Proteome Profiler™ Array

Human Chemokine Array Kit

Catalog Number ARY017

For the parallel determination of the relative levels of selected human 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

Analyzing the expression profile of chemokines is essential for understanding their roles in regulating migration of monocytes, neutrophils, and lymphocytes. The Human Chemokine Array Kit is a rapid, sensitive, and economical tool to simultaneously detect chemokine differences between samples. The relative expression levels of 31 human chemokines can be determined without performing numerous immunoprecipitations or Western blots. Each capture and detection antibody was carefully selected using both natural and recombinant proteins.

PRINCIPLE OF THE ASSAY

Capture and control antibodies have been spotted in duplicate on nitrocellulose membranes. Cell culture supernates, cell lysates, serum, plasma, urine, saliva, or tissue lysates are diluted, mixed with a cocktail of biotinylated detection antibodies, and incubated overnight with the Proteome Profiler Human Chemokine Array Kit. 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 Chemokine 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.
- Soluble receptors and other proteins present in biological samples do not necessarily interfere with the measurement of analytes in samples. Until these proteins have been tested with the Human Chemokine Array, 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 Chemokine Array	894153	4 nitrocellulose membranes each containing 31 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.	
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 Chemokine Array	894154	1 vial of a 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 Rectangular Multi-dish	607544	Clear 4-well rectangular multi-dish.	Store at room temperature.
Transparency Overlay Template	607753	1 transparency overlay template for coordinate reference.	

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

OTHER SUPPLIES REQUIRED

- Aprotinin (Sigma, Catalog # A6279)
- Leupeptin (Tocris, Catalog # 1167)
- Pepstatin (Tocris, Catalog # 1190)
- Igepal® CA-630 (Sigma, Catalog # I3021)
- Pipettes and pipette tips
- Gloves
- Deionized or distilled water
- 1000 mL graduated cylinder
- 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 (KimWipes® or equivalent)
- 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

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)

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 Chemokine 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 ranges are: 200-500 µL for cell culture supernates, 100-200 µg for cell and tissue lysates, and 50-200 µL for serum 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 concentration using a total protein assay is recommended. Assay immediately or aliquot and store at ≤ -70 °C. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 30 miutes 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.

Urine - Collect urine and centrifuge to remove particulate matter. Assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Saliva - Collect saliva in a tube and centrifuge for 5 minutes at 10,000 x g. Collect the aqueous layer, and assay immediately or aliquot and store samples at ≤ -20 °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.

Note: *High levels of some proteins are found in saliva. It is recommended that a mask and gloves be used to protect kit reagents from contamination.*

Human Chemokine Array - Four nitrocellulose membranes each containing 31 different capture antibodies and 3 sample controls 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 Chemokine 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. Add 40 mL of Wash Buffer Concentrate to 960 mL of deionized or distilled water to prepare 1000 mL of 1X Wash Buffer.

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.**

PRECAUTIONS

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

High levels of some proteins are found in saliva. It is recommended that a mask and gloves be used to protect kit reagents from contamination.

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.

ARRAY PROCEDURE

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

Note: *High levels of some proteins are found in saliva. It is recommended that a mask and gloves be used to protect kit reagents from contamination.*

1. Prepare all reagents and samples as directed in the previous sections.
2. Pipette 2.0 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 15 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 2.0 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.

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

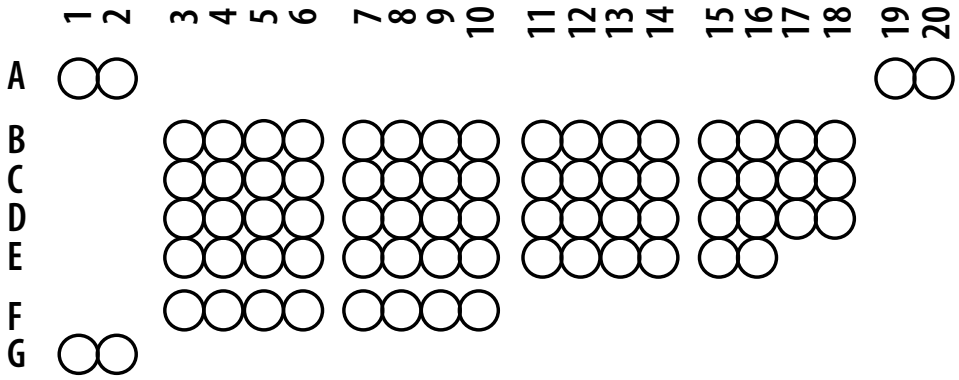
The positive signals seen on developed film can be quickly identified by placing the transparency overlay template on the array image and aligning it with the pairs of reference spots in three corners of each array. The stamped identification number on the array should be placed on the left hand side.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

Human Chemokine 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 CULTURE SUPERNATES

