Proteome Profiler™ Array

Mouse Chemokine Array Kit

Catalog Number ARY020

For the parallel determination of the relative levels of selected mouse 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 Mouse Chemokine Array Kit is a rapid, sensitive, and economical tool to simultaneously detect chemokine differences between samples. The relative expression levels of 25 mouse chemokines can be determined without performing numerous immunoassays. Each capture and detection antibody was carefully selected using cell culture supernate, cell and tissue lysates, and serum samples.

PRINCIPLE OF THE ASSAY

Capture and control antibodies have been spotted in duplicate on nitrocellulose membranes. Cell culture supernates, cell lysates, serum samples, or tissue lysates are diluted, mixed with a cocktail of biotinylated detection antibodies, and incubated overnight with the Proteome Profiler Mouse 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 Mouse 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.
- Other proteins present in biological samples do not necessarily interfere with the measurement of chemokines in samples. Until these proteins have been tested with the Mouse 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
Mouse Chemokine Array	894289	4 nitrocellulose membranes each containing 25 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, Mouse Chemokine Array	894290	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	607763	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
- 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

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)

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 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.

SAMPLE COLLECTION & STORAGE

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

Since the Mouse 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 concentrations using a total protein assay is recommended. Use the lysates immediately or aliquot and store at ≤ -70 °C. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 15 minutes at approximately 2000 x g. Remove serum 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 concentrations 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.

Mouse Chemokine Array - Four nitrocellulose membranes each containing 25 different capture antibodies and 3 sample controls printed in duplicate. **Handle membranes only with gloved hands and flat-tipped tweezers.**

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

1X Wash Buffer - If crystals have formed in the concentrate, warm bottles to room temperature and mix gently until 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 Reagent 1 and 2 should be mixed in equal volumes within 15 minutes of use. **Protect from light. 1 mL of 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 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 the 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 a 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 at room temperature.

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

    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

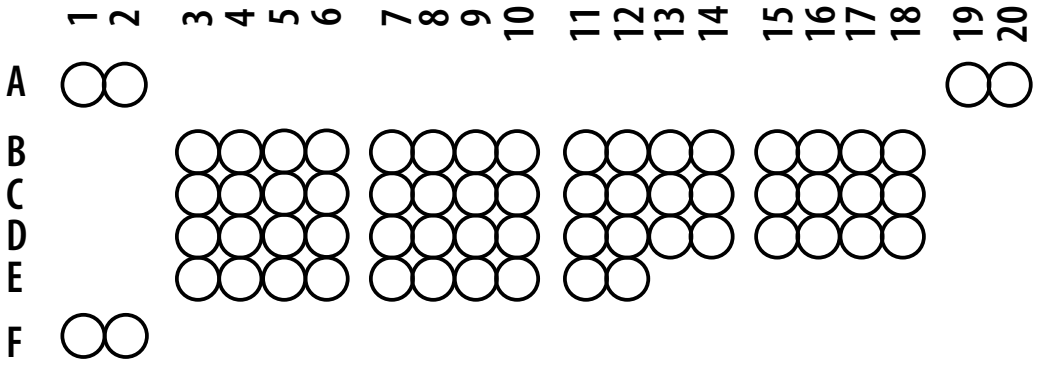
The positive signals seen on developed film can be quickly identified by placing the transparency overlay on the array image and aligning it with the pairs of reference spots in three corners of each array. The stamped identification number on the array should be placed on the left hand side. The location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

Mouse Chemokine Array Coordinates



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

PROFILING CHEMOKINES IN CELL CULTURE SUPERNATES

The Mouse Chemokine Array detects multiple analytes in cell culture supernates. Cells were either 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.

