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R&D SYSTEMS

Proteome Profiler™ Array

Human Cell Stress Array Kit

Catalog Number ARY018

For the parallel determination of the relative levels of selected human cell stress related proteins.

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 expression profile of cell stress related proteins is essential for understanding the roles these molecules play in the cellular response to stress and the development of disease states. The Proteome Profiler™ Human Cell Stress Array is a rapid, sensitive, and economical tool to simultaneously detect changes between samples. The relative expression levels of 26 cell stress related proteins can be determined without performing numerous immunoprecipitations or Western blots. Each capture and detection antibody was carefully selected using lysate samples prepared from both cell lines and tissues known to express the target proteins.

PRINCIPLE OF THE ASSAY

Capture and control antibodies have been spotted in duplicate on nitrocellulose membranes. Cell lysates or tissue lysates are diluted, mixed with a cocktail of biotinylated detection antibodies, and incubated overnight with the Proteome Profiler Human Cell Stress 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 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 Cell Stress 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 arrays 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.
- For a procedure demonstration video, visit: www.RnDSystems.com/ProteomeProfilerVideo.

MATERIALS PROVIDED & STORAGE CONDITIONS

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

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human Cell Stress Array	893405	4 nitrocellulose membranes each containing 26 different capture antibodies printed in duplicate.	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 4	895022	21 mL of a buffered protein base with preservatives. *May contain a precipitate. Mix well before and during use.*	May be stored for up to 3 months at 2-8 °C.*
Array Buffer 6	893573	2 vials (21 mL/vial) of a buffered protein base with preservatives. *May contain a precipitate. Mix well before and during use.*	
Lysis Buffer 6	895561	21 mL of a denaturing buffered solution.	
Wash Buffer Concentrate	895003	2 vials (21 mL/vial) of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Detection Antibody Cocktail, Human Cell Stress Array	893965	1 vial of biotinylated antibody cocktail; lyophilized.	
Streptavidin-HRP	893019	200 µL of streptavidin conjugated to horseradish-peroxidase.	
Chemi Reagent 1	894287	1 vial (2.5 mL)	
Chemi Reagent 2	894288	1 vial (2.5 mL)	
4-Well Multi-dish	607544	Clear 4-well rectangular multi-dish.	Store at room temperature.
Transparency Overlay Template	607745	1 transparency overlay template for coordinate reference.	

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

Note: *Additional wash buffer is available for purchase (R&D Systems®, # WA126).*

PRECAUTIONS

Chemi Reagents 1 and 2 contain Boric Acid which is suspected of damaging fertility or the unborn child.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

OTHER SUPPLIES REQUIRED

- Aprotinin (Tocris™, # 4139)
- Leupeptin hemisulfate (Tocris, # 1167)
- Pepstatin A (Tocris, # 1190)
- Pipettes and pipette tips
- Gloves
- Phosphate-Buffered Saline (PBS) (R&D Systems®, # RB01)
- Deionized or distilled water
- Rocking platform shaker
- Microcentrifuge
- A plastic container 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
- Paper towels
- Absorbent lab wipes
- Autoradiography cassette
- Film developer
- X-ray film
- Flat-tipped tweezers
- Flatbed scanner with transparency adapter capable of transmission mode
- Computer capable of running image analysis software and Microsoft® Excel

If using tissue lysate samples, the following are also required:

- PBS with protease inhibitors (10 µg/mL Aprotinin, 10 µg/mL Leupeptin hemisulfate, and 10 µg/mL Pepstatin A)
- Triton™ X-100 (Sigma, # T9284)

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 Cell Stress Array detects relative expression 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. Suggested starting range is: 100-300 µg for cell and tissue lysates.*

Cell Lysates - Rinse cells with PBS, making sure to remove any remaining PBS before adding lysis buffer. Solubilize cells at 1 x 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 concentration using a total protein assay is recommended. Assay immediately or aliquot and store at ≤ -70 °C. Avoid repeated freeze-thaw cycles.

Tissue Lysates - Excise tissue and homogenize in PBS with protease inhibitors. After homogenization, add Triton X-100 to a final concentration of 1%. Freeze samples at ≤ -70 °C, thaw, and centrifuge at 10,000 x g for 5 minutes to remove cellular debris. Quantitation of sample protein concentration using a total protein assay is recommended. Assay immediately or aliquot and store samples at ≤ -70 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Human Cell Stress 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 antibodies. Before use, reconstitute the Human Cell Stress Detection Antibody Cocktail in 100 µL of deionized or distilled water.

Lysis Buffer 6 - Add 10 µg/mL of Aprotinin, 10 µg/mL of Leupeptin hemisulfate, and 10 µg/mL of Pepstatin A to the volume of lysis buffer required for cell lysate preparation. **Prepare fresh for each use.**

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 Reagents 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 per 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 mL of Array Buffer 6 into each well of the 4-Well Multi-dish to be used. Array Buffer 6 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 6, 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 1 mL of each sample to 0.5 mL of Array Buffer 4 in separate tubes. Adjust to a final volume of 1.5 mL with Array Buffer 6 as necessary.
6. Add 20 µL of reconstituted Detection Antibody Cocktail to each prepared sample. Mix and incubate at room temperature for one hour.
7. Aspirate Array Buffer 6 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 6 using the dilution factor on the vial label. Pipette 2 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.

ARRAY PROCEDURE *CONTINUED*

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 the top and sides of the 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

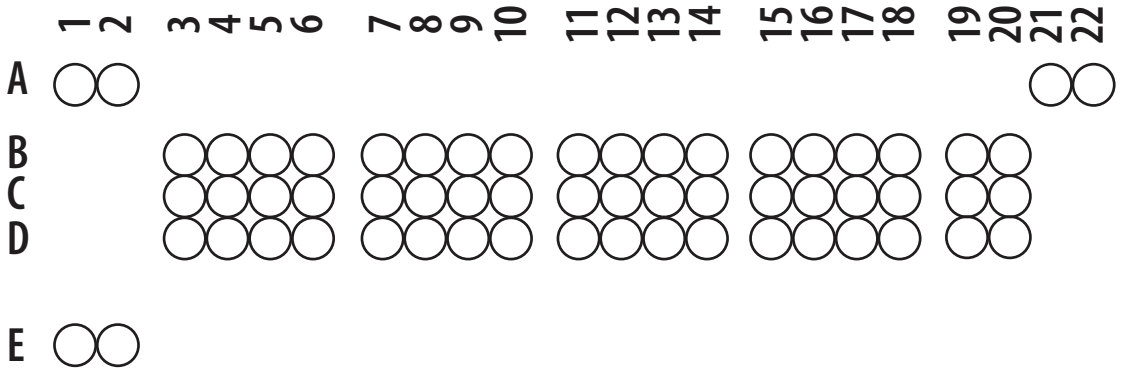
The positive signals seen on developed film can be quickly identified by placing the transparency overlay template on the array image and aligning it with the pairs of reference spots in three corners of each array. The stamped identification number on the array should be placed on the left hand side. 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 cell stress related protein.
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 cell stress related protein levels between samples.

Human Cell Stress Array Coordinates



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

PROFILING PROTEINS IN CELL LYSATES

The Human Cell Stress Array detects multiple proteins in cell lysates. Cells were untreated or treated as indicated below. 300 µg of cell lysate was run on each array. Data shown are from a 5 minute exposure to X-ray film.

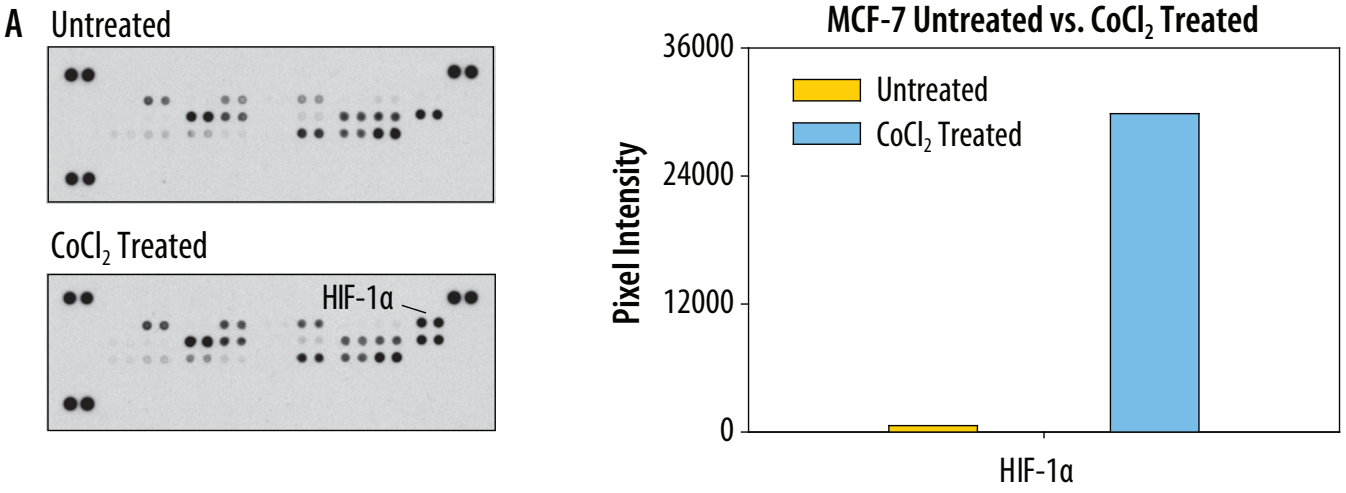


Figure 1A: MCF-7 human breast cancer cells were either untreated or treated with 150 mM $CoCl_2$ for 6 hours.

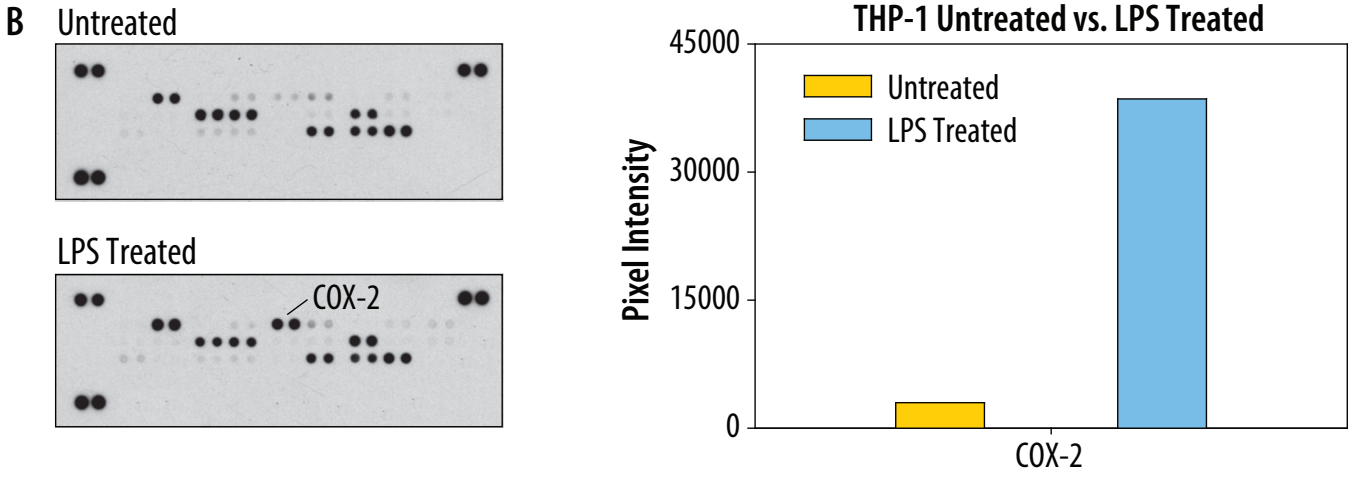


Figure 1B: THP-1 human monocytic leukemia cells were either untreated or treated with 1 µg/mL lipopolysaccharide (LPS) for 24 hours.

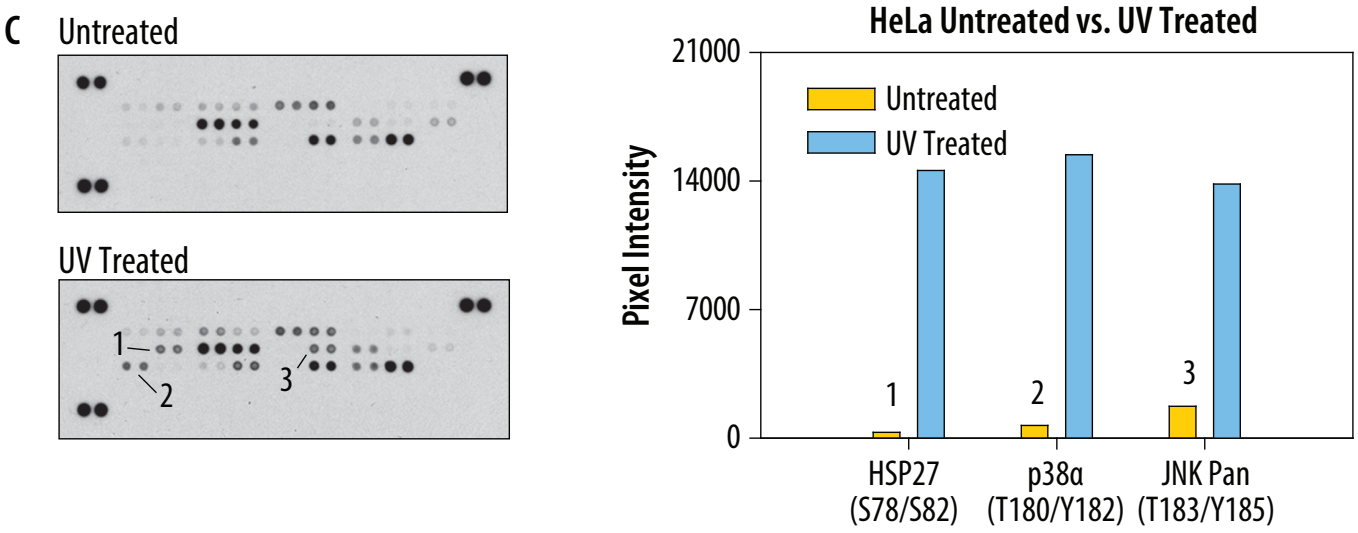


Figure 1C: HeLa human cervical epithelial carcinoma cells were either untreated or exposed to 150 J/m^2 of UV light followed by a 30 minute recovery period before lysis.

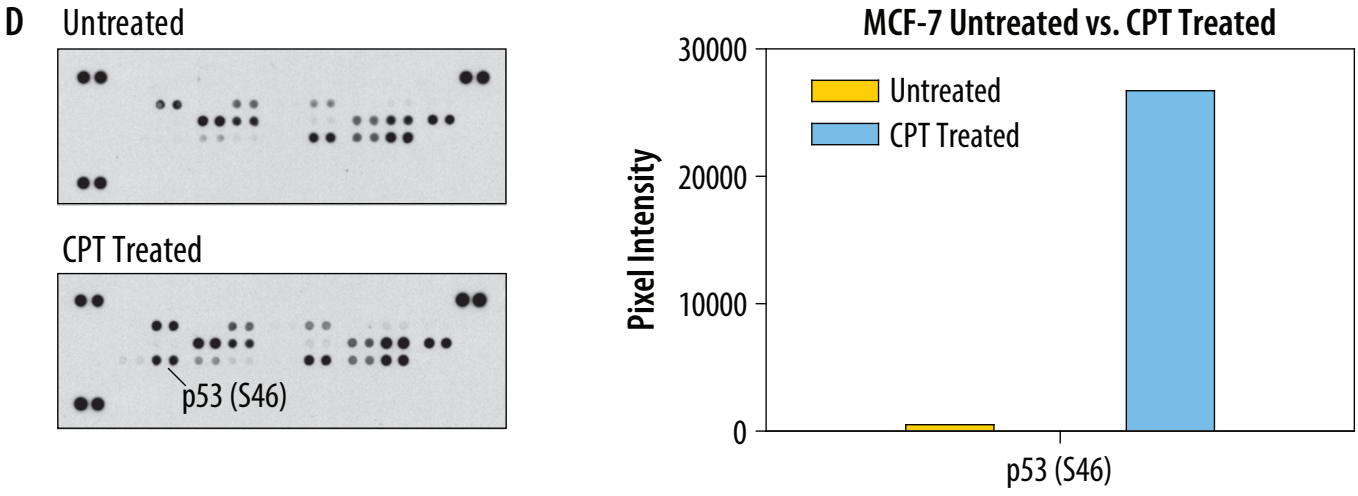


Figure 1D: MCF-7 human breast cancer cells were either untreated or treated with 1 µM camptothecin (CPT) for 4 hours.

PROFILING PROTEINS IN TISSUE LYSATES

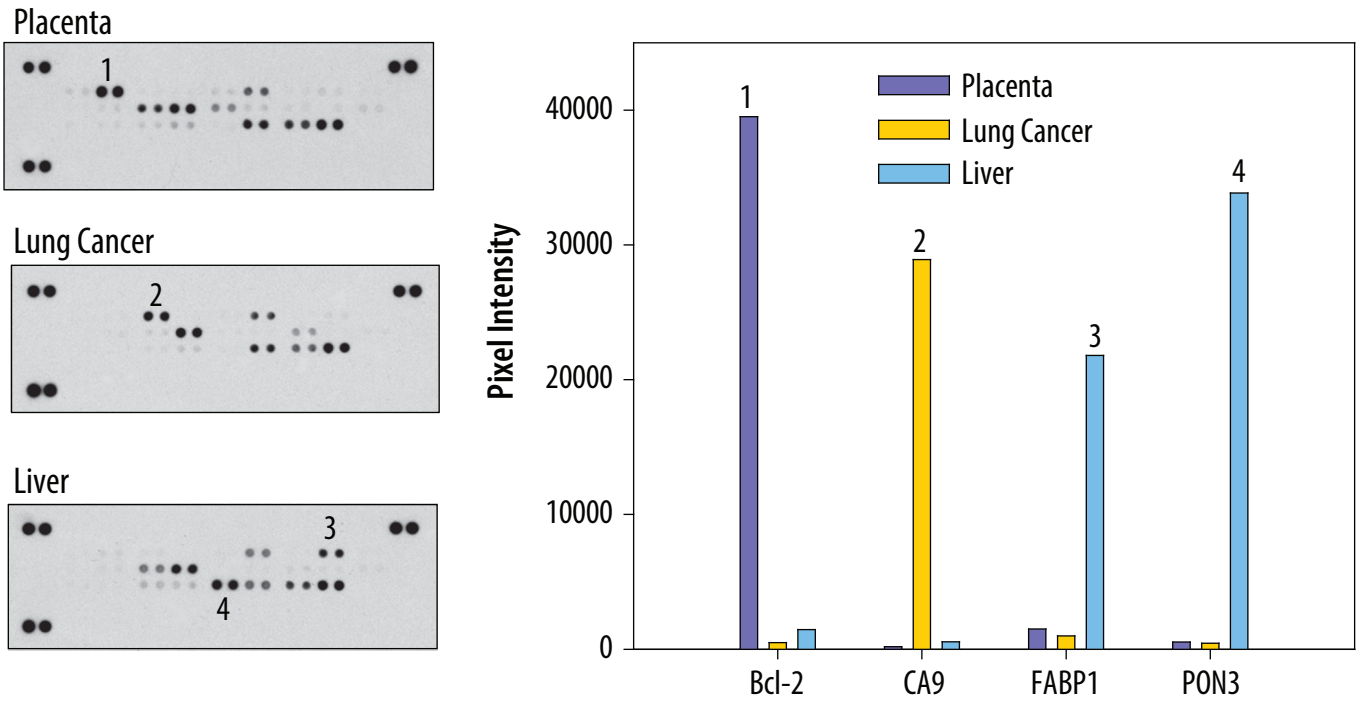


Figure 2: The Human Cell Stress Array detects multiple analytes in tissue lysates.
300 µg of tissue lysate was run on each array. Data shown are from a 5 minute exposure to X-ray film.

APPENDIX

Refer to the table below for the Human Cell Stress Array coordinates.

Coordinate	Analyte/Control	Alternate Nomenclature
A1, A2	Reference Spots	——
A21, A22	Reference Spots	——
B3, B4	ADAMTS1	——
B5, B6	Bcl-2	——
B7, B8	Carbonic Anhydrase IX	CA9
B9, B10	Cited-2	——
B11, B12	COX-2	——
B13, B14	Cytochrome c	——
B15, B16	Dkk-4	——
B17, B18	FABP-1	L-FABP
B19, B20	HIF-1α	——
C3, C4	HIF-2α	EPAS1
C5, C6	Phospho-HSP27 (S78/S82)	——
C7, C8	HSP60	——
C9, C10	HSP70	——
C11, C12	IDO	Indoleamine 2,3-dioxygenase
C13, C14	Phospho-JNK Pan (T183/Y185)	——
C15, C16	NFκB1	——
C17, C18	p21/CIP1	CDNK1A
C19, C20	p27	Kip1
D3, D4	Phospho-p38α (T180/Y182)	——
D5, D6	Phospho-p53 (S46)	——
D7, D8	PON1	——
D9, D10	PON2	——
D11, D12	PON3	——
D13, D14	Thioredoxin-1	——
D15, D16	SIRT2	Sirtuin 2
D17, D18	SOD2	Mn-SOD
D19, D20	Negative Control	Control (-)
E1, E2	Reference Spots	——

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