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

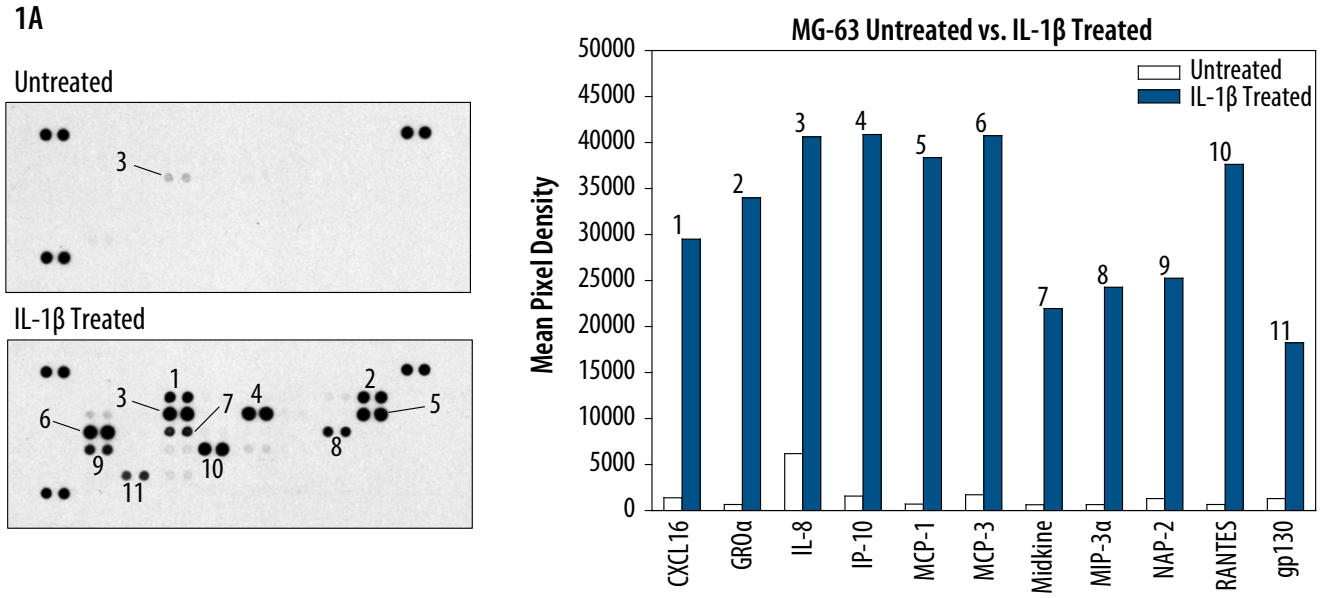


Figure 1A: MG-63 human osteosarcoma cells were untreated or treated with 1 ng/mL recombinant human IL-1β (R&D Systems, Catalog # 201-LB) for 72 hours.

