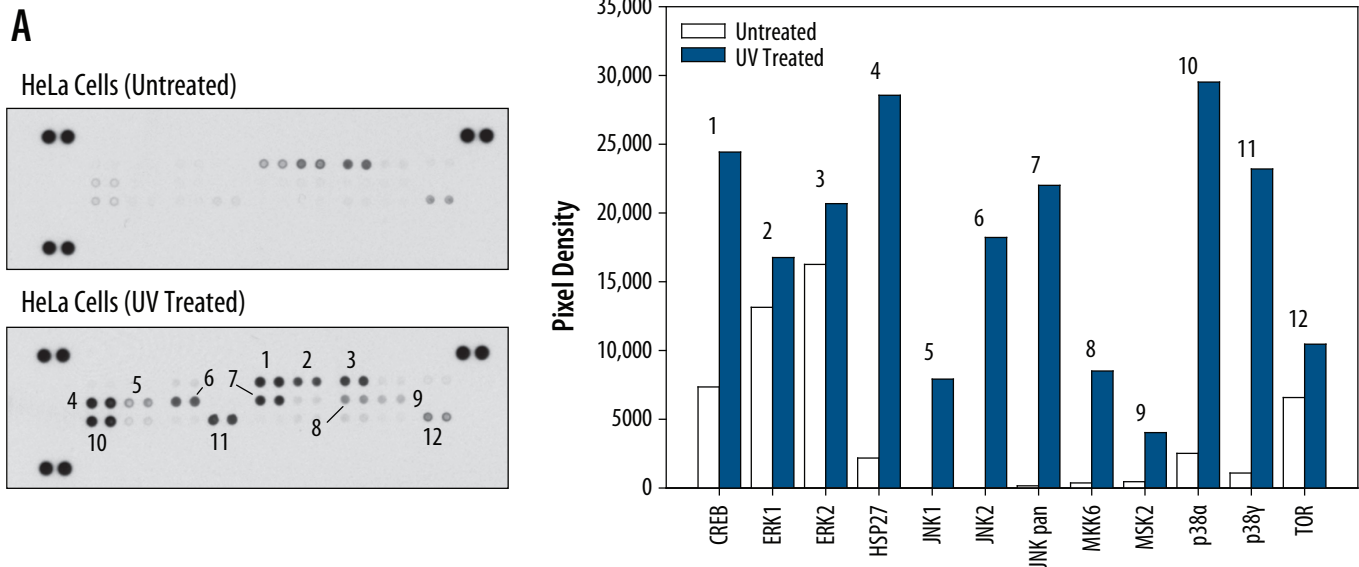
1. Create a template to analyze pixel density in each spot of the array.
2. Export signal values to a spreadsheet file for manipulation in a program such as Microsoft Excel.
3. Determine the average signal (pixel density) of the pair of duplicate spots representing each kinase.
4. Subtract an averaged background signal from each spot. Use a signal from a clear area of the array or negative control spots as a background value.
5. Compare corresponding signals on different arrays to determine the relative change in protein phosphorylation between samples.



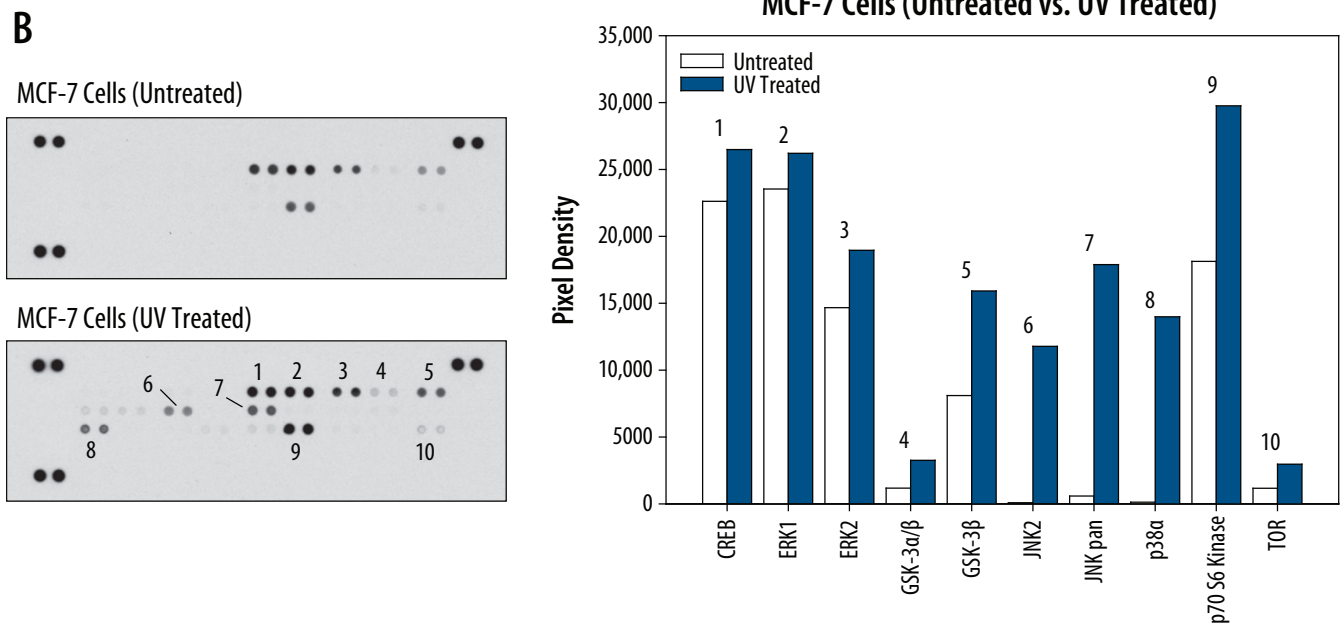
This image is not to scale. It is for coordinate reference only.  
Please use the transparency overlay for analyte identification.

