Proteome Profiler™ Array

Human Cytokine Array Panel A

Catalog Number ARY005

For the parallel determination of the relative levels of selected human cytokines and chemokines.

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

Cytokines and chemokines are extracellular signaling molecules that mediate cell-cell communication. They are released from cells and have critical roles in many biological processes such as cellular growth, differentiation, gene expression, migration, immunity and inflammation. In most biological processes, multiple cytokines operate in a large network, where the action of one cytokine is regulated by the presence or absence of other cytokines. The Human Cytokine Array is a rapid, sensitive, and economic tool to simultaneously detect cytokine differences between samples. The relative expression levels of 36 human cytokines can be determined without performing numerous immunoassays.

PRINCIPLE OF THE ASSAY

Carefully selected capture antibodies have been spotted in duplicate on nitrocellulose membranes. Cell culture supernates, lysates, serum, or plasma samples are diluted and mixed with a cocktail of biotinylated detection antibodies. The sample/antibody mixture is then incubated with the Human Cytokine Array Panel A membrane. Any cytokine/detection antibody complex present is bound by its cognate immobilized capture antibody on the membrane. Following a wash to remove unbound material, Streptavidin-HRP and chemiluminescent detection reagents are added sequentially. Light is produced at each spot in proportion to the amount of cytokine 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 Cytokine Array Panel A 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.
- Soluble receptors and other proteins present in biological samples do not necessarily interfere with the measurement of cytokines in samples. Until these proteins have been tested with the Human Cytokine Array Panel A, the possibility of interference cannot be excluded.
- For a procedure demonstration video, please 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 Cytokine Array Panel A	893194	4 nitrocellulose membranes each containing 36 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 be fore and during use.*	May be stored for up to 3 months at 2-8 °C.*
Array Buffer 5	895876	21 mL of a buffered protein base 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 Cytokine Array Panel A	893195	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	607575	1 transparency overlay template for coordinate reference.	

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

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 MSDS have been read and understood.

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

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

OTHER SUPPLIES REQUIRED

- Aprotinin (Sigma, Catalog # A6279)
- Leupeptin (Sigma, Catalog # L8511)
- Pepstatin (Sigma, Catalog # P4265)
- Igepal® CA-630 (Sigma, Catalog # I3021)
- Pipettes and pipette tips
- Gloves
- 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
- Absorbent lab wipes (KimWipes® or equivalent)
- Paper towels
- Autoradiography cassette
- Film developer
- X-ray film (Kodak® BioMax™ Light-1, Catalog # 1788207) or equivalent
- Flat-tipped tweezers
- Flatbed scanner with transparency adapter capable of transmission mode
- Computer capable of running image analysis software and Microsoft® Excel

OTHER SUPPLIES REQUIRED FOR CELL LYSATE SAMPLES

- Phosphate-Buffered Saline (PBS)
- Lysis buffer (1% Igepal CA-630, 20 mM Tris-HCl (pH 8.0), 137 mM NaCl, 10% glycerol, 2 mM EDTA, 10 µg/mL Aprotinin, 10 µg/mL Leupeptin, and 10 µg/mL Pepstatin)

OTHER SUPPLIES REQUIRED FOR TISSUE LYSATE SAMPLES

- PBS with protease inhibitors (10 µg/mL Aprotinin, 10 µg/mL Leupeptin, and 10 µg/mL Pepstatin)
- Triton™ X-100 (Sigma, Catalog # 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 Cytokine Array Panel A 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 ranges are 200-700 µL for cell culture supernates, 100-300 µg for cell and tissue lysates, and 50-200 µL for serum and plasma samples.*

Cell Culture Supernates - Remove particulates by centrifugation. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

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. 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 ≤ -70 °C. Thawed lysates should be kept on ice prior to use. 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 concentrations using a total protein assay is recommended. Assay immediately or aliquot and store samples at ≤ -70 °C. Thawed lysates should be kept on ice prior to use. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 30 minutes at room temperature before centrifuging for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at approximately 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Human Cytokine Array Panel A - Four nitrocellulose membranes each containing 36 different anti-cytokine antibodies printed in duplicate. **Handle arrays only with gloved hands and flat-tipped tweezers.**

Detection Antibody Cocktail - One vial of lyophilized biotinylated antibodies. Before use, reconstitute 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 completey dissolved. Add 40 mL of 25X Wash Buffer Concentrate to 960 mL of deionized or distilled water to prepare 1000 mL of 1X Wash Buffer.