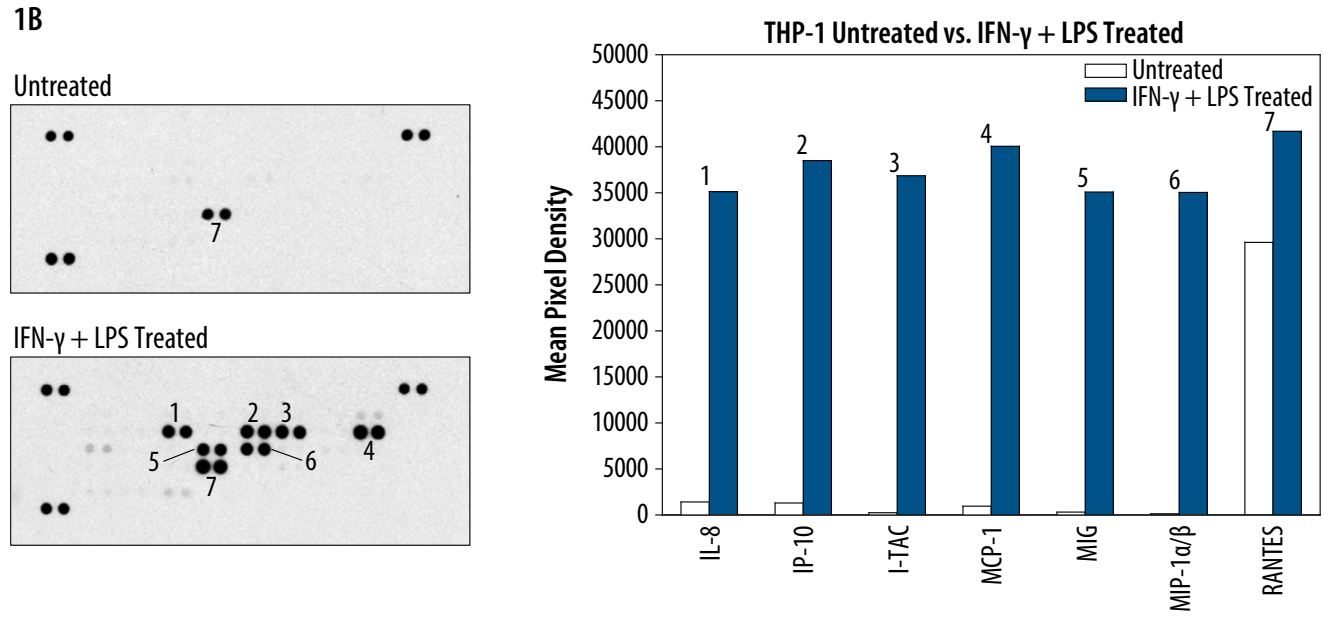


Figure 1B: THP-1 human acute monocytic leukemia cells were untreated or treated with 1 µg/mL recombinant human IFN-γ (R&D Systems, Catalog # 285-IF) for 8 hours, followed by the addition of 1 µg/mL LPS for 16 hours.

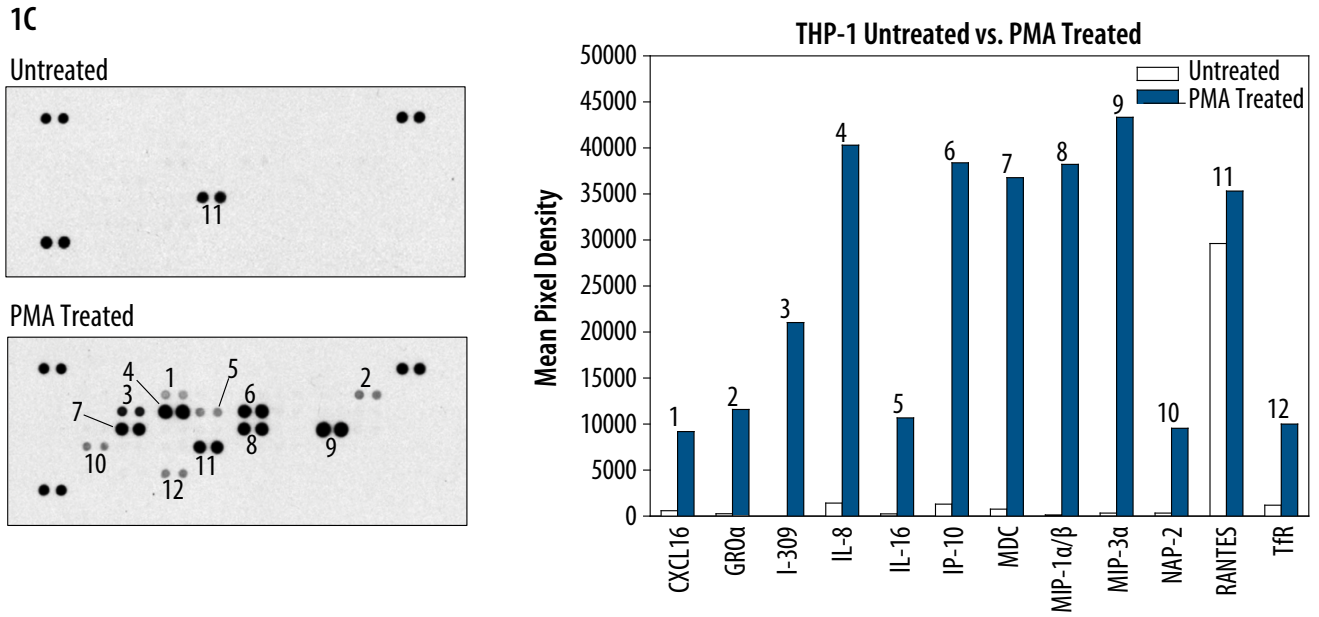


Figure 1C: THP-1 human acute monocytic leukemia cells were untreated or treated with 200 nM PMA for 24 hours.