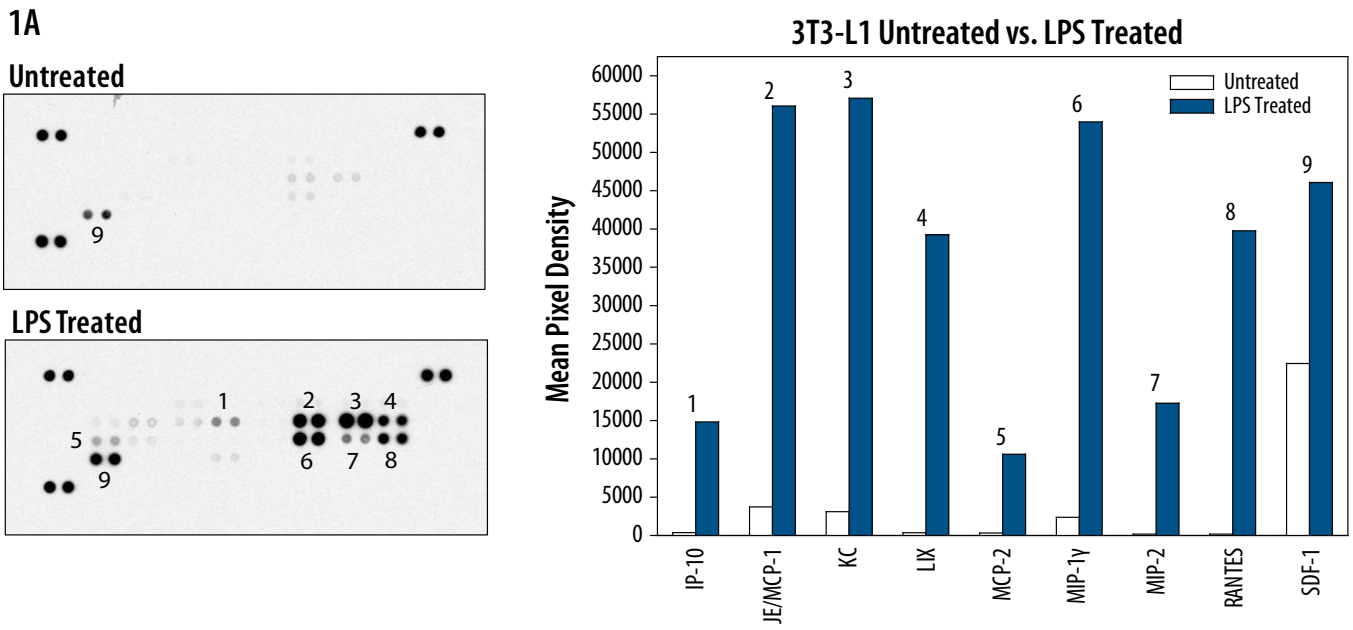


Figure 1A: 3T3-L1 mouse embryonic fibroblast adipose-like cells were untreated or treated with 100 ng/mL LPS for 24 hours.