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 per membrane.** Discard any remaining after use.

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 4 into each well of the 4-Well Multi-dish to be used. Array Buffer 4 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 array number should be facing upward.

   Note: *Upon contact with Array Buffer 4, 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 array 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 5 as necessary.
6. Add 15 µL of reconstituted Human Cytokine Array Panel A Detection Antibody Cocktail to each prepared sample. Mix and incubate at room temperature for one hour.
7. Aspirate Array Buffer 4 from the wells of the 4-Well Multi-dish and add sample/antibody mixtures prepared in steps 5 and 6. Place the lid on the 4-Well Multi-dish.
8. Incubate overnight at 2-8 °C on a rocking platform.

   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 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 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 the 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.

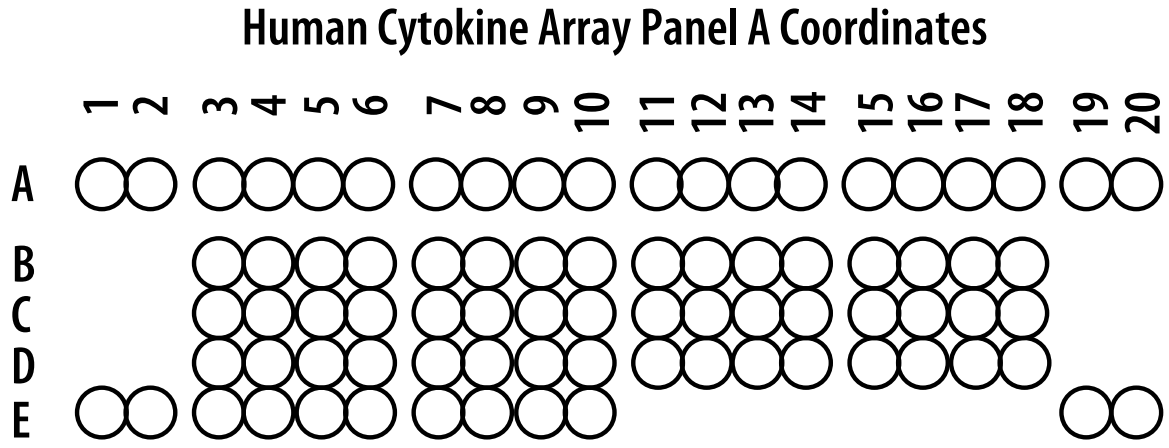
DATA ANALYSIS

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 cytokine 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 cytokine.
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 cytokine levels between samples.