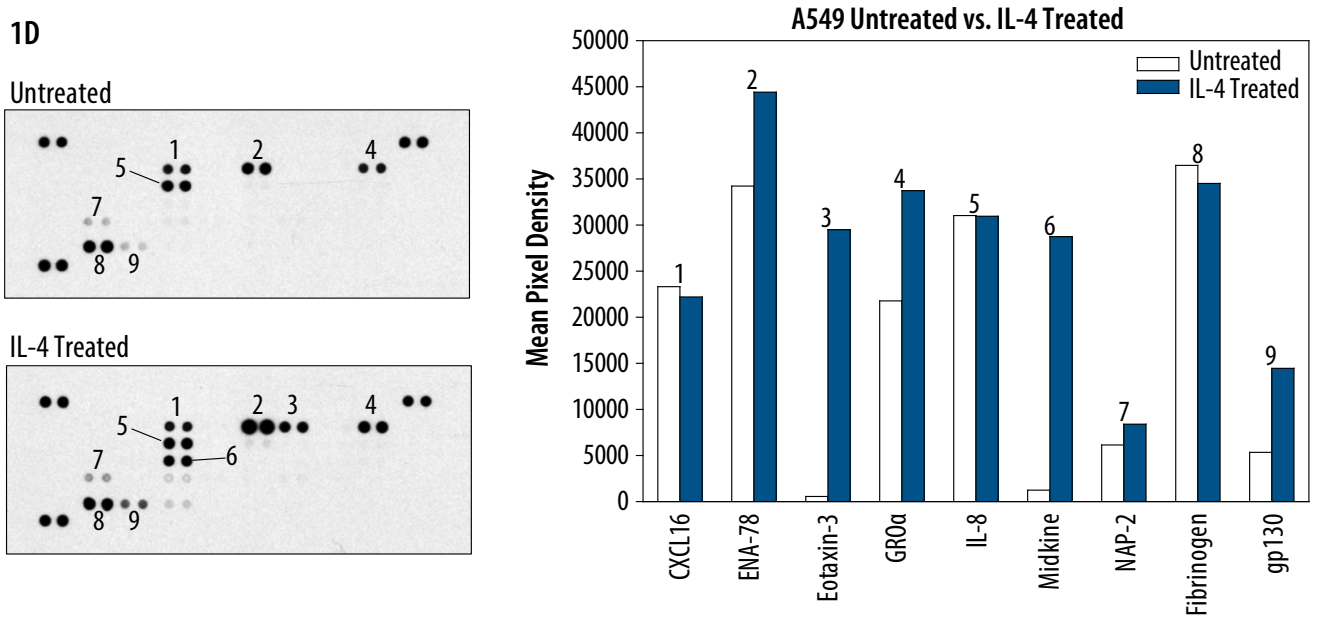


Figure 1D: A549 human lung carcinoma cells were untreated or treated with 20 ng/mL recombinant human IL-4 (R&D Systems, Catalog # 204-IL) for 72 hours.

PROFILING PROTEINS IN CELL LYSATES

The Human Chemokine Array detects multiple analytes in cell lysates. Cells were either untreated or treated as indicated below. 200 µg of cell lysate was run on each array. Data shown are from a 5 minute exposure to X-ray film.

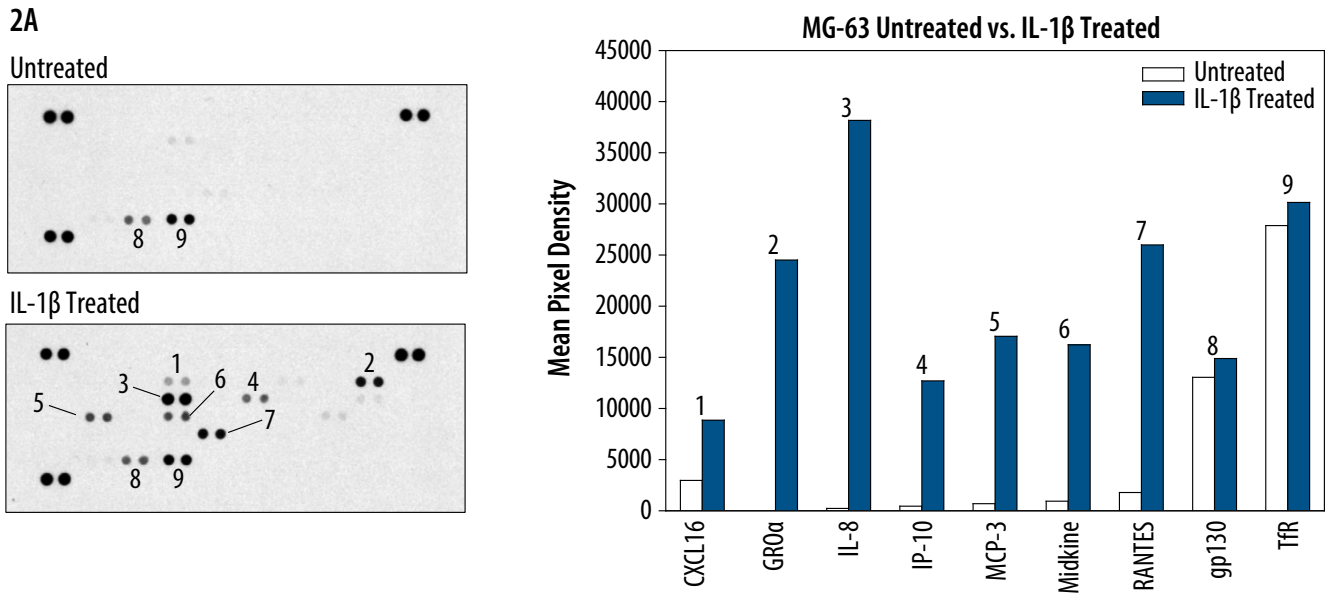


Figure 2A: MG-63 human osteosarcoma cells were untreated or treated with 1 ng/mL recombinant human IL-1β (R&D Systems, Catalog # 201-LB) for 72 hours.