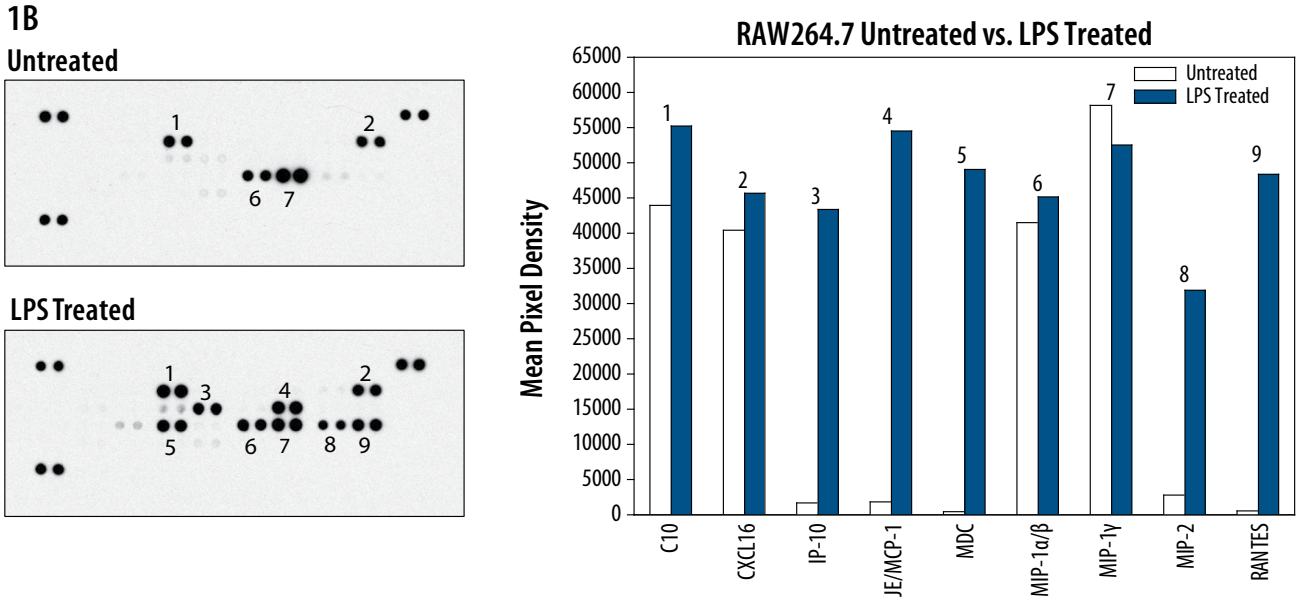


Figure 1B: RAW264.7 mouse monocyte/macrophage cells were untreated or treated with 100 ng/mL LPS for 24 hours.

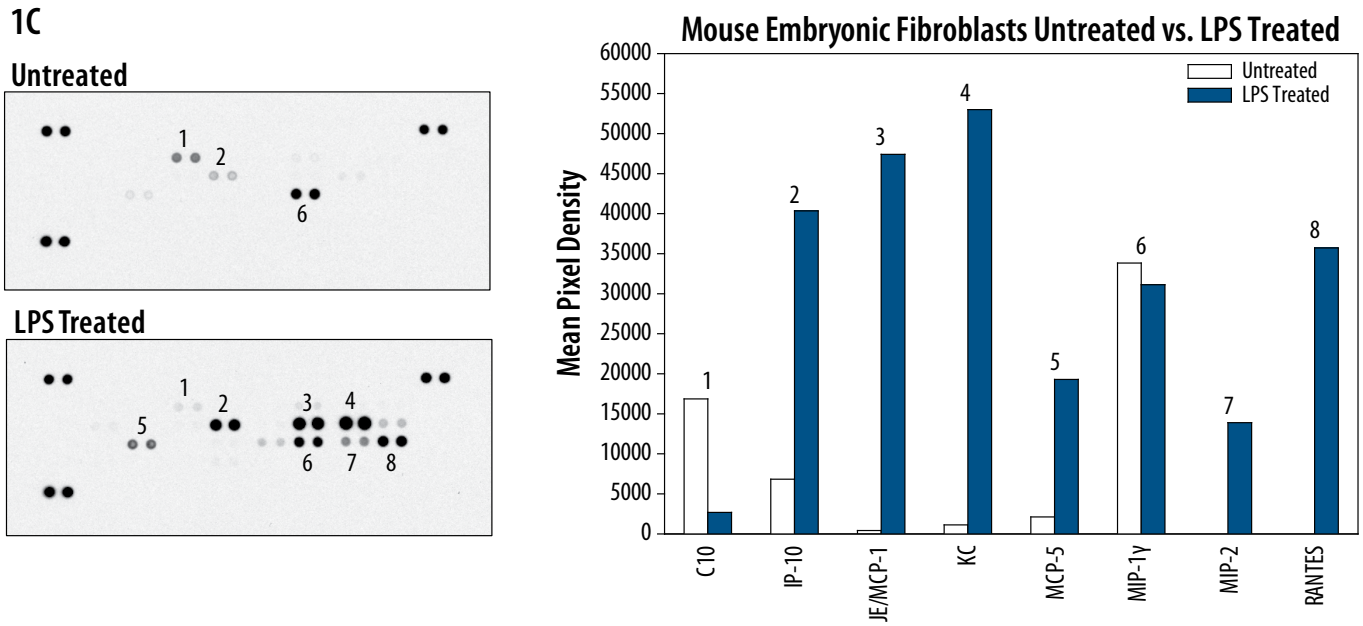


Figure 1C: Mouse embryonic fibroblasts (E14) were untreated or treated with 100 ng/mL LPS for 19 hours.

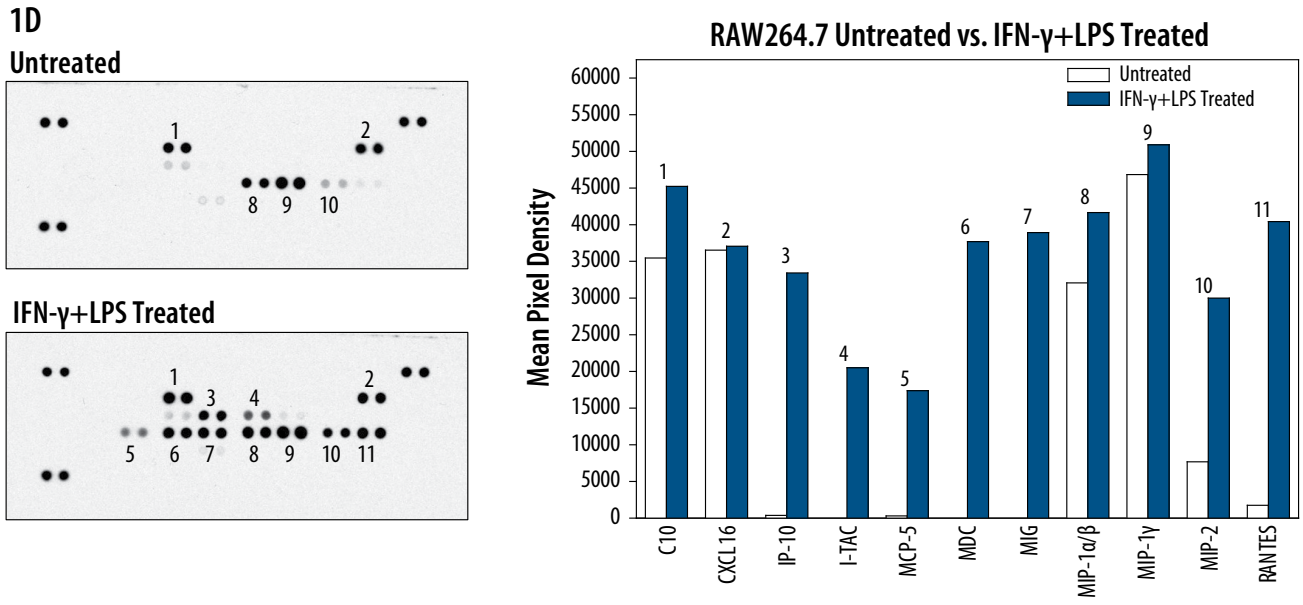


Figure 1D: RAW264.7 mouse monocyte/macrophage cells were untreated or treated with 1 µg/mL recombinant mouse IFN-γ (R&D Systems, Catalog # 485-MI) for 8 hours, followed by 1 µg/mL LPS for 16 hours.

PROFILING CHEMOKINES IN CELL LYSATES

The Mouse 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

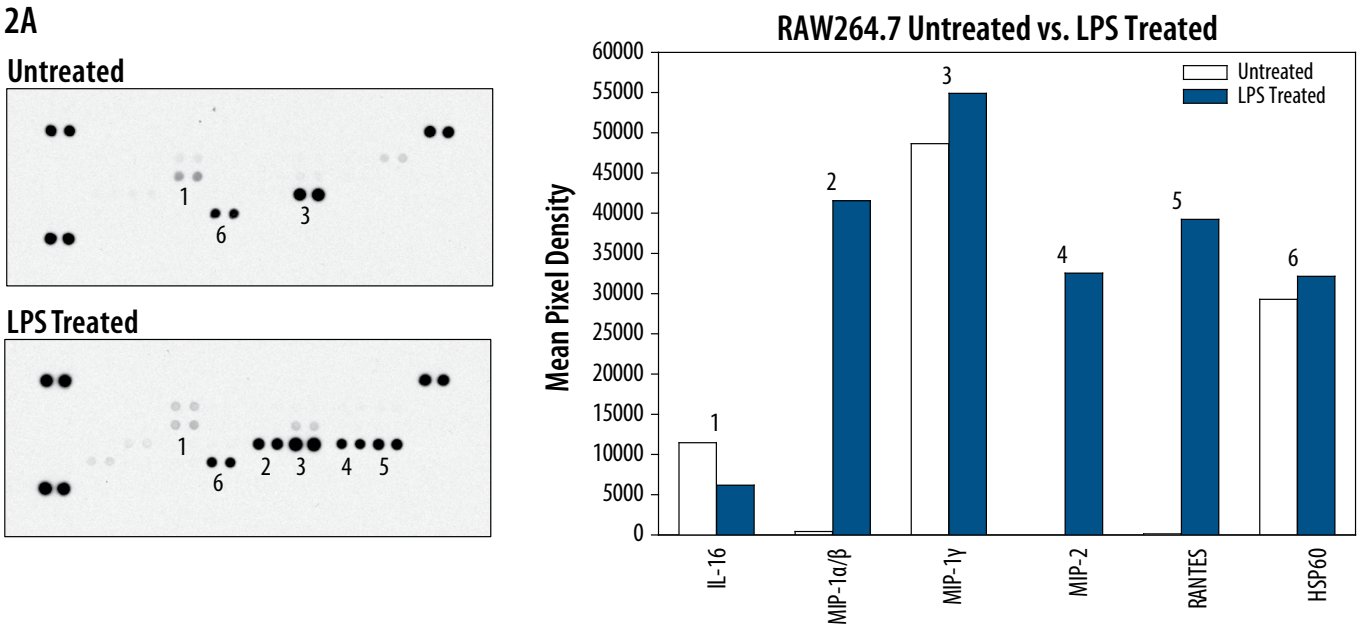


Figure 2A: RAW264.7 mouse monocyte/macrophage cells were untreated or treated with 100 ng/mL LPS for 24 hours.

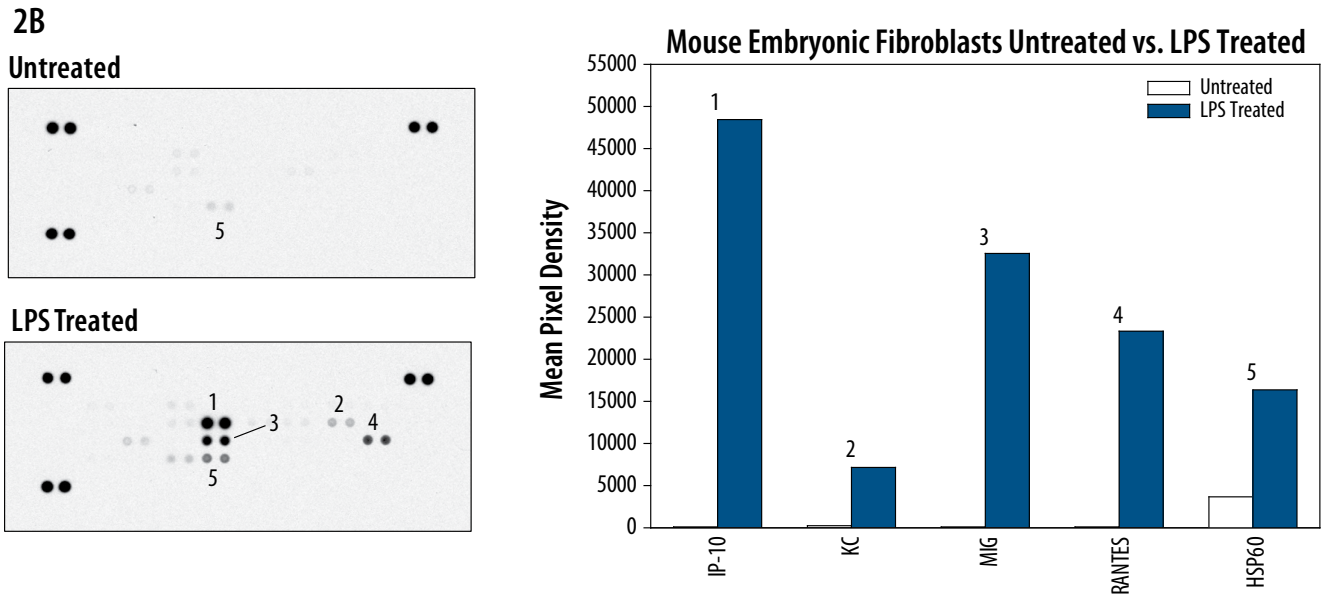


Figure 2B: Mouse embryonic fibroblasts (E14) were untreated or treated with 100 ng/mL LPS for 19 hours.

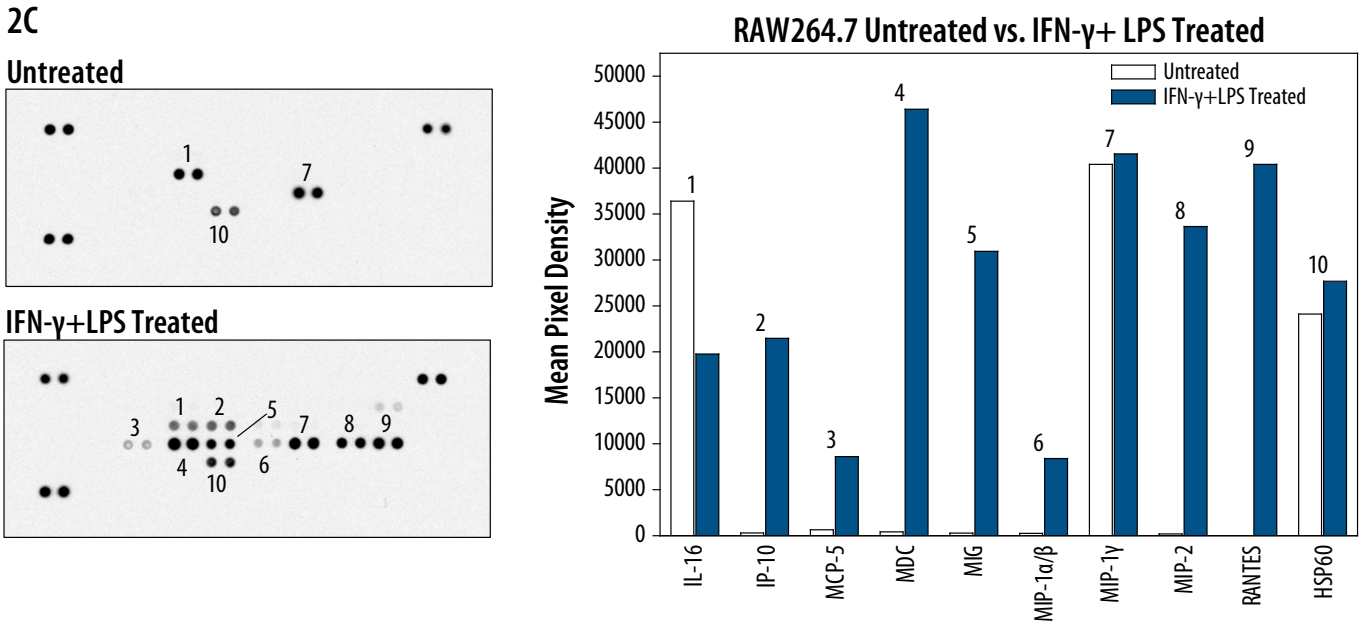


Figure 2C: RAW264.7 mouse monocyte/macrophage cells were untreated or treated with 1 µg/mL recombinant mouse IFN-γ (R&D Systems, Catalog # 485-MI) for 8 hours, followed by addition of 1 µg/mL LPS for 16 hours.

PROFILING CHEMOKINES IN SERUM AND TISSUE LYSATES

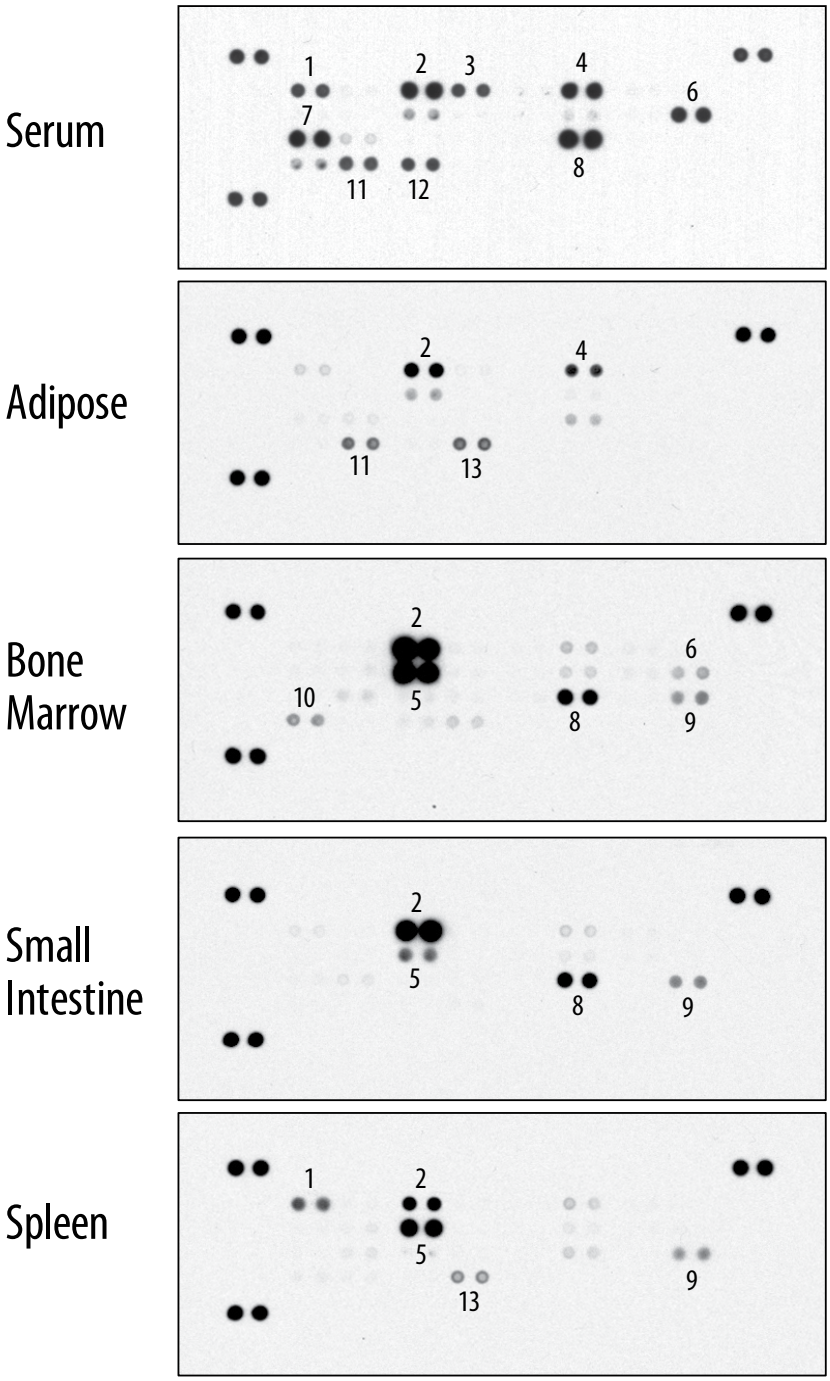


Figure 3: The Mouse Chemokine Array detects multiple analytes in tissue lysates and serum. 100 µL of normal mouse serum from Non-Swiss Albino mice was run on the array. Data shown are from a 2 minute exposure to X-ray film.

Tissue lysates were prepared as described in the Sample Collection and Storage section. 200 µg of tissue lysate was run on each array. Data shown are from a 5 minute exposure to X-ray film.

PROFILING CHEMOKINES IN SERUM AND TISSUE LYSATES *CONTINUED*

		MEAN PIXEL DENSITY				
		Serum	Adipose	Bone Marrow	Small Intestine	Spleen
1	6Ckine	22,382	3,155	528	1,312	23,007
2	C10	42,848	36,544	61,231	62,099	33,882
3	C5/C5a	21,858	395	1,242	1,478	239
4	Chemerin	39,535	21,754	5,559	3,501	4,469
5	IL-16	6486	7,811	61,337	22,327	52,135
6	LIX	33,299	ND	8,056	12	401
7	MCP-2	41,253	784	120	611	ND
8	MIP-1γ	51,657	5,845	41,126	40,629	3,419
9	RANTES	ND	61	14,211	13,378	12,273
10	SDF-1	7200	265	10,978	168	749
11	Complement Factor D	21,795	16,773	87	28	327
12	gp130	21,485	422	1,747	70	119
12	HSP60	ND	19,277	4,013	541	12,240

ND=Non-detectable

APPENDIX

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

Coordinate	Analyte/Control	Entrez Gene ID	Alternate Nomenclature
A1, A2, A19, A20	Reference Spots	N/A	RS
B3, B4	6Ckine	18829	CCL21, SLC, Exodus-2
B5, B6	BLC	55985	CXCL13, BCA-1
B7, B8	C10	20305	CCL6, MRP-1
B9, B10	Complement Component C5/C5a	15139	C5/C5a
B11, B12	CCL28	56838	MEC
B13, B14	Chemerin	71660	RARRES2
B15, B16	CTACK	20301	CCL27, ALP, ILC, ESkine
B17, B18	CXCL16	66102	SRPSOX
C3, C4	Eotaxin	20292	CCL11
C5, C6	Fractalkine	20312	CX3CL1
C7, C8	IL-16	16170	———
C9, C10	IP-10	15945	CXCL10, CRG-2, C7
C11, C12	I-TAC	56066	CXCL11, H174, SCYB9B
C13, C14	JE	20296	CCL2, MCP-1
C15, C16	KC	14825	CXCL1
C17, C18	LIX	20311	GCP-2, ENA-78
D3, D4	MCP-2	20307	CCL8, HC14
D5, D6	MCP-5	20293	CCL12
D7, D8	MDC	20299	CCL22, ABCD-1
D9, D10	MIG	17329	CXCL9, CRG-10, CMK
D11, D12	MIP-1α/β (pan)	20302/20303	CCL3/CCL4
D13, D14	MIP-1γ	20308	CCL9/10, CCF18, MRP-2
D15, D16	MIP-2	20310	CXCL2, GROβ, GRO2, CINC-3
D17, D18	RANTES	20304	CCL5, SISd
E3, E4	SDF-1	20315	CXCL12, PBSF
E5, E6	Complement Factor D (Sample Control)*	11537	Adipsin, DF, Adn
E7, E8	gp130 (Sample Control)*	16195	IL-6ST, CD130
E9, E10	HSP60 (Sample Control)*	15510	Hspd1
E11, E12	Negative Control	N/A	Control (-)
F1, F2	Reference Spots	N/A	RS

*Sample controls are included to allow for the detection of proteins commonly present in cell culture supernates, cell and tissue lysates, and serum. 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.