Proteome Profiler™ Array

Human Apoptosis Array Kit

Catalog Number ARY009

For the parallel determination of the relative levels of human apoptosis-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 profiles of apoptosis-related proteins is helpful for understanding the roles these signaling molecules play in mechanisms related to programmed cell death and disease states. The Human Apoptosis Array is a rapid, sensitive, and economical tool to simultaneously detect the relative levels of expression of 35 apoptosis-related proteins without performing numerous immunoprecipitations and Western Blots. Each capture antibody was carefully selected using cellular extracts prepared from cell lines known to express the target protein.

PRINCIPLE OF THE ASSAY

Capture and control antibodies have been spotted in duplicate on nitrocellulose membranes. Cell lysates are diluted and incubated overnight with the Human Apoptosis Array. The array is washed to remove unbound proteins, followed by incubation with a cocktail of biotinylated detection antibodies. 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 Apoptosis 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, please visit: www.RnDSystems.com/ProteomeProfilerVideo.

PRECAUTIONS

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 SDS have been read and understood.

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.

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 Apoptosis Array	893900	4 nitrocellulose membranes each containing 35 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 1	895477	21 mL of a buffered protein base with preservatives.	May be stored for up to 3 months at 2-8 °C.*
Array Buffer 2 5X Concentrate	895478	21 mL of a buffered protein base with preservatives.	
Array Buffer 3	895008	21 mL of a buffered protein base with preservatives.	
Lysis Buffer 17	895943	21 mL of a non-denaturing buffered solution with preservatives.	
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 Apoptosis Array	893333	1 vial of biotinylated antibody cocktail; lyophilized.	
Streptavidin-HRP	893019	200 µL of streptavidin conjugated to horseradish-peroxidase.	
Chemi Reagent 1	894287	2.5 mL of stabilized hydrogen peroxide with preservative.	
Chemi Reagent 2	894288	2.5 mL of stabilized luminol with preservative.	
4-Well Multi-dish	607544	Clear 4-well rectangular multi-dish.	Store at room temperature.
Transparency Overlay Template	607593	1 transparency overlay template for coordinate reference.	

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

OTHER SUPPLIES REQUIRED

- Aprotinin (Tocris®, Catalog # 4139)
- Leupeptin (Tocris®, Catalog # 1167)
- Pepstatin (Tocris®, Catalog # 1190)
- 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 wrap
- Plastic transparent sheet protector (trimmed to 10 cm x 12 cm and open on three sides)
- 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 Apoptosis 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. The suggested starting range for cell lysates is 200-400 µg.*

Cell Lysates - Rinse cells with PBS and remove any remaining PBS before adding lysis buffer. Solubilize the cells at 1 x 10^7 cells/mL in Lysis Buffer 17. 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. The maximum allowable lysate volume is 250 µL/array. Cell lysates should be used immediately or aliquoted and stored at ≤ -70 °C. Avoid repeated freeze-thaw cycles. Thawed lysates should be kept on ice prior to use.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Human Apoptosis Array - Four nitrocellulose membranes each containing 35 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 Apoptosis Detection Antibody Cocktail in 100 µL of deionized or distilled water.

Lysis Buffer 17 - Add 10 µg/mL Aprotinin, 10 µg/mL Leupeptin, and 10 µg/mL Pepstatin to the volume of Lysis Buffer 17 required for cell lysate preparation. **Prepare fresh for each use.**

1X Array Buffer 2/3 - Dilute 2.0 mL of 5X Array Buffer 2 Concentrate into 8.0 mL of Array Buffer 3. **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 completey 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. 1.0 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 2.0 mL of Array Buffer 1 into each well of the 4-Well Multi-dish which will be used. Array Buffer 1 serves as a block buffer.
3. Using flat-tip tweezers, remove each array to be used from between the protective sheets.
4. Place one array into each well of the 4-Well Multi-dish and place the lid on the 4-Well Multi-dish. The array number should be facing upward.