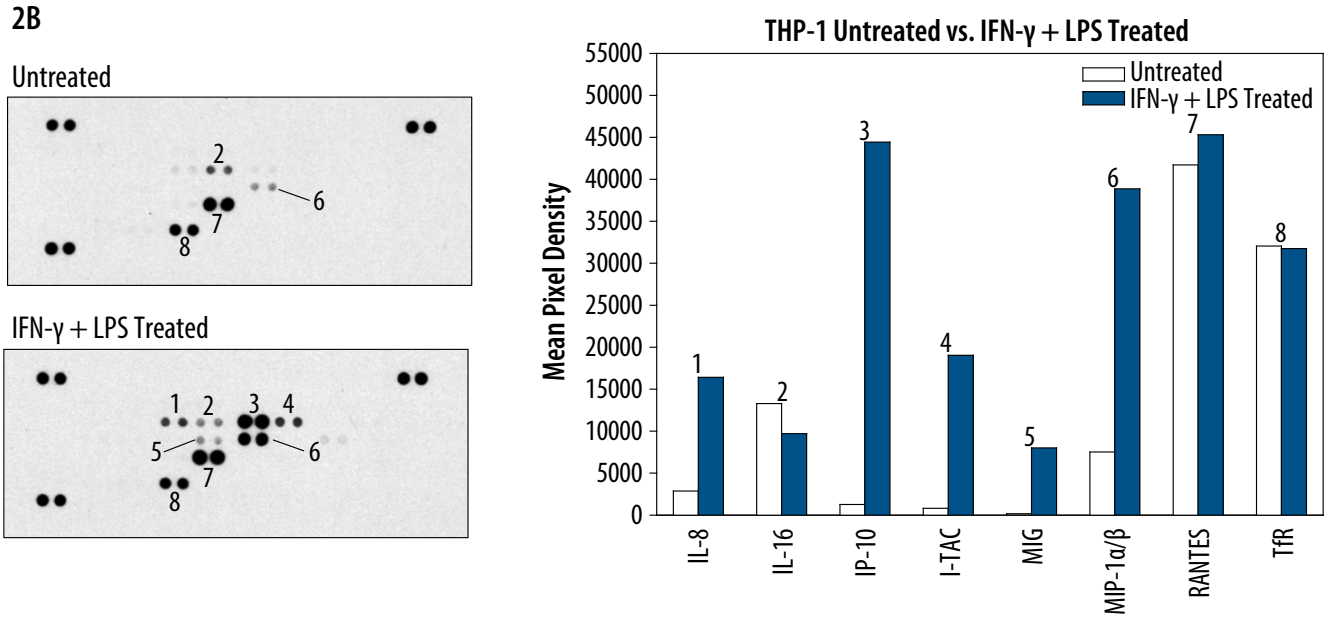


Figure 2B: THP-1 human acute monocytic leukemia cells were untreated or treated with 1 µg/mL recombinant human IFN-γ (R&D Systems, Catalog # 285-IF) for 8 hours followed by addition of 1 µg/mL LPS for 16 hours.

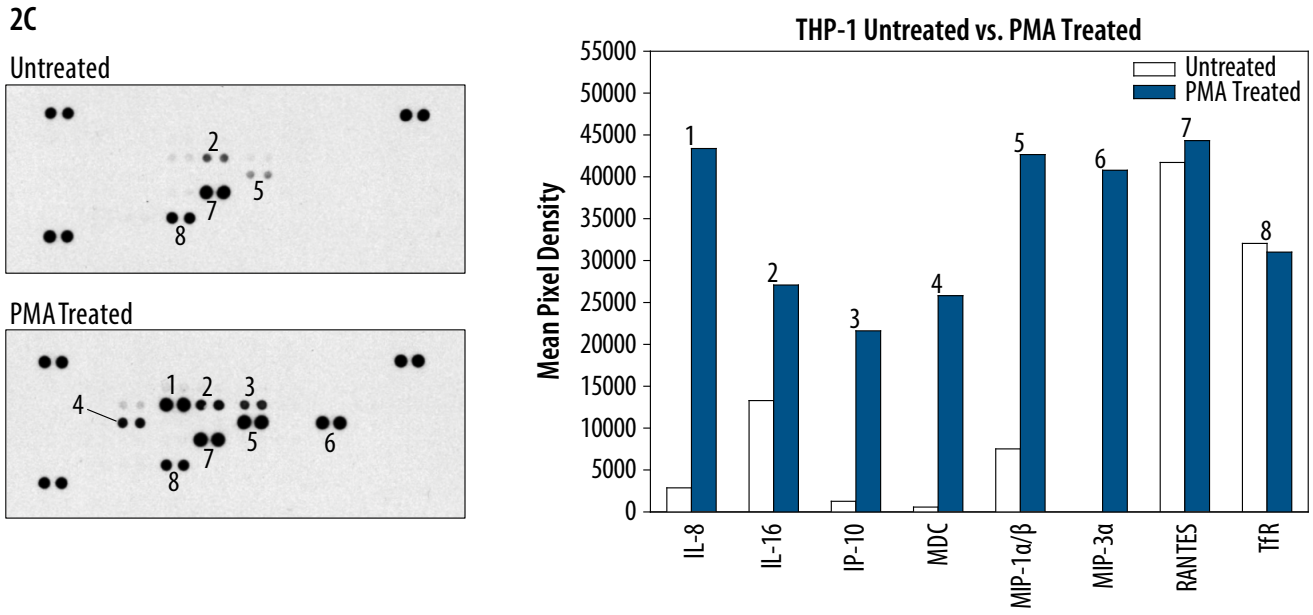


Figure 2C: THP-1 human acute monocytic leukemia cells were untreated or treated with 200 nM PMA for 24 hours.

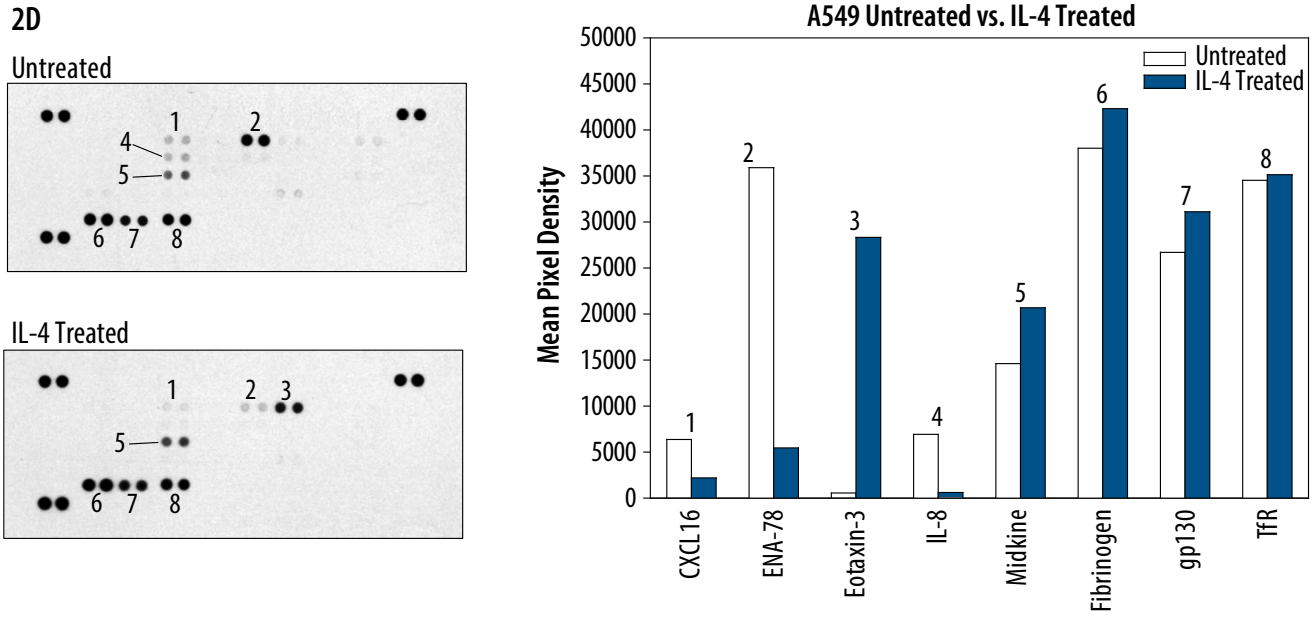
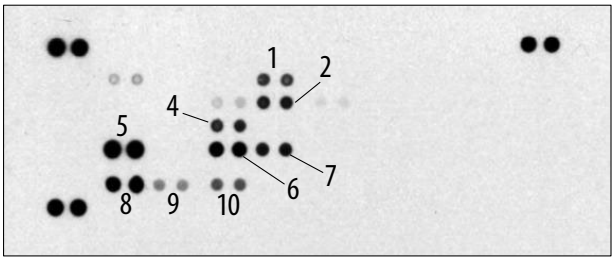


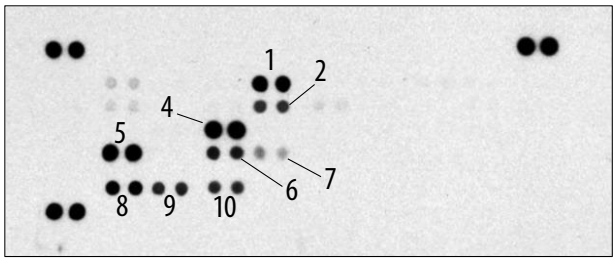
Figure 2D: A549 human lung carcinoma cells were untreated or treated with 20 ng/mL recombinant human IL-4 (R&D Systems, Catalog # 204-IL) for 72 hours.

PROFILING PROTEINS IN TISSUE LYSATES

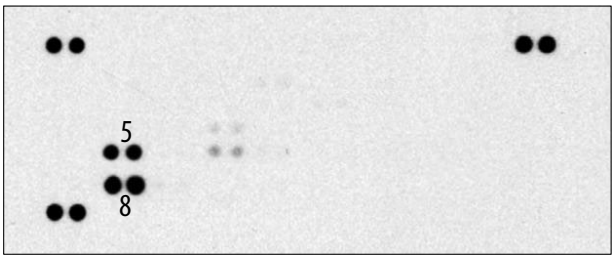
Liver



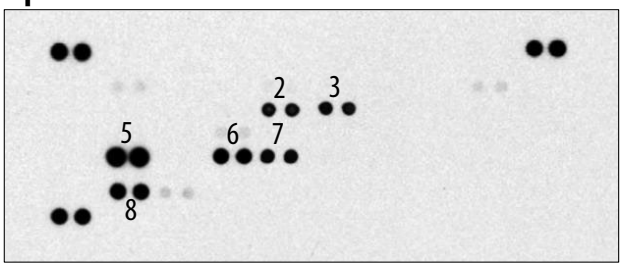
Kidney, Medulla



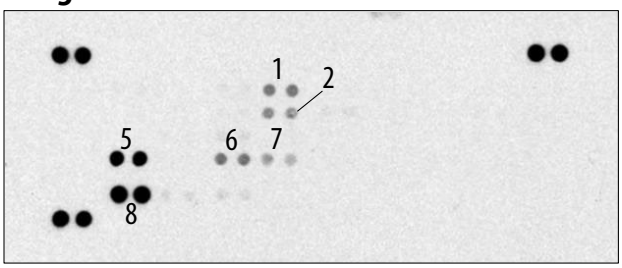
Heart



Spleen



Lung



1	Chemerin
2	IL-16
3	IP-10
4	Midkine
5	NAP-2
6	PF4
7	RANTES
8	Fibrinogen
9	gp130
10	TfR

Figure 4: The Human Chemokine Array detects multiple analytes in tissue lysates.
200 µg of tissue lysate was run on each array. Data shown are from a 5 minute exposure to X-ray film

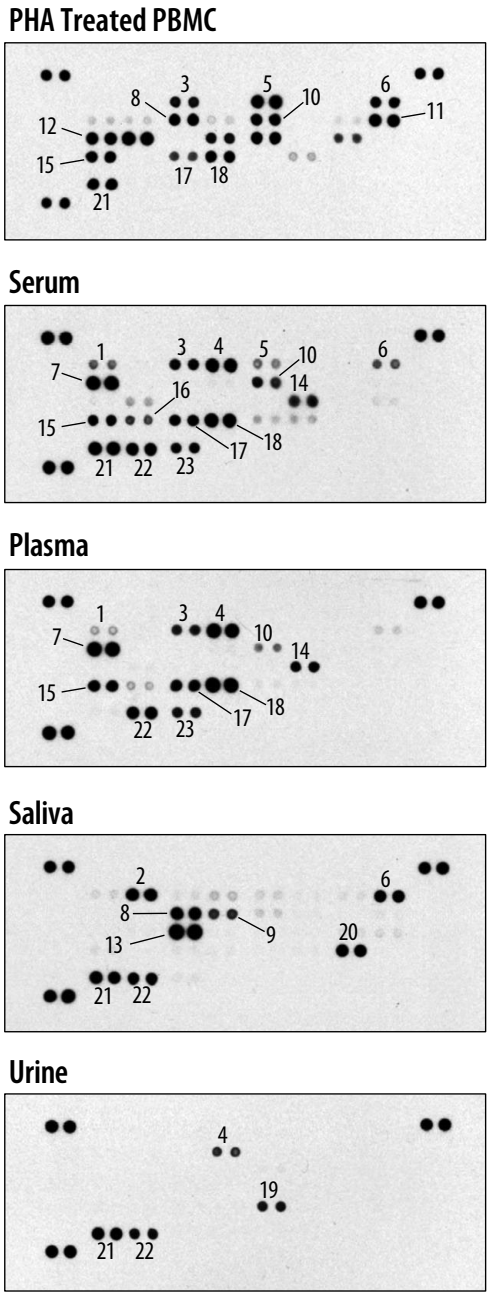


Figure 3: The Human Chemokine Array detects multiple analytes in PBMCs, serum, plasma, saliva, and urine samples. The sample type, quantity used per array, and duration of exposure to X-ray film are listed below.

A. PBMCs were treated with 10 µg/mL PHA for 48 hours; 500 µL of cell culture supernate per array (2 minute exposure).

B. Serum; 100 µL per array (5 minute exposure).

C. Heparin plasma; 100 µL per array (5 minute exposure).

D. Saliva; 250 µL per array (5 minute exposure).

E. Urine; 500 µL per array (5 minute exposure).

PROFILING PROTEINS IN PBMCs AND BODY FLUIDS *CONTINUED*

		MEAN PIXEL DENSITY				
		PBMCs	Serum	Plasma	Saliva	Urine
1	6Ckine	195	14,417	9280	3935	0
2	CCL28	654	758	902	38,081	0
3	CXCL16	31,594	26,973	24,739	3666	0
4	Chemerin	592	38,052	42,554	7552	17,457
5	ENA-78	43,721	12,459	255	3954	0
6	GROα	32,770	13,837	2709	34,015	0
7	HCC-1	10,337	41,109	43,439	1608	0
8	IL-8	37,330	1228	532	38,920	0
9	IL-16	9669	3022	2325	22,619	337
10	IP-10	37,205	25,469	10,793	4146	299
11	MCP-1	40,631	0	0	675	0
12	MCP-3	39,889	1843	1546	1668	0
13	Midkine	0	1759	709	46,842	814
14	MIP-1δ	0	28,895	26,218	82	0
15	NAP-2	36,853	26,328	31,853	3303	875
16	PARC	1154	16,195	7598	871	567
17	PF4	24,526	28,438	32,142	3148	763
18	RANTES	34,967	36,935	43,410	1045	524
19	SDF-1	0	6458	706	0	22,184
20	VCC-1	0	0	0	34,254	0
21	Fibrinogen	37,011	39,219	1846	37,166	34,103
22	gp130	1529	34,136	36,102	33,319	25,407
23	TfR	713	25,708	25,599	2175	268

APPENDIX

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

Coordinate	Analyte/Control	Alternate Nomenclature
A1, A2, A19, A20	Reference Spots	——
B3, B4	6Ckine	CCL21, Exodus-2
B5, B6	CCL28	MEC
B7, B8	CXCL16	SRPSOX
B9, B10	Chemerin	TIG-2, RARRES2
B11, B12	ENA-78	CXCL5
B13, B14	Eotaxin-3	CCL26
B15, B16	Fractalkine	CX3CL1, Neurotactin
B17, B18	GROα	CXCL1
C3, C4	HCC-1	CCL14, HCC-3
C5, C6	I-309	CCL1, TCA3
C7, C8	IL-8	CXCL8
C9, C10	IL-16	LCF
C11, C12	IP-10	CXCL10
C13, C14	I-TAC	CXCL11
C15, C16	Lymphotactin	XCL1, Lptn, ATAC, SCM-1α
C17, C18	MCP-1	CCL2, MCAF
D3, D4	MCP-3	CCL7
D5, D6	MDC	CCL22, STCP-1, ABCD-1
D7, D8	Midkine	——
D9, D10	MIG	CXCL9
D11, D12	MIP-1α/β	CCL3, CCL4
D13, D14	MIP-1δ	CCL15, Leukotactin 1, MIP-5, HCC-2
D15, D16	MIP-3α	CCL20, LARC, Exodus-1
D17, D18	MIP-3β	CCL19, ELC, Exodus-3
E3, E4	NAP-2	CXCL7, CTAP III
E5, E6	PARC	CCL18, MIP-4, AMAC-1
E7, E8	PF4	CXCL4
E9, E10	RANTES	CCL5, SISd
E11, E12	SDF-1	CXCL12, PBSF
E13, E14	TARC	CCL17
E15, E16	VCC-1	CXCL17, DMC

APPENDIX *CONTINUED*

Coordinate	Analyte/Control	Alternate Nomenclature
F3, F4	Fibrinogen (Sample Control)	——
F5, F6	gp130 (Sample Control)	IL6ST, CD130
F7, F8	Transferrin R (Sample Control)	TfR, CD71
F9, F10	Negative Control	Control (-)
G1, G2	Reference Spots	——

*Sample controls are included to allow for the detection of proteins commonly present in cell culture supernates, cell and tissue lysates, serum, plasma, saliva, and urine. If these endogenous proteins are present in a particular sample, positive signals indicate that the sample has been incubated with the array and the assay procedure has been performed correctly.

NOTES