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

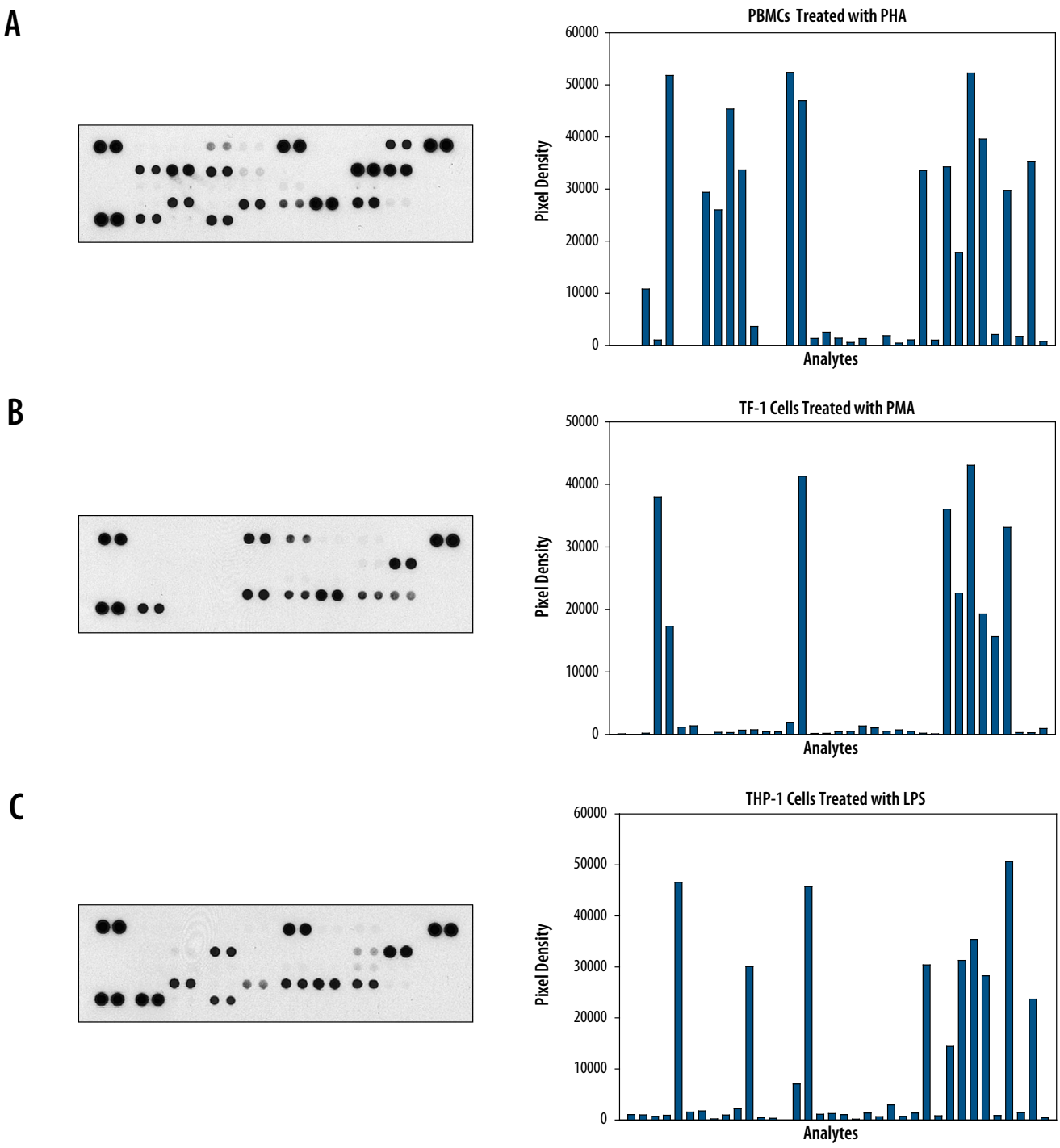
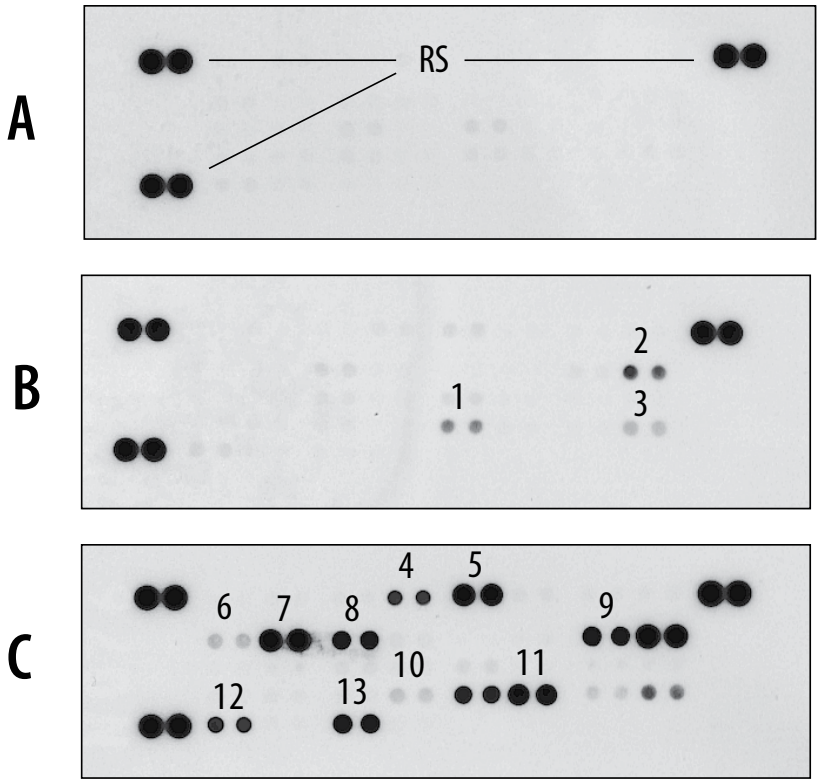


Figure 1: The Human Cytokine Array detects multiple analytes in cell culture supernates. Cells were treated as below. 500 µL of cell culture supernate was run on each array. Data shown are from a one minute exposure to X-ray film.

A. PBMCs treated for 24 hours with 10 µg/mL PHA.

B. TF-1 human erythroleukemic cells treated for 24 hours with 50 ng/mL PMA.

C. THP-1 human acute monocytic leukemia cells treated for 16 hours with 1 µg/mL LPS.



RS	Reference Spot
1	MIF
2	IL-8
3	Serpin E1
4	GM-CSF
5	GROα
6	IL-1α
7	IL-1β
8	IL-1ra
9	IL-6
10	MCP-1
11	MIP-1α
12	RANTES
13	TNF-α

Figure 2: The Human Cytokine Array detects multiple analytes in cell culture supernates. Cells were treated as below. 500 µL of cell culture supernate was run on each array. Data shown are from a one minute exposure to X-ray film.

A. Media only, RPMI 1640, 10% FBS.

B. Untreated PBMCs.

C. PBMCs treated for 24 hours with 50 ng/mL PMA.

PROFILING CYTOKINES IN SERUM SAMPLES

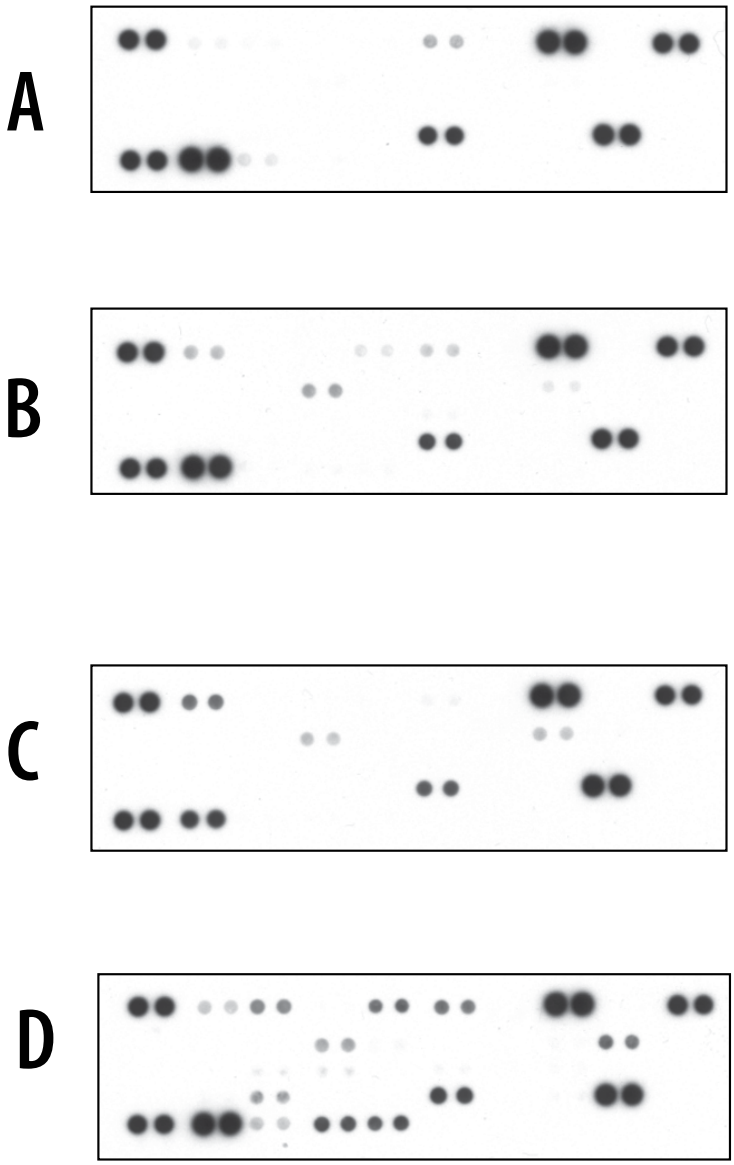


Figure 3: The Human Cytokine Array detects multiple analytes in human serum samples. 250 µL of human serum from 4 donors (A-D) was run on each array. Images are from five minute exposures to X-ray film.