   Note: *Upon contact with Array Buffer 1, the blue dye will disappear from the spots. The capture antibodies are retained in their specific locations.*
5. Incubate for 1 hour on a rocking platform shaker. Orient the tray so that each array rocks end to end in its well.
6. While arrays are blocking, prepare samples by adding the desired quantity of lysate to 1.25 mL of Array Buffer 1. Adjust to a final volume of 1.5 mL with Lysis Buffer 17 as necessary. The maximum allowable lysate volume is 250 µL/array.
7. Aspirate Array Buffer 1 from the 4-Well Multi-dish. Add prepared samples and 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 array 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 array with 1X Wash Buffer for 10 minutes on a rocking platform shaker. Repeat two times for a total of three washes.
11. For each array, dilute 15 µL of reconstituted Detection Antibody Cocktail to 1.5 mL with 1X Array Buffer 2/3. Pipette 1.5 mL per well of diluted Detection Antibody Cocktail into the 4-Well Multi-dish.
12. Carefully remove each array from its wash container. Allow excess Wash Buffer to drain from the array. Return the array to the 4-Well Multi-dish containing the diluted Detection Antibody Cocktail, and cover with the lid.
13. Incubate for 1 hour on a rocking platform shaker.

ARRAY PROCEDURE *CONTINUED*

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

15. Dilute the Streptavidin-HRP in 1X Array Buffer 2/3 using the dilution factor on the vial label. Pipette 2.0 mL into each well of the 4-Well Multi-dish.

16. Carefully remove each membrane from the wash container. Allow excess Wash Buffer to drain from the membrane. Return the array to the 4-Well Multi-dish containing the diluted Streptavidin-HRP, and cover with the lid. Incubate for 30 minutes on a rocking platform shaker.

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

    Note: *Complete the remaining steps without interruption.*

18. 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.

19. Pipette 1.0 mL of the prepared Chemi Reagent Mix evenly onto each membrane.

    Note: *Using less than 1.0 mL of Chemi Reagent Mix per membrane may result in incomplete membrane coverage.*

20. 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.

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

22. 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.

23. 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.

24. 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.*

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

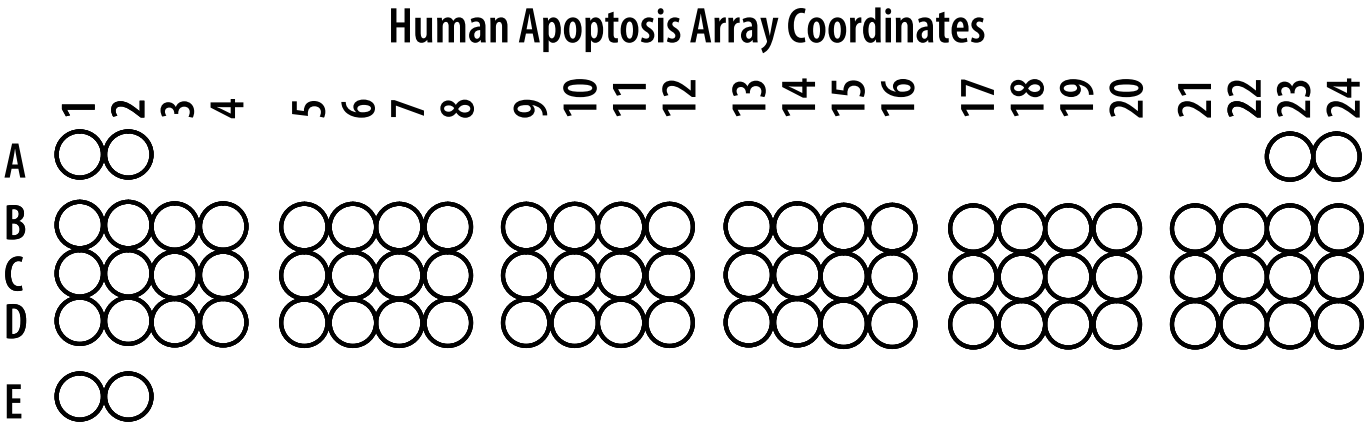
DATA ANALYSIS

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 apoptosis-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 apoptosis-related protein levels between samples.

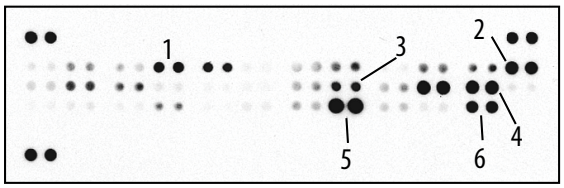


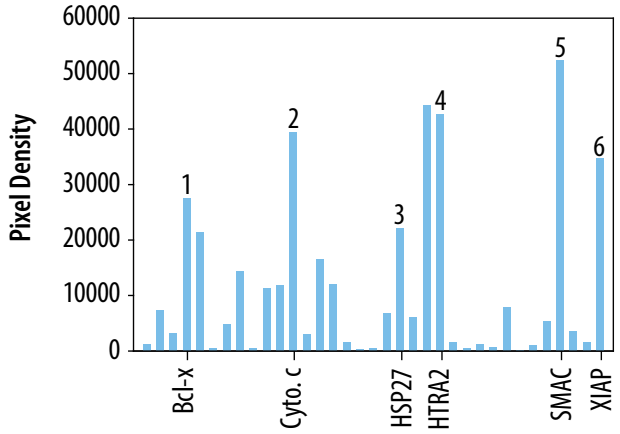
This image is not to scale. It is for coordinate reference only.
Please use the transparency overlay for analyte identification.

PROFILING APOPTOSIS PROTEINS IN CELL LYSATES

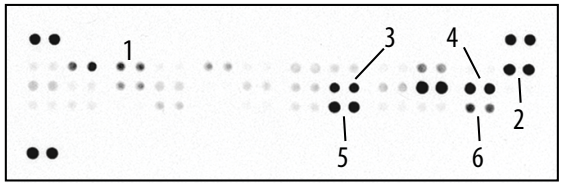
The Human Apoptosis Array detects multiple apoptosis-related proteins in cell lysates. Arrays were incubated with 400 µg of each cell lysate shown below. Data shown are from a 2 minute exposure to X-ray film.

