Proteome Profiler™ Array

Rat Cytokine Array Panel A

Catalog Number ARY008

For the parallel determination of the relative levels of selected rat 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. Measuring cytokines present in biological samples one at a time is tedious and requires large sample volumes. R&D Systems' Rat Cytokine Array Panel A is a rapid, sensitive, and economical tool for simultaneously profiling the relative levels of multiple cytokines between samples.

PRINCIPLE OF THE ASSAY

Capture and control antibodies have been spotted in duplicate on nitrocellulose membranes. Cell culture supernates, cell lysates, serum, plasma, or tissue lysates are diluted and mixed with a cocktail of biotinylated detection antibodies. The sample/antibody mixture is then incubated with the Rat 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 on page 12 for a list and coordinates of target capture antibodies.

TECHNICAL HINTS AND LIMITATIONS

- 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.
- Any variation in buffers, operator, pipetting technique, washing technique, and incubation time or temperature can alter the performance of the kit.
- The Rat 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 membrane 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. However, until these proteins have been tested with the Proteome Profiler Array, the possibility of interference cannot be excluded.
- For recommended chemiluminescent detection reagents, see the Other Materials Required section. The use of some high intensity chemiluminescent reagents may cause either increased background or diminished signal depending on the reagent.

MATERIALS PROVIDED

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

Component	Part #	Storage Conditions	Amount Provided
Rat Cytokine Array Panel A	893587	2-8° C	4 membranes
Array Buffer 4	895022	2-8° C	1 vial (21 mL)
Array Buffer 6	893573	2-8° C	2 vials (21 mL each)
Wash Buffer Concentrate, 25X	895003	2-8° C	2 vials (21 mL each)
Detection Antibody Cocktail, Rat Cytokine Array Panel A	893586	2-8° C	1 vial
Streptavidin-HRP	890803	2-8° C	1 vial
4-Well Rectangular Multi-dish	607544	Room Temperature	1 dish
Transparency Overlay Template	607590	Room Temperature	1 template

OTHER MATERIALS REQUIRED

- Aprotinin (Sigma, Catalog # A6279)
- Leupeptin (Sigma, Catalog # L8511)
- Pepstatin (Sigma, Catalog # P4265)
- Igepal® CA-630 (Sigma, Catalog # I3021)
- 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 transparent sheet protector
- Plastic wrap
- 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
- Chemiluminescent detection substrate (Pierce®, Catalog # 32106 or Amersham, Catalog # RPN2132)

If using cell lysate samples, the following buffer is also required:

- 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)

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

- 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 AND STORAGE

Since the Rat Cytokine 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: 200-700 μL for cell culture supernates, 100-400 μg for cell and tissue lysates, and 50-200 μL for serum and plasma samples.*

Cell Culture Supernates - Remove particulates by centrifugation and 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. For the initial experiment, use a quantity of lysate similar to that used for Western blot. 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 20 minutes at approximately 2000 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 20 minutes at approximately 2000 x g within 30 minutes of collection. 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.

Rat Cytokine Array Panel A - Four nitrocellulose membranes each containing 29 different anti-cytokine antibodies printed in duplicate. **Handle membranes only with gloved hands and flat-tipped tweezers.** After opening, reseal unused membranes in the foil pouch with desiccant and store at 2-8° C for up to 3 months.*

Detection Antibody Cocktail - One vial of lyophilized biotinylated antibodies. Before use, reconstitute the Detection Antibody Cocktail in 100 μL of deionized or distilled water. Store at 2-8° C for up to 3 months after reconstitution.*

Array Buffer 4 - Ready for use. Store at 2-8° C for up to 3 months after initial use.*
May contain a precipitate. Mix well before and during use.

Array Buffer 6 - Ready for use. Store at 2-8° C for up to 3 months after initial use.*

1X Wash Buffer - Dilute 40 mL of 25X Wash Buffer Concentrate into 960 mL of deionized or distilled water. Store at 2-8° C for up to 3 months after initial use.*

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

ARRAY PROTOCOL

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. 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 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.