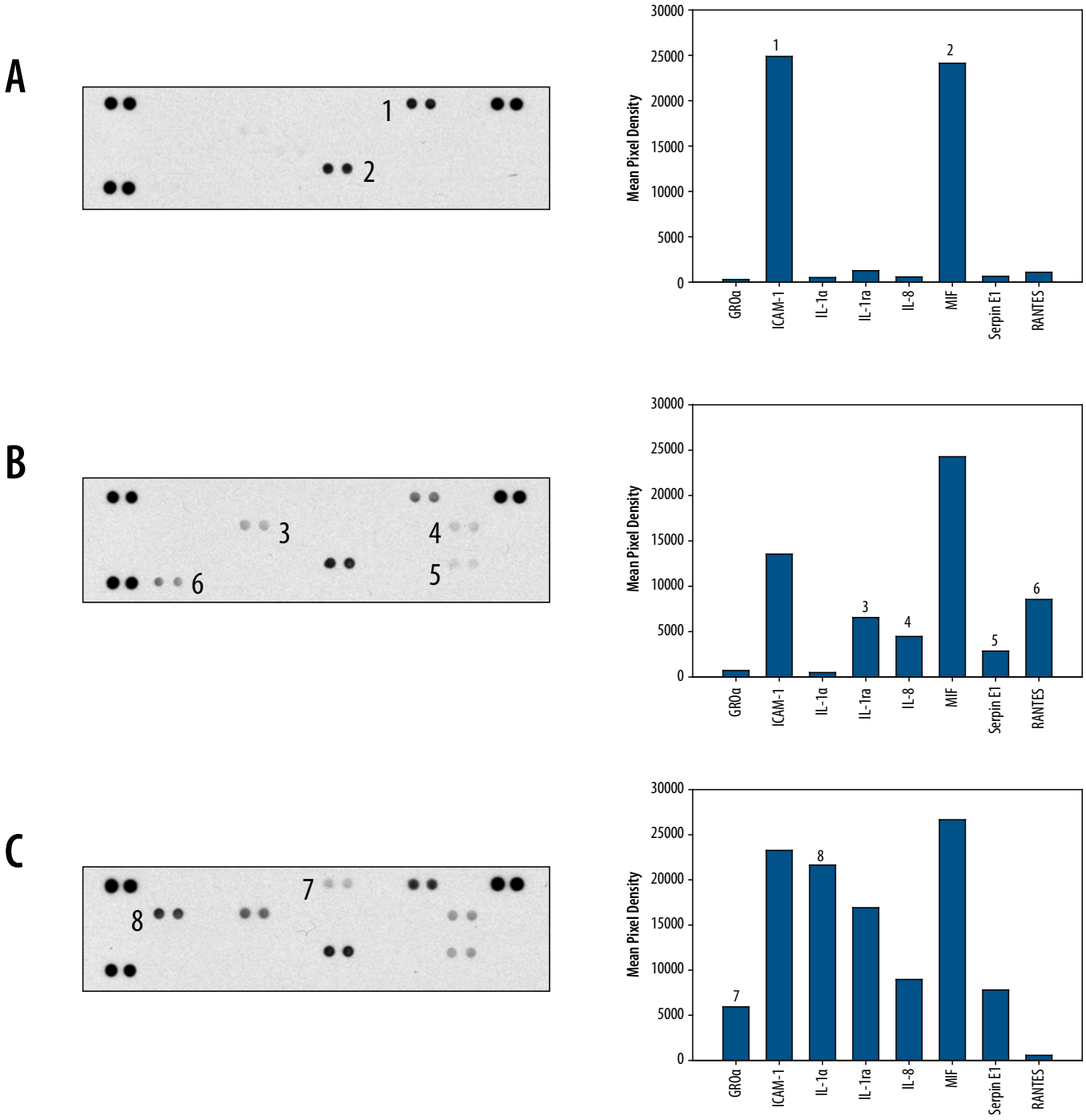


Figure 4: The Human Cytokine Array detects multiple analytes in tissue and cell lysates. 200 µg of tissue or cell lysate was run on each array. Data shown are from a ten minute exposure to X-ray film.

A. Lung tissue (normal)

B. Lung tissue (malignant)

C. A431 human epithelial carcinoma cells

APPENDIX

Refer to the table below for the Human Cytokine Array Panel A coordinates.

Coordinate	Target/Control	Alternate Nomenclature
A1, A2	Reference Spot	——
A3, A4	C5/C5a	Complement Component 5/5a
A5, A6	CD40 Ligand	CD154
A7, A8	G-CSF	CSFβ, CSF-3
A9, A10	GM-CSF	CSFα, CSF-2
A11, A12	GROα	CXCL1
A13, A14	I-309	CCL1
A15, A16	sICAM-1	CD54
A17, A18	IFN-γ	Type II IFN
A19, A20	Reference Spot	——
B3, B4	IL-1α	IL-1F1
B5, B6	IL-1β	IL-1F2
B7, B8	IL-1ra	IL-1F3
B9, B10	IL-2	——
B11, B12	IL-4	——
B13, B14	IL-5	——
B15, B16	IL-6	——
B17, B18	IL-8	CXCL8
C3, C4	IL-10	——
C5, C6	IL-12 p70	——
C7, C8	IL-13	——
C9, C10	IL-16	LCF
C11, C12	IL-17	——
C13, C14	IL-17E	——
C15, C16	IL-23	——
C17, C18	IL-27	——

continued on next page...

APPENDIX *CONTINUED*

Coordinate	Target/Control	Alternate Nomenclature
D3, D4	IL-32α	——
D5, D6	IP-10	CXCL10
D7, D8	I-TAC	CXCL11
D9, D10	MCP-1	CCL2
D11, D12	MIF	GIF, DER6
D13, D14	MIP-1α	CCL3
D15, D16	MIP-1β	CCL4
D17, D18	Serpin E1	PAI-1
E1, E2	Reference Spot	——
E3, E4	RANTES	CCL5
E5, E6	SDF-1	CXCL12
E7, E8	TNF-α	TNFSF1A
E9, E10	sTREM-1	——
E19, E20	Negative Control	——