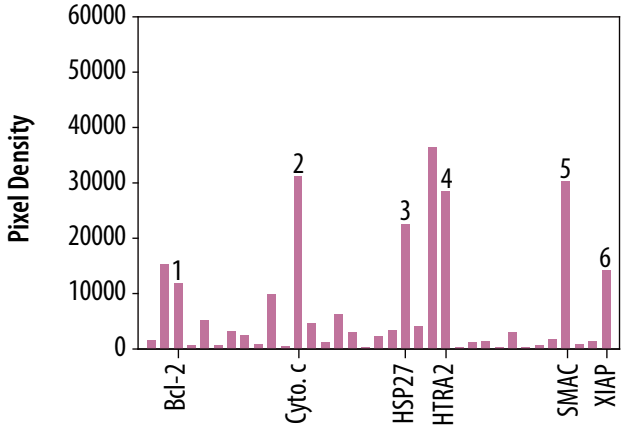
1A



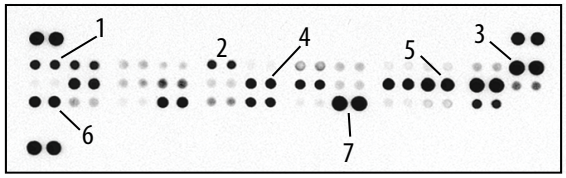


1B





1C



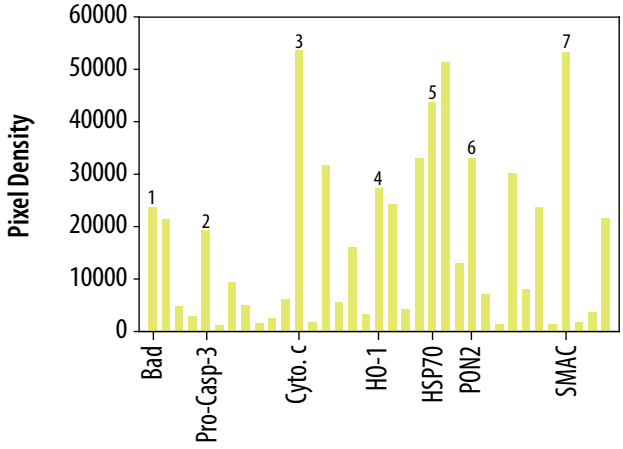


Figure 1A: K562 human chronic myelogenous leukemia cells.
Figure 1B: THP-1 human acute monocytic leukemia cells.
Figure 1C: SK-Mel-28 human malignant melanoma cells.

PROFILING APOPTOSIS PROTEINS IN TREATED AND UNTREATED SAMPLES

The Human Apoptosis Array detects multiple apoptosis-related proteins in untreated and treated cell lysates. Arrays were incubated with 400 µg of cell lysate. Data shown are from a 2 minute exposures to X-ray film.

2A

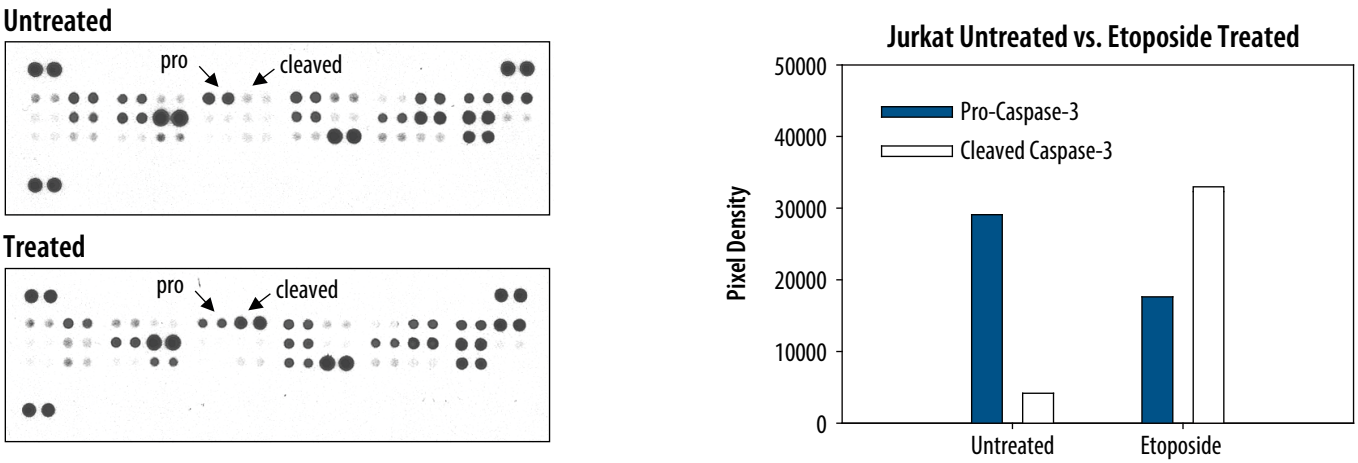


Figure 2A: Jurkat human acute T cell leukemia cells were either untreated or treated with 25 µM etoposide for 6 hours. Etoposide is a topoisomerase II inhibitor that causes caspase-3 cleavage as apoptosis is induced.

2B

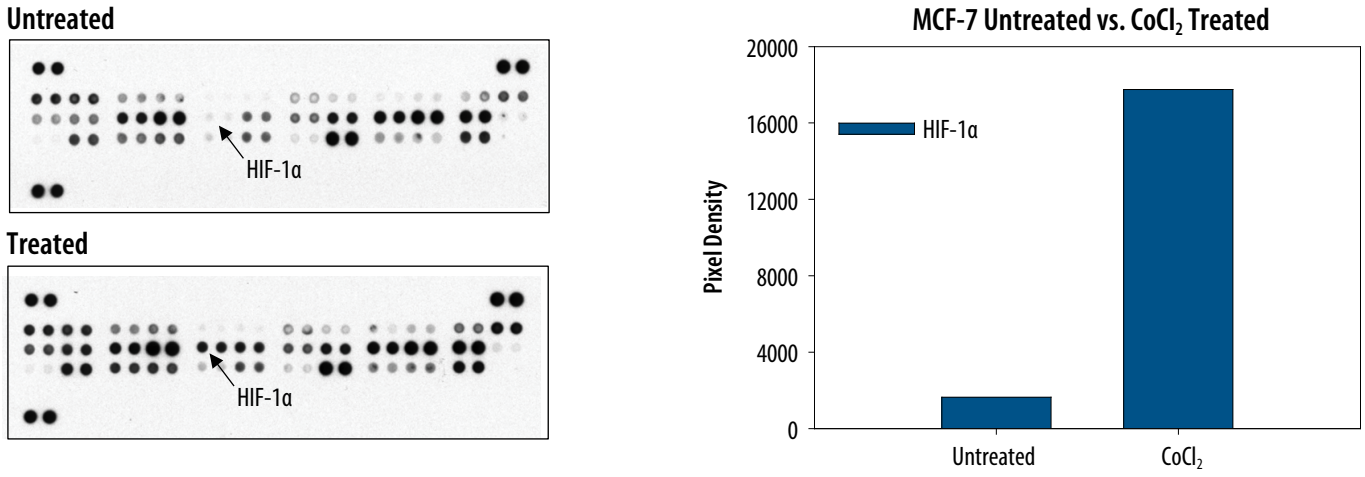


Figure 2B: MCF-7 human breast cancer cells were either untreated or treated with 150 µM $CoCl_2$ for 6 hours. $CoCl_2$ is a hypoxia mimetic that stabilizes HIF-1α expression.

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SPECIFICITY-PATHWAY INHIBITION

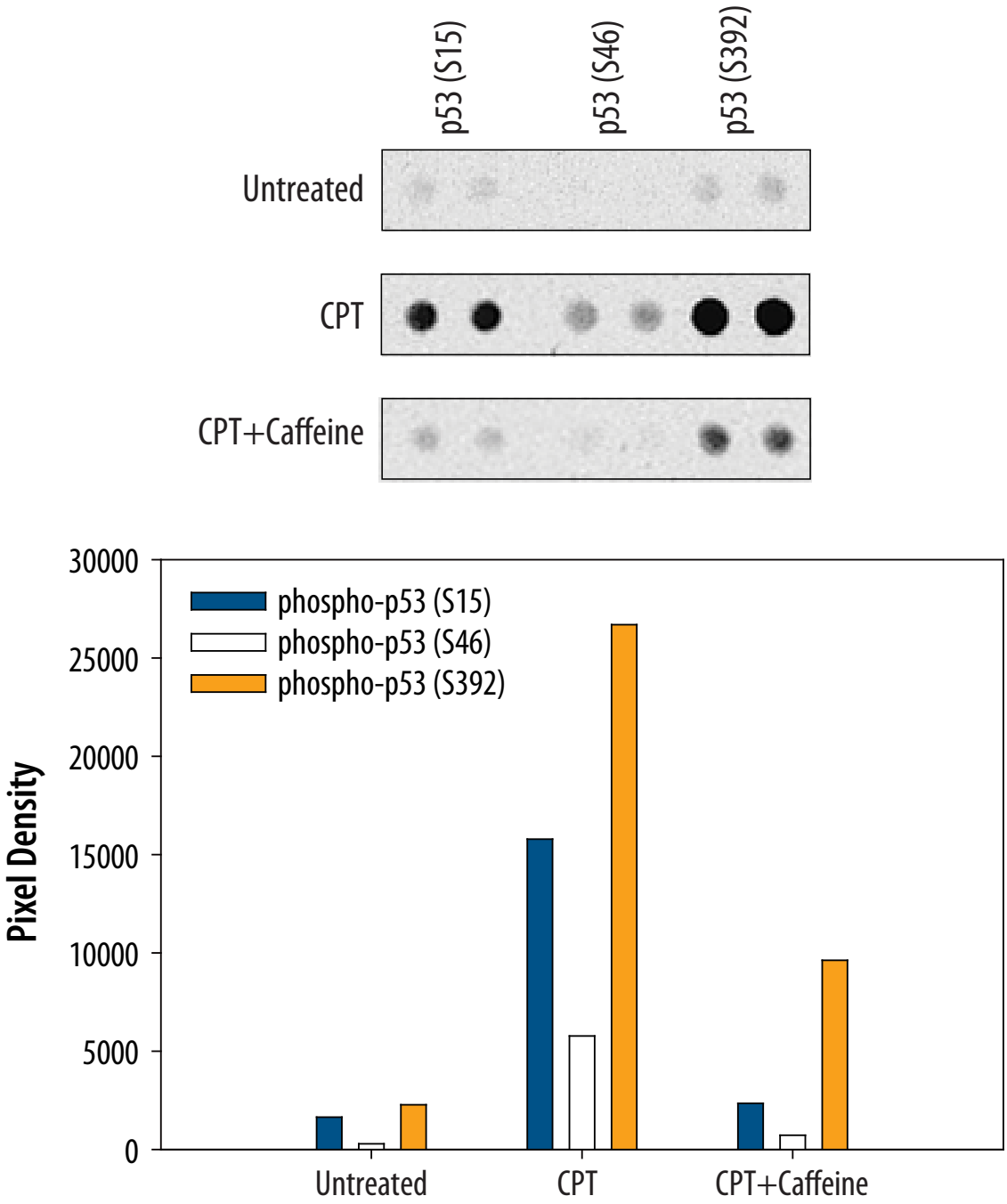

Figure 3: The Human Apoptosis Array may also be used to screen inhibitors. MCF-7 human breast cancer cells were either untreated, treated with 1 µM camptothecin (CPT) for 4 hours, or treated with 1 µM CPT and 10 mM caffeine for 4 hours. Arrays were incubated with 400 µg of each cell lysate.

Data shown are from a 1 minute exposure to X-ray film. Data analysis shows the increase of p53 phosphorylation in MCF-7 human breast cancer cells treated with CPT, a topoisomerase I inhibitor that causes DNA breaks. This response is reduced in the presence of caffeine, which inhibits the catalytic activity of ATM and ATR kinases.

APPENDIX

Refer to the table below for the Human Apoptosis Array coordinates.

Coordinate	Target/Control	Coodinate	Target/Control
A1, A2	Reference Spots	C13, C14	HO-2/HMOX2
A23, A24	Reference Spots	C15, C16	HSP27
B1, B2	Bad	C17, C18	HSP60
B3, B4	Bax	C19, C20	HSP70
B5, B6	Bcl-2	C21, C22	HTRA2/Omi
B7, B8	Bcl-x	C23, C24	Livin
B9, B10	Pro-Caspase-3	D1, D2	PON2
B11, B12	Cleaved Caspase-3	D3, D4	p21/CIP1/CDKN1A
B13, B14	Catalase	D5, D6	p27/Kip1
B15, B16	cIAP-1	D7, D8	Phospho-p53 (S15)
B17, B18	cIAP-2	D9, D10	Phospho-p53 (S46)
B19, B20	Claspin	D11, D12	Phospho-p53 (S392)
B21, B22	Clusterin	D13, D14	Phospho-Rad17 (S635)
B23, B24	Cytochrome c	D15, D16	SMAC/Diablo
C1, C2	TRAIL R1/DR4	D17, D18	Survivin
C3, C4	TRAIL R2/DR5	D19, D20	TNF RI/TNFRSF1A
C5, C6	FADD	D21, D22	XIAP
C7, C8	Fas/TNFRSF6/CD95	D23, D24	PBS (Negative Control)
C9, C10	HIF-1α	E1, E2	Reference Spots
C11, C12	HO-1/HMOX1/HSP32		

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