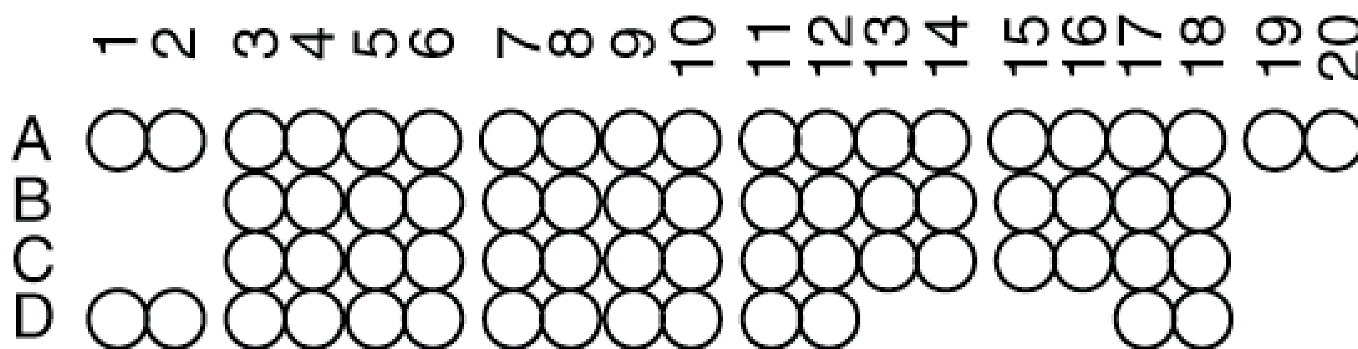
   Note: *A shorter incubation time may be used if optimal sensitivity is not required.*
9. Carefully remove each membrane and place into individual plastic containers with 20 mL of 1X Wash Buffer. Rinse the 4-Well Multi-dish with deionized or distilled water and dry thoroughly.
10. Wash each membrane with 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 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 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.
14. Wash each array as described in steps 9 and 10.
15. Carefully remove each membrane from the wash container. Allow excess Wash Buffer to drain from the membrane. Place each membrane on a transparent sheet protector.
16. Expose membranes to chemiluminescent reagents as directed by the manufacturer. The recommended volume of chemiluminescent reagent is 0.5 mL per array.
17. Cover the membranes with plastic wrap taking care to smooth out any air bubbles between the plastic wrap and the membranes. Place membranes, with the identification number up, in an X-ray film cassette.
18. Expose to X-ray film for 1-10 minutes. Multiple exposures are recommended.

DATA ANALYSIS

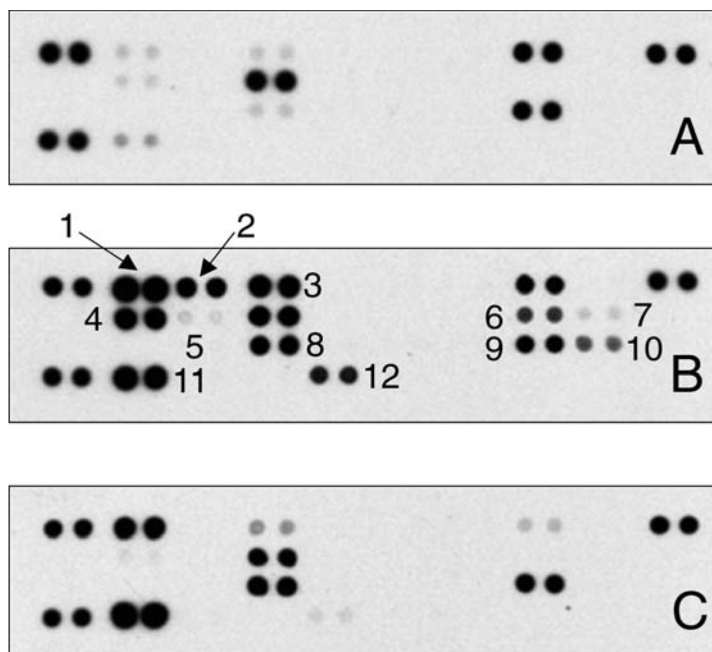
The positive signals seen on developed film can be quickly identified by placing the transparency overlay on the array image and aligning it with the three pairs of positive control spots in the corners of each array. The stamped identification number on the array should be placed on the left hand side. The location of controls and cytokine capture antibodies is listed in the Appendix on page 12.

Array data on developed X-ray film can be quantitated by scanning the film on a transmission-mode scanner and analyzing the array image file using 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.
See the Appendix on page 12.



		Mean Pixel Density		
		A	B	C
1	CINC-1	10,132	53,770	50,001
2	CINC-2α/β	794	45,826	721
3	CINC-3	9175	51,769	18,158
4	IL-1α/IL-1F1	7883	50,730	4734
5	IL-1β/IL-1F2	694	6987	905
6	IL-6	535	28,269	820
7	IL-10	453	7630	915
8	IP-10	10,642	45,370	46,015
9	MIP-1α/CCL3	42,556	37,520	41,834
10	MIP-3α	731	26,063	1446
11	RANTES/CCL5	16,491	51,258	53,724
12	TNF-α	206	36,230	6739

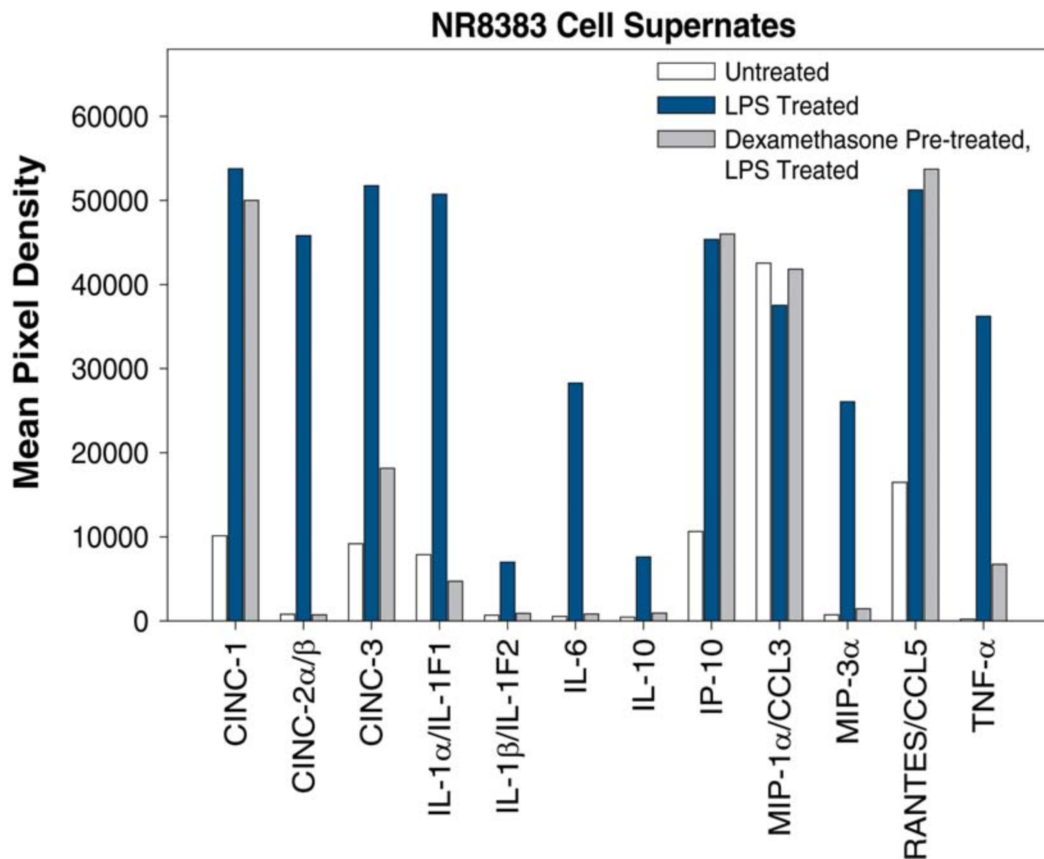


Figure 1: This Rat Cytokine Array detects multiple analytes in cell culture supernates.

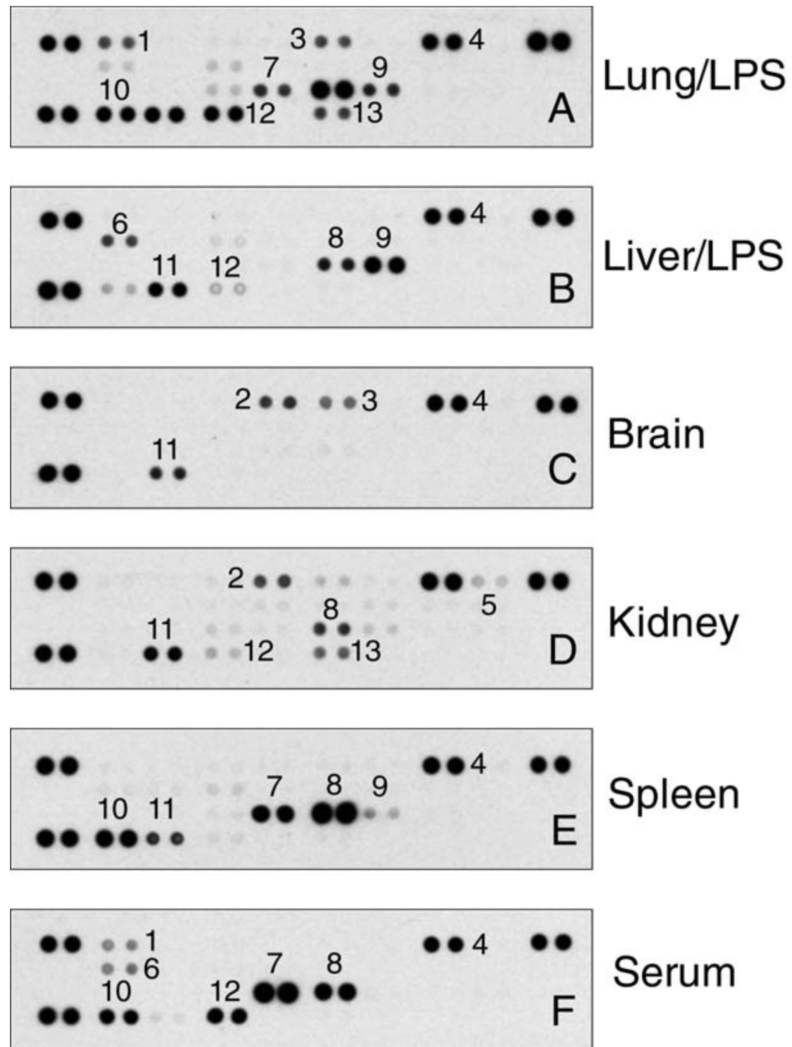
A) Untreated NR8383 rat alveolar macrophage cells.

B) NR8383 cells treated with 100 ng/mL LPS for 24 hours.

C) NR8383 cells pre-treated with 100 nM Dexamethasone for 72 hours followed by 100 ng/mL LPS for 24 hours.

500 μL of cell culture supernate was run on each array. Images and Mean Pixel Densities from a 2 minute exposure to film are shown.

PROFILING PROTEINS IN TISSUE LYSATES AND SERUM



		Mean Pixel Density					
		A	B	C	D	E	F
		Lung/LPS	**Liver/LPS**	**Brain**	**Kidney**	**Spleen**	**Serum**
1	CINC-1	16,812	1096	406	2284	844	10,792
2	CNTF	399	287	19,226	19,226	1183	1226
3	Fractalkine	15,631	450	13,245	4998	1733	1544
4	ICAM-1/CD54	38,203	38,243	38,416	42,109	42,834	36,505
5	IFN-γ	590	1049	1277	5614	1936	1190
6	IL-1α/IL-1F1	3695	15,835	768	1343	1624	12,050
7	LIX	19,682	984	1118	2172	43,605	51,272
8	L-Selectin	46,309	22,458	1568	19,403	52,406	44,565
9	MIG/CXCL9	20,979	41,455	723	4224	6818	1966
10	RANTES/CCL5	34,020	6041	635	1873	41,514	33,135
11	Thymus Chemokine	36,064	30,758	20,324	30,315	21,319	2709
12	TIMP-1	33,931	5988	661	6003	2562	37,417
13	VEGF	17,487	1012	801	14,013	2739	1892

Figure 2: This Rat Cytokine Array detects multiple analytes in tissue lysates and serum.

A-B) A rat was injected with 0.1 mg/kg LPS for 24 hours. Tissues were excised and prepared as described in the Sample Collection and Storage section on page 4. 400 μg of lysate was run on each array. Images and Mean Pixel Densities from a 7 minute exposure to film are shown.

C-E) Tissues were excised from untreated rats and prepared as described in the Sample Collection and Storage section on page 4. 400 μg of lysate was run on each array. Images and Mean Pixel Densities from a 7 minute exposure to film are shown.

F) Serum samples from 15 week old male rats were prepared as described in the Sample Collection and Storage section on page 4. 100 μL of serum was run on the array. Image and Mean Pixel Densities from a 5 minute exposure to film are shown.

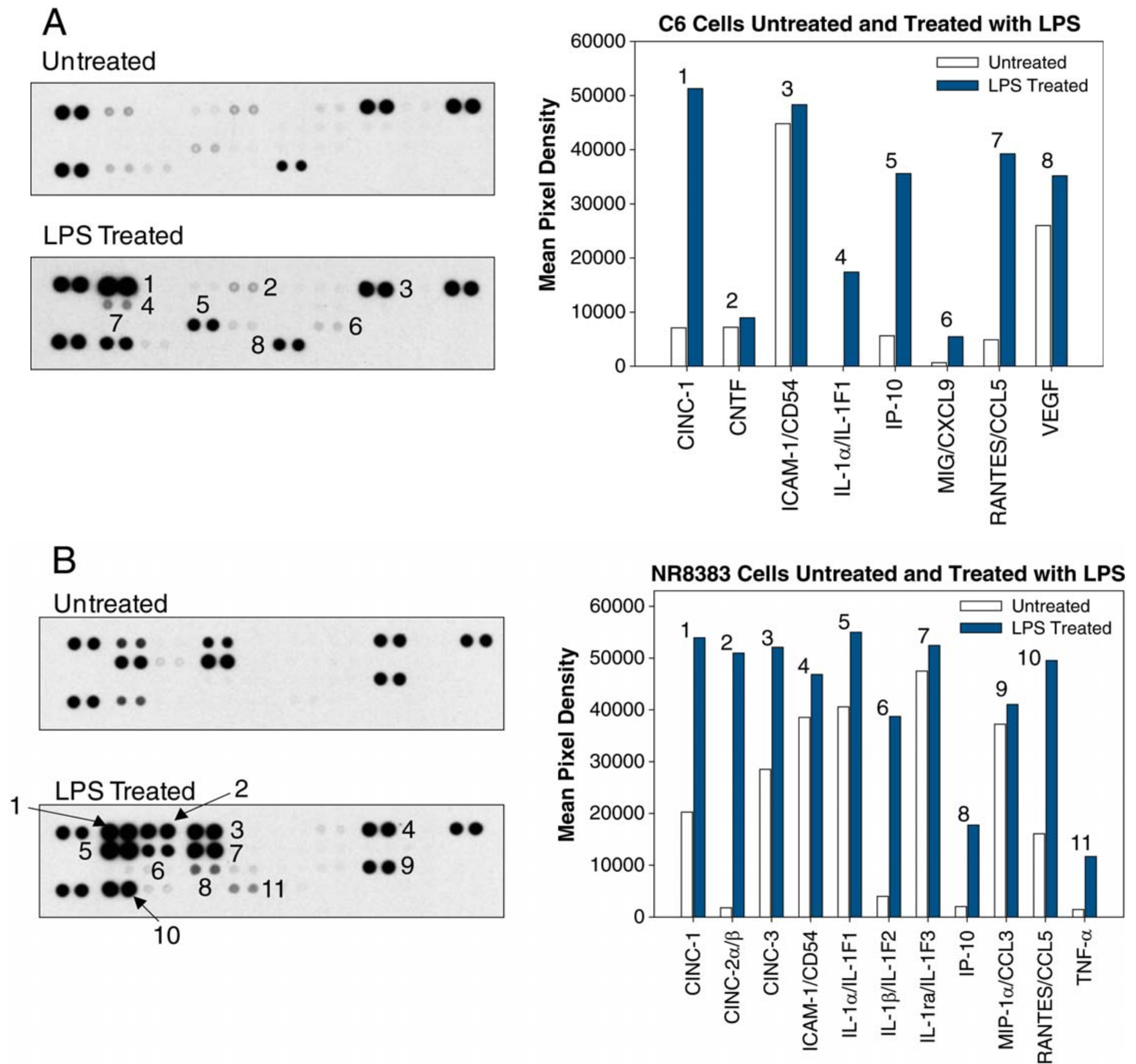


Figure 3: The Rat Cytokine Array detects multiple analytes in untreated and treated cell culture lysates.

A) C6 rat glioma cells were either untreated or treated with 100 ng/mL LPS for 24 hours. 200 μg of lysate was run on each array. A 5 minute exposure to film is shown.

B) NR8383 rat alveolar macrophage cells were either untreated or treated with 50 ng/mL LPS for 24 hours. 200 μg of lysate was run on each array. A 5 minute exposure to film is shown.

APPENDIX

Refer to the table below for the Cytokine Array coordinates.

Coordinate	Target/Control	Alternate Nomenclature
A1, A2	Positive Control	Control (+)
A3, A4	CINC-1	——
A5, A6	CINC-2α/β	——
A7, A8	CINC-3	——
A9, A10	CNTF	——
A11, A12	Fractalkine	CX3CL1
A13, A14	GM-CSF	——
A15, A16	sICAM-1	CD54
A17, A18	IFN-γ	——
A19, A20	Positive Control	Control (+)
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-3	——
B13, B14	IL-4	——
B15, B16	IL-6	——
B17, B18	IL-10	——
C3, C4	IL-13	——
C5, C6	IL-17	——
C7, C8	IP-10	CXCL10
C9, C10	LIX	——
C11, C12	L-Selectin	CD62L/LECAM-1
C13, C14	MIG	CXCL9
C15, C16	MIP-1α	CCL3
C17, C18	MIP-3α	CCL20
D1, D2	Positive Control	Control (+)
D3, D4	RANTES	CCL5
D5, D6	Thymus Chemokine	CXCL7
D7, D8	TIMP-1	——
D9, D10	TNF-α	TNFSF2
D11, D12	VEGF	VEGF-A/Vasculotropin
D17, D18	Negative Control	Control (-)

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