## PROFILING PROTEIN PHOSPHORYLATION IN CELL LYSATES

The Human Phospho-MAPK Array detects multiple phosphorylated proteins in untreated and treated cell lysates. All arrays were incubated with 200 µg of lysate.



**Figure 1A:** HeLa human cervical epithelial carcinoma cells were either untreated or exposed to 150 J/m<sup>2</sup> of UV light followed by a 30 minute recovery period before lysis. Data shown are from a 2 minute exposure to X-ray film.

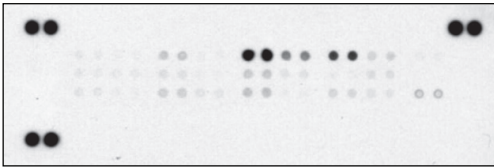


**Figure 1B:** MCF-7 human breast cancer cells were either untreated or exposed to 50 J/m<sup>2</sup> of UV light followed by a 4 hour recovery period before lysis. Data shown are from a 1 minute exposure to X-ray film.

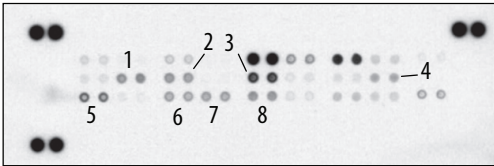
## PROFILING PROTEIN PHOSPHORYLATION IN CELL LYSATES *CONTINUED*

C

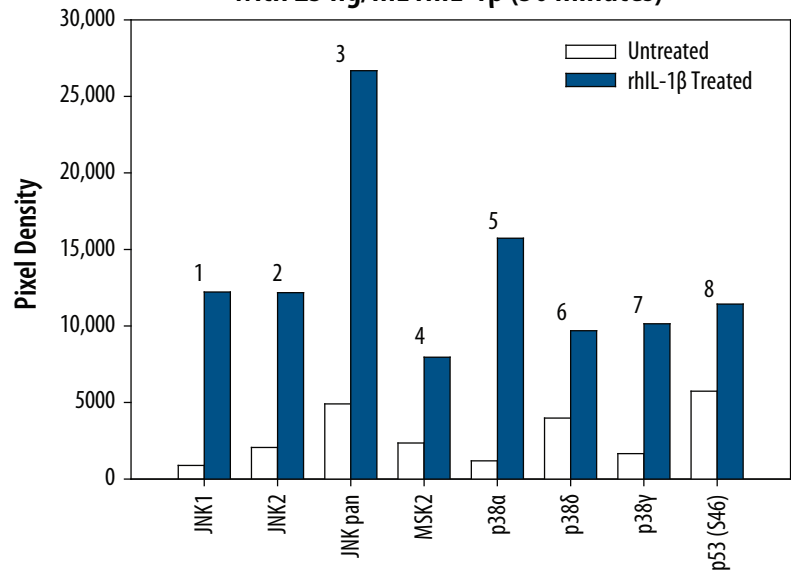
HepG2 Cells (Untreated)



HepG2 Cells (rhIL-1 $\beta$  Treated)



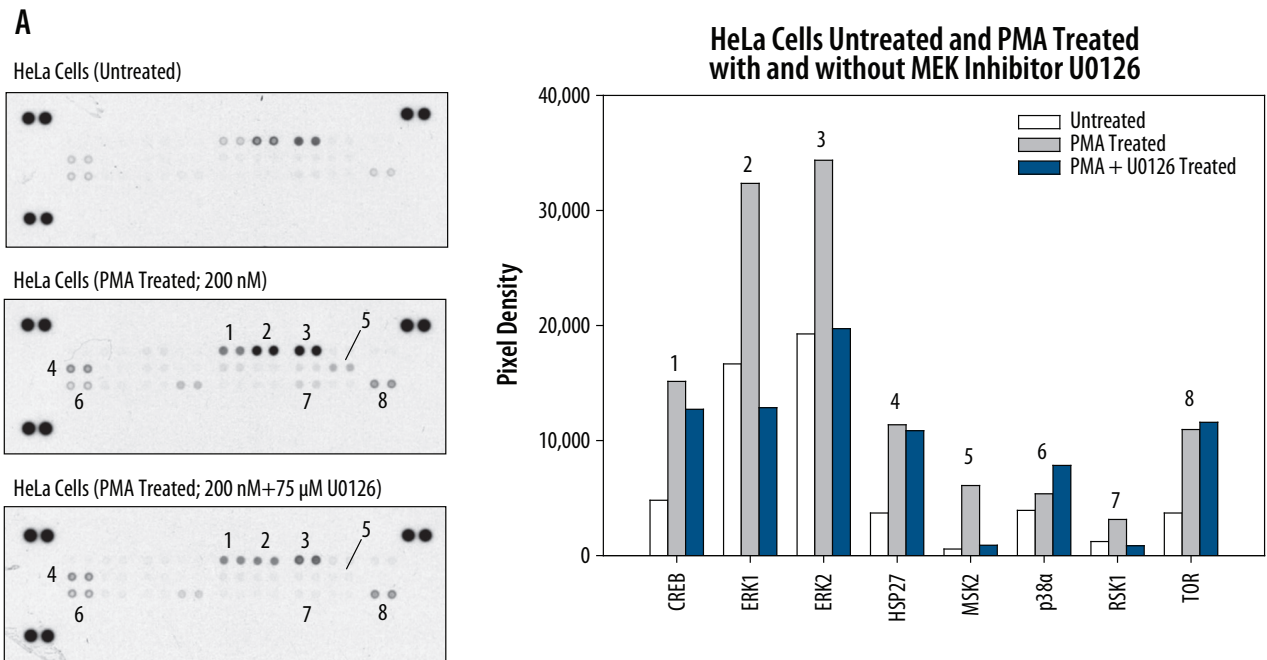
HepG2 Cells Untreated and Treated with 25 ng/mL rhIL-1 $\beta$  (30 minutes)



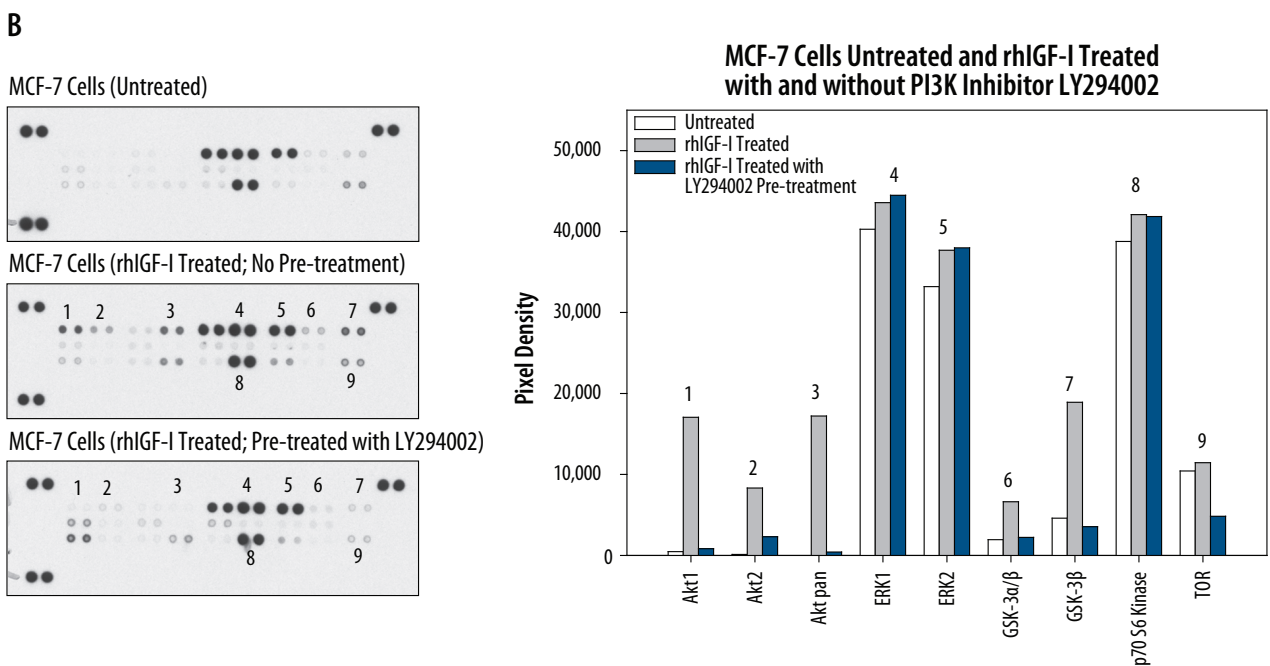
**Figure 1C:** HepG2 human hepatocellular carcinoma cells were either untreated or treated with 25 ng/mL of recombinant human (rh) IL-1 $\beta$  (R&D Systems, Catalog # 201-LB) for 30 minutes. Data shown are from a 2 minute exposure to X-ray film.

## PATHWAY INHIBITION

The Human Phospho-MAPK Array shows the effect of inhibitors on specific pathways. All arrays were incubated with 200 µg of lysate.



**Figure 2A:** HeLa human cervical epithelial carcinoma cells were either untreated or treated with 200 nM PMA for 20 minutes either with or without the MEK inhibitor U0126. Data shown are from a 5 minute exposure to X-ray film.



**Figure 2B:** MCF-7 human breast cancer cells were either untreated or treated with 100 ng/mL of rhIGF-I (R&D Systems, Catalog # 291-G1) for 1 hour. Cells for rhIGF-I treatment either received a 1 hour pre-treatment with the PI3K inhibitor, LY294002, or received no pre-treatment. Data shown are from a 6 minute exposure to X-ray film.

## APPENDIX

Refer to the table below for the Human Phospho-MAPK Array coordinates.

Coordinate	Target/Control	Alternate Nomenclature	Phosphorylation Site Detected
A1, A2	Reference Spots	—	—
A21, A22	Reference Spots	—	—
B3, B4	Akt1	PKB $\alpha$ , RAC $\alpha$	S473
B5, B6	Akt2	PKB $\beta$ , RAC $\beta$	S474
B7, B8	Akt3	PKB $\gamma$ , RAC $\gamma$	S472
B9, B10	Akt pan	—	S473, S474, S472
B11, B12	CREB	—	S133
B13, B14	ERK1	MAPK3, p44 MAPK	T202/Y204
B15, B16	ERK2	MAPK1, p42 MAPK	T185/Y187
B17, B18	GSK-3 $\alpha/\beta$	GSK3A/GSK3B	S21/S9
B19, B20	GSK-3 $\beta$	GSK3B	S9
C3, C4	HSP27	HSPB1, SRP27	S78/S82
C5, C6	JNK1	MAPK8, SAPK1 $\gamma$	T183/Y185
C7, C8	JNK2	MAPK9, SAPK1 $\alpha$	T183/Y185
C9, C10	JNK3	MAPK10, SAPK1 $\beta$	T221/Y223
C11, C12	JNK pan	—	T183/Y185, T221/Y223
C13, C14	MKK3	MEK3, MAP2K3	S218/T222
C15, C16	MKK6	MEK6, MAP2K6	S207/T211
C17, C18	MSK2	RSK $\beta$ , RPS6KA4	S360
D3, D4	p38 $\alpha$	MAPK14, SAPK2A, CSBP1	T180/Y182
D5, D6	p38 $\beta$	MAPK11, SAPK2B, p38-2	T180/Y182
D7, D8	p38 $\delta$	MAPK13, SAPK4	T180/Y182
D9, D10	p38 $\gamma$	MAPK12, SAPK3, ERK6	T183/Y185
D11, D12	p53	—	S46
D13, D14	p70 S6 Kinase	S6K1, p70 $\alpha$ , RPS6KB1	T421/S424
D15, D16	RSK1	MAPKAPK1 $\alpha$ , RPS6KA1	S380
D17, D18	RSK2	ISPK-1, RPS6KA3	S386
D19, D20	TOR	—	S2448
E19, E20	PBS	Control (-)	—
F1, F2	Reference Spots	—	—

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