Proteome Profiler™ Array

Mouse XL Cytokine Array Kit

Catalog Number ARY028

For the parallel determination of the relative levels of selected mouse cytokines.

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, chemokines and growth factors are extracellular signaling molecules that mediate cell to cell communication. These molecules 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 Mouse XL Cytokine Array Kit is a rapid, sensitive, and economic tool to simultaneously detect cytokine differences between samples. The relative expression levels of 111 soluble mouse proteins can be determined without performing numerous immunoassays.

PRINCIPLE OF THE ASSAY

Capture and control antibodies have been spotted in duplicate on nitrocellulose membranes. Cell culture supernates, cell lysates, serum, urine, or tissue lysates are diluted and incubated overnight with the Proteome Profiler Mouse XL Cytokine Array. The membrane is washed to remove unbound material followed by incubation with a cocktail of biotinylated detection antibodies. Streptavidin-HRP and chemiluminescent detection reagents are then 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 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 analytes in samples. Until these proteins have been tested with the Proteome Profiler Array kit, 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 XL Cytokine Array	894886	4 nitrocellulose membranes each containing 111 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 XL Cytokine Array	894887	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	607911	1 transparency overlay template for coordinate reference.	

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

OTHER SUPPLIES REQUIRED

- Pipettes and pipette tips
- Gloves
- Deionized or distilled water
- Rocking platform shaker
- Microcentrifuge
- Plastic containers 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 17 (R&D Systems, Catalog # 895943)
- Aprotinin (Sigma, Catalog # A6279)
- Leupeptin (Tocris, Catalog # 1167)
- Pepstatin (Tocris, Catalog # 1190)

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.

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.

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 XL 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: 200-500 µL for cell culture supernates, 100-200 µg for cell and tissue lysates, 50-200 µL for serum, and 25-50 µL for urine 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 17 supplemented with 10 µg/mL Aprotinin, 10 µg/mL Leupeptin, and 10 µg/mL Pepstatin. Solubilize cells at 1 x 10^7 cells/mL in this 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 2000 x g. Remove serum and 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.

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.

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.*

Mouse XL Cytokine Array - Immediately before use, remove each membrane to be used from between the protective sheets with a flat-tipped tweezers. **Handle the membranes with gloved hands and flat-tipped tweezers only.**

Detection Antibody Cocktail - Before use, reconstitute the Mouse XL Cytokine Detection Antibody Cocktail in 200 µL of deionized or distilled water.

1X Array Buffer 4/6 - *Array Buffer 4 may contain a precipitate. Mix well before and during use.* Add 4 mL of Array Buffer 4 to 8 mL of Array Buffer 6. 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 completely dissolved. Add 40 mL of Wash Buffer Concentrate to 960 mL of deionized or distilled water.

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.**

1X Streptavidin-HRP - Immediately before use, dilute the Streptavidin-HRP in Array Buffer 6. See vial label for dilution factor.

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. Pipet 2.0 mL of Array Buffer 6 into each well of the 4-Well Multi-dish. Array Buffer 6 serves as a block buffer.
3. Place each membrane in a separate well. 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 4-Well Multi-dish so that each membrane rocks end to end in its well
5. While the arrays 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. Aspirate Array Buffer 6 from the wells of the 4-Well Multi-dish and add the prepared samples. Place the lid on the 4-Well Multi-dish.
7. 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.*
8. 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.
9. Wash each membrane with 1X Wash Buffer for 10 minutes on a rocking platform shaker. Repeat two times for a total of three washes.
10. For each array, add 30 µL of Detection Antibody Cocktail to 1.5 mL of 1X Array Buffer 4/6. Pipette 1.5 mL per well of diluted Detection Antibody Cocktail into the 4-Well Multi-dish.
11. 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.
12. Incubate for 1 hour at room temperature on a rocking platform shaker.
13. Wash each array as described in steps 8 and 9.

ARRAY PROCEDURE *CONTINUED*

14. Pipette 2.0 mL of 1X Streptavidin-HRP into each well of the 4-Well Multi-dish.

15. 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 1X Streptavidin-HRP. Cover the wells with the lid.

16. Incubate for 30 minutes at room temperature on a rocking platform shaker.

17. Wash each array as described in steps 8 and 9.

    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. 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.

23. 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.*

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

DATA ANALYSIS

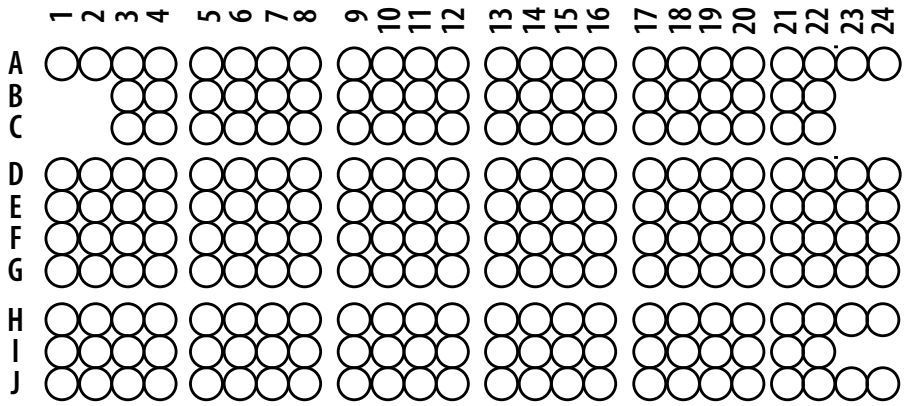
The positive signals seen on developed film can be quickly identified by placing the transparency overlay template on the array image and aligning it with the pairs of reference spots in three corners of each array. The stamped identification number on the array should be placed on the left hand side.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

Mouse XL Cytokine 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 LYSATES

The Mouse XL Cytokine Array detects multiple cytokines, chemokines, growth factors and other soluble proteins in cell lysates. The amount of cell lysate used on each array and the duration of exposure to X-ray film is indicated below. Profiles of mean spot pixel density were created using a transmission-mode scanner and image analysis software.

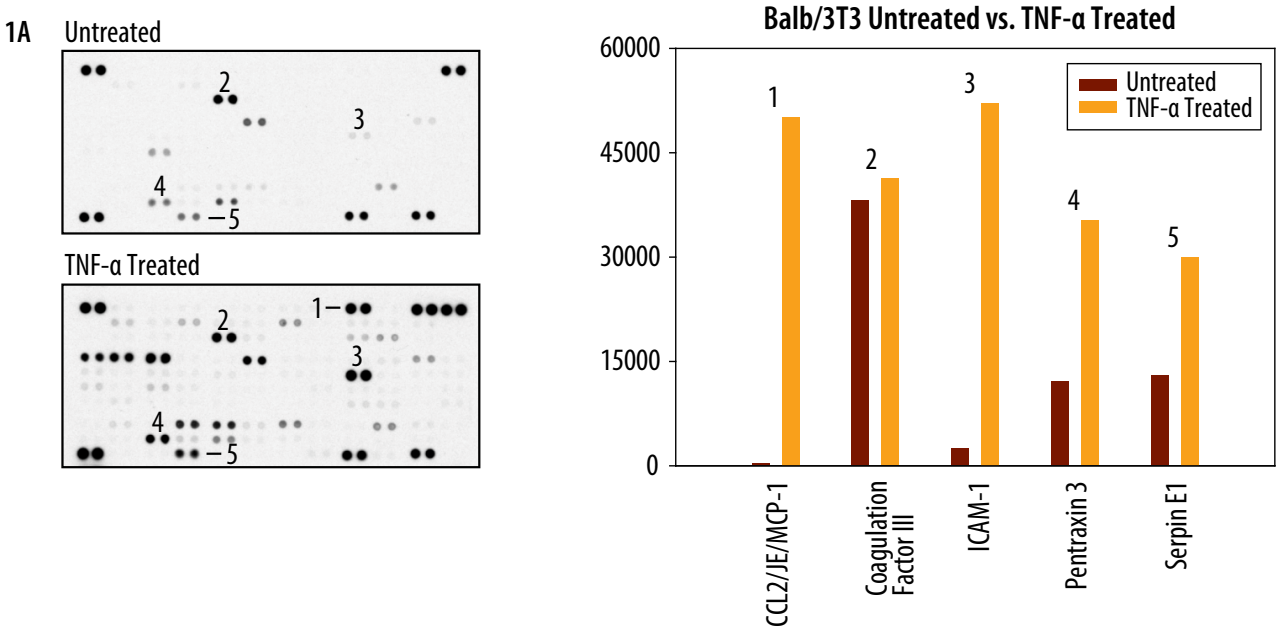


Figure 1A: Lysates from Balb/3T3 mouse embryonic fibroblast cells were untreated or treated with 100 ng/mL of recombinant mouse TNF-α (R&D Systems, Catalog # 410-MT) for 24 hours (200 µg lysate, 10 minute exposure).

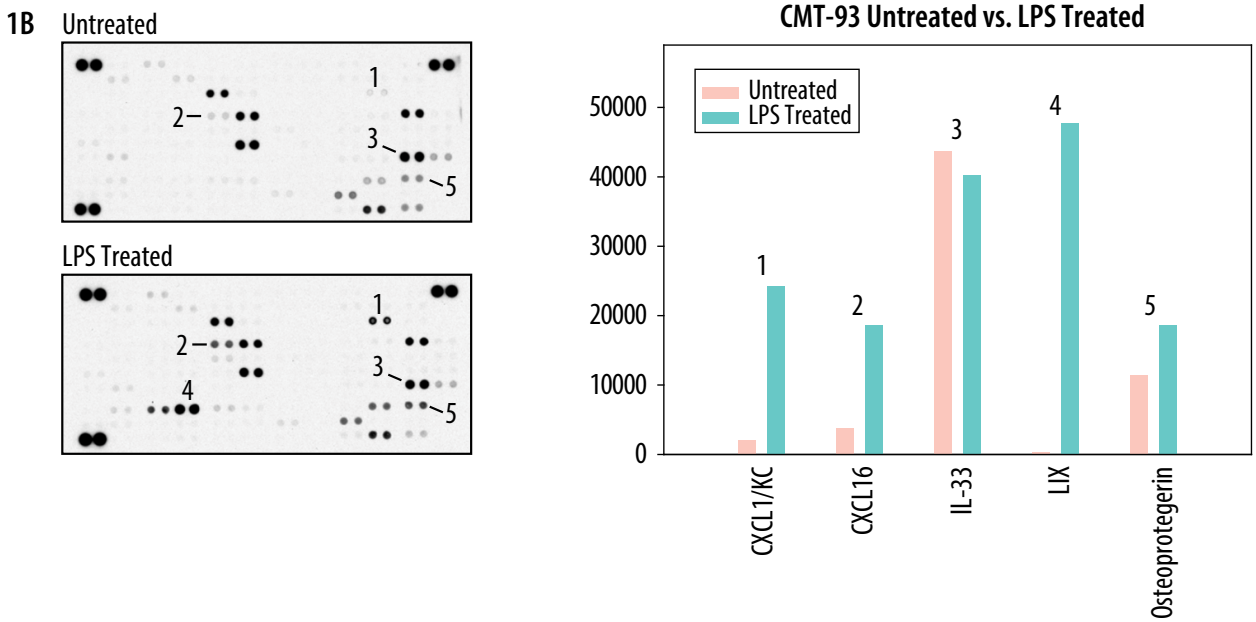


Figure 1B: Lysates from CMT-93 mouse rectal carcinoma cells were untreated or treated with 100 ng/mL LPS for 24 hours (200 µg lysate, 10 minute exposure).

PROFILING PROTEINS IN CELL CULTURE SUPERNATES

The Mouse XL Cytokine Array detects multiple cytokines, chemokines, growth factors and other soluble proteins in cell culture supernates. The amount of cell culture supernate used on each array and the duration of exposure to X-ray film is indicated below. Profiles of mean spot pixel density were created using a transmission-mode scanner and image analysis software.

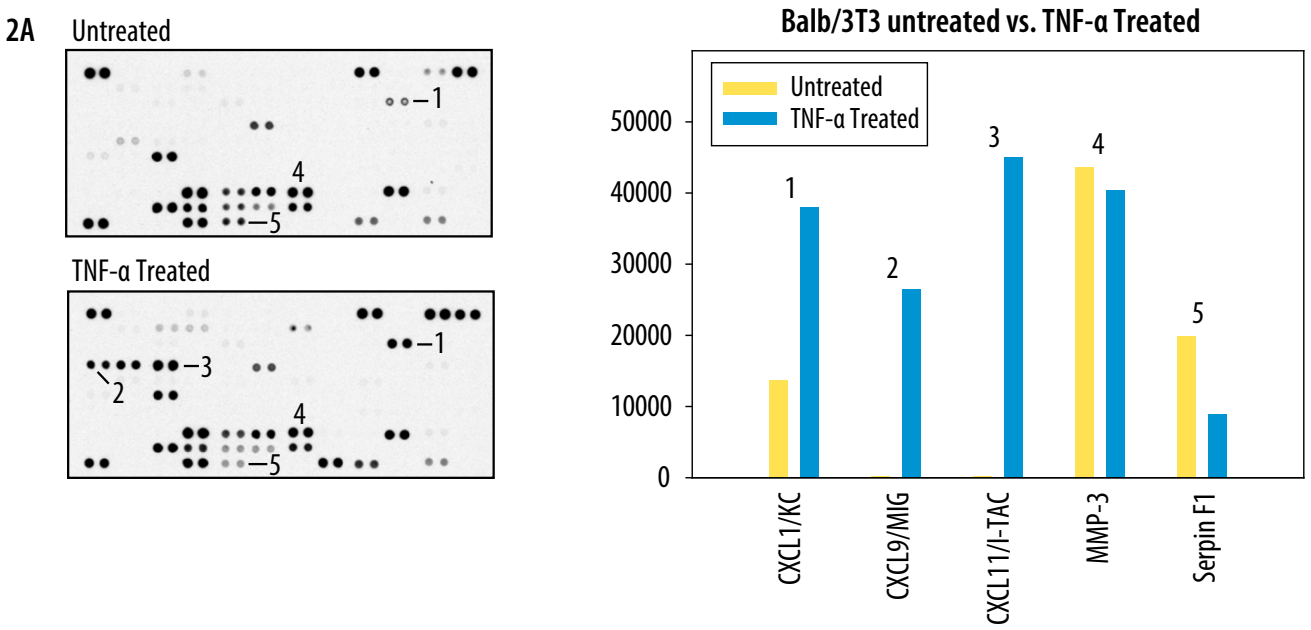


Figure 2A: Balb/3T3 mouse embryonic fibroblast cells were untreated or treated with 100 ng/mL of recombinant mouse TNF-α (R&D Systems, Catalog # 410-MT) for 24 hours (500 µL of cell culture supernate, 5 minute exposure).

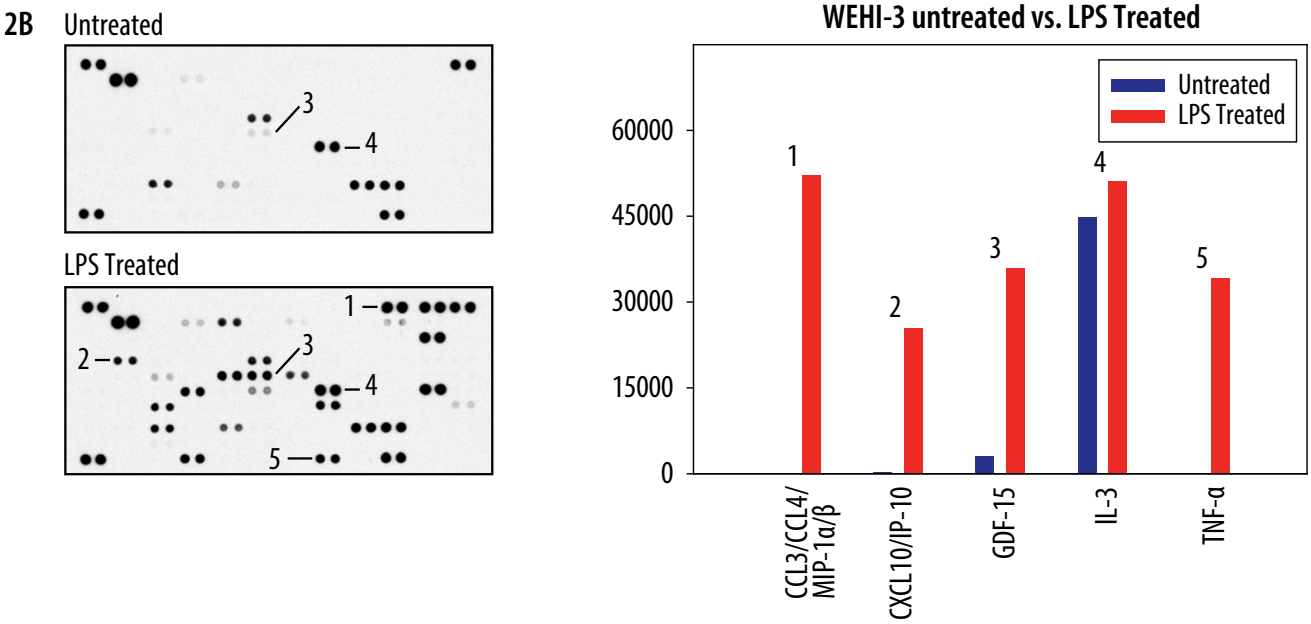


Figure 2B: WEHI-3 myelomonocytic leukemia cells were untreated or treated with 100 ng/mL LPS for 24 hours (500 µL of cell culture supernate, 5 minute exposure).

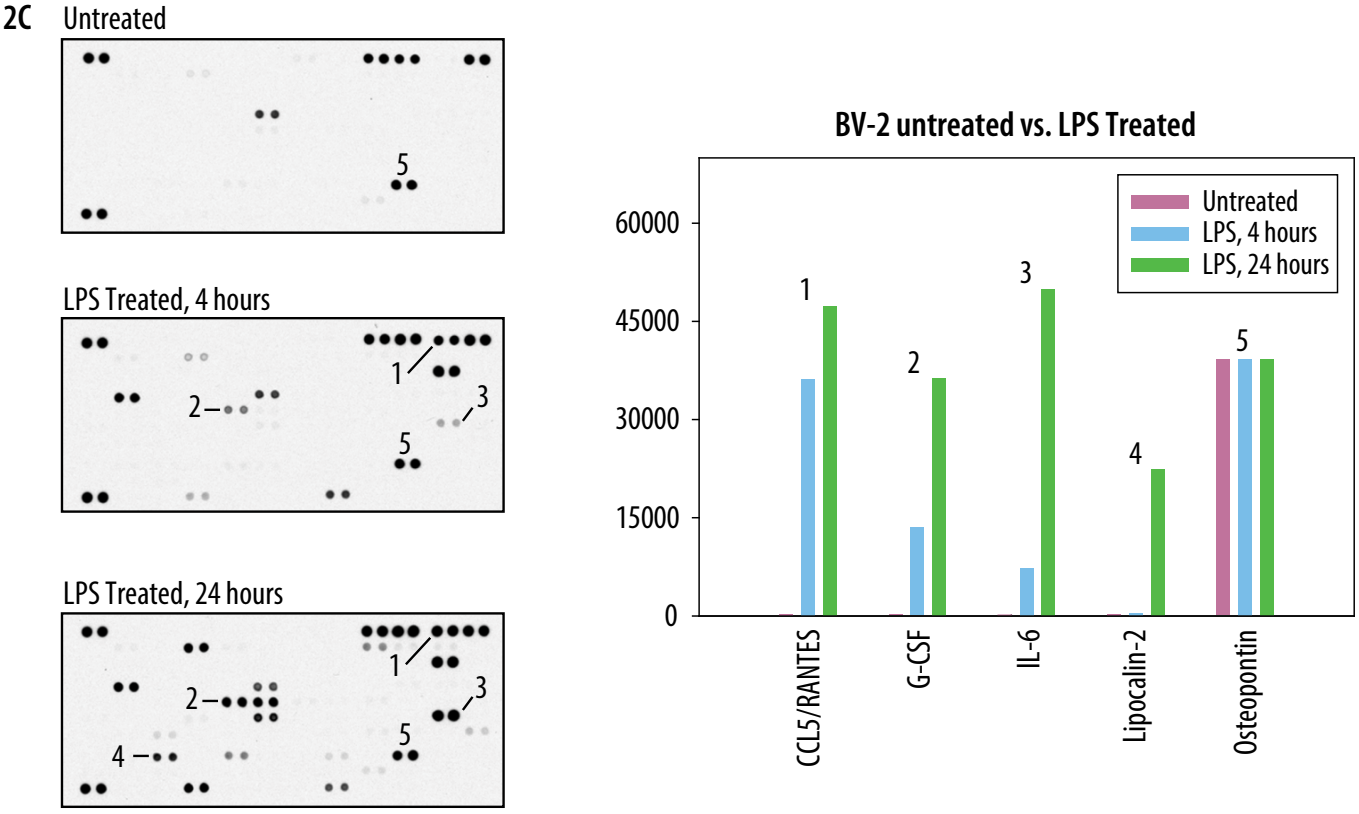
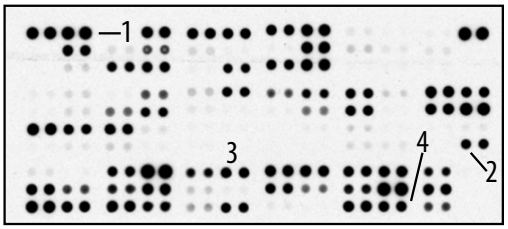


Figure 2C: BV-2 mouse microglial cells were untreated or treated with 100 ng/mL LPS for 4 or 24 hours (200 µL of cell culture supernate, 5 minute exposure).

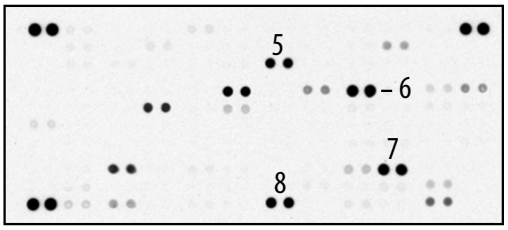
PROFILING PROTEINS IN BODY FLUIDS & TISSUE LYSATES

The Mouse XL Cytokine Array detects multiple cytokines, chemokines, growth factors and other soluble proteins in serum, urine, lung tissue lysate, uterus tissue lysate and spleen tissue lysate samples. Tissue lysates were generated from Balb/C mice. The sample type and quantity used are listed below. Data shown are from a five minute exposure to X-ray film.

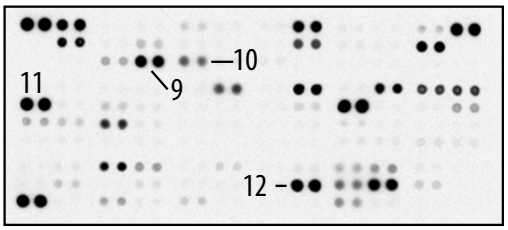
A. Serum, 200 µL per array



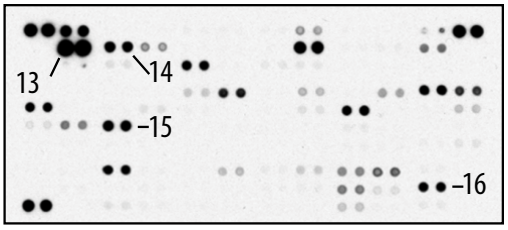
B. Urine, 50 µL per array



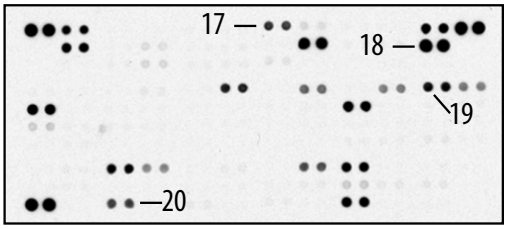
C. Lung Tissue Lysate, 200 µg per array



D. Uterus Tissue Lysate, 200 µg per array



E. Spleen Tissue Lysate, 200 µg per array



PROFILING PROTEINS IN BODY FLUIDS & TISSUE LYSATES *CONTINUED*

		MEAN PIXEL DENSITY				
		Serum	Urine	Lung	Uterus	Spleen
1	Adiponectin/Acrp30	50,148	1089	40,816	44,981	24,429
2	LDL R	30,796	395	1329	1455	367
3	MMP-2	35,305	279	2558	5429	980
4	VEGF	35,948	409	1336	388	434
5	Complement Factor D	32,461	30,769	1113	1029	1344
6	EGF	30,732	42,662	1261	241	489
7	Osteopontin (OPN)	39,921	39,986	10,746	16,890	348
8	TIM-1/KIM-1/HAVCR	1293	34,280	361	333	899
9	Chitinase 3-like 1	34,671	21	42,349	512	2466
10	Coagulation Factor III/ Tissue Factor	821	185	17,599	30,036	670
11	FGF acidic	406	0	50,864	28,363	35,714
12	RAGE	13,402	769	46,775	914	985
13	CCL6/C10	36,546	785	27,473	62,006	29,248
14	CCL11/Eotaxin	2417	0	599	37,283	344
15	IGFBP-6	36,325	0	22,416	37,574	763
16	Resistin	38,209	4314	3616	28,794	1140
17	BAFF/BLyS/TNFSF13B	35,294	460	1047	427	19,483
18	CD40/TNFRSF5	1677	132	35,547	13,531	50,316
19	Endostatin	41,295	2611	21,625	31,799	26,485
20	P-Selectin/CD62P	40,209	7632	6820	1823	19,324

APPENDIX

Refer to the table below for the Mouse XL Cytokine Array coordinates.

Coordinate	Analyte/Control	Entrez Gene ID	Alternate Nomenclature
A1, A2	Reference Spots	N/A	———
A3, A4	Adiponectin/Acrp30	11450	AdipoQ
A5, A6	Amphiregulin	11839	AR, SDGF
A7, A8	Angiopoietin-1	11600	Ang-1, Angpt1
A9, A10	Angiopoietin-2	11601	Ang-2, Angpt2
A11, A12	Angiopoietin-like 3	30924	ANGPT-L3
A13, A14	BAFF/BLyS/TNFSF13B	24099	CD257, TALL1, THANK, ZTNF4
A15, A16	C1q R1/CD93	17064	AA4 Antigen, C1q Rp, CD93
A17, A18	CCL2/JE/MCP-1	20296	MCAF
A19, A20	CCL3/CCL4/MIP-1α/β	20302/20303	———
A21, A22	CCL5/RANTES	20304	SISd
A23, A24	Reference Spots	N/A	———
B3, B4	CCL6/C10	20305	MRP-1
B5, B6	CCL11/Eotaxin	20292	———
B7, B8	CCL12/MCP-5	20293	———
B9, B10	CCL17/TARC	20295	———
B11, B12	CCL19/MIP-3β	24047	ELC
B13, B14	CCL20/MIP-3α	20297	exodus-1, LARC
B15, B16	CCL21/6Ckine	18829	exodus-2, SCYA21, SLC, TCA-4
B17, B18	CCL22/MDC	20299	ABCD-1, MDC, STCP-1
B19, B20	CD14	12475	———
B21, B22	CD40/TNFRSF5	21939	———
C3, C4	CD160	54215	Natural killer cell receptor BY55, NK1; NK28
C5, C6	Chemerin	71660	RARRES2, TIG-2
C7, C8	Chitinase 3-like 1	12654	CHI3L1, Cgp39, YKL40
C9, C10	Coagulation Factor III/Tissue Factor	14066	TF, CD142, Thromboplastin
C11, C12	Complement Component C5/C5a	15139	C5/C5a
C13, C14	Complement Factor D	11537	Adipsin, C3 convertase activator, Properdin factor D
C15, C16	C-Reactive Protein/CRP	12944	———
C17, C18	CX3CL1/Fractalkine	20312	FKN, Neurotactin
C19, C20	CXCL1/KC	14825	CINC-1; GRO alpha; KC; MGSA-alpha
C21, C22	CXCL2/MIP-2	20310	GROβ, GRO2, CINC-3
D1, D2	CXCL9/MIG	17329	CRG-10, CMK
D3, D4	CXCL10/IP-10	15945	CRG-2, C7
D5, D6	CXCL11/I-TAC	56066	H174, SCYB9B
D7, D8	CXCL13/BLC/BCA-1	55985	———
D9, D10	CXCL16	66102	SRPSOX
D11, D12	Cystatin C	13010	ARMD11, CST3, Gamma-trace

APPENDIX *CONTINUED*

Coordinate	Analyte/Control	Entrez Gene ID	Alternate Nomenclature
D13, D14	DKK-1	13380	Dickkopf-1
D15, D16	DPPIV/CD26	13482	Dpp4, Dipeptidyl-peptidase IV
D17, D18	EGF	13645	Epidermal Growth Factor
D19, D20	Endoglin/CD105	13805	ENG
D21, D22	Endostatin	12822	Col18a1
D23, D24	Fetuin A/AHSG	11625	AHSG, alpha-2-HS-glycoprotein
E1, E2	FGF acidic	14164	FGF-1
E3, E4	FGF-21	56636	———
E5, E6	Flt-3 Ligand	14256	Flt3lg
E7, E8	Gas 6	14456	Growth Arrest Specific
E9, E10	G-CSF	12985	Csf3
E11, E12	GDF-15	23886	MIC-1
E13, E14	GM-CSF	12981	Csf2
E15, E16	HGF	15234	Scatter Factor, SF, Hepatopoietin-A
E17, E18	ICAM-1/CD54	15894	———
E19, E20	IFN-γ	15978	IFNG
E21, E22	IGFBP-1	16006	———
E23, E24	IGFBP-2	16008	———
F1, F2	IGFBP-3	16009	———
F3, F4	IGFBP-5	16011	———
F5, F6	IGFBP-6	16012	———
F7, F8	IL-1α/IL-1F1	16175	———
F9, F10	IL-1β/IL-1F2	16176	———
F11, F12	IL-1ra/IL-1F3	16181	IL1RN
F13, F14	IL-2	16183	———
F15, F16	IL-3	16187	———
F17, F18	IL-4	16189	B cell-stimulatory factor-1
F19, F20	IL-5	16191	———
F21, F22	IL-6	16193	———
F23, F24	IL-7	16196	———
G1, G2	IL-10	16153	CSIF
G3, G4	IL-11	16156	———
G5, G6	IL-12 p40	16160	———
G7, G8	IL-13	16163	———
G9, G10	IL-15	16168	———
G11, G12	IL-17A	16171	———
G13, G14	IL-22	50929	IL-TIF
G15, G16	IL-23	83430	———
G17, G18	IL-27 p28	246779	———

APPENDIX *CONTINUED*

Coordinate	Analyte/Control	Entrez Gene ID	Alternate Nomenclature
G19, G20	IL-28A/B	330496/338374	———
G21, G22	IL-33	77125	NF HEV, DVS 27
G23, G24	LDL R	16835	low density lipoprotein receptor
H1, H2	Leptin	16846	OB
H3, H4	LIF	16878	———
H5, H6	Lipocalin-2/NGAL	16819	Siderocalin, 24p3
H7, H8	LIX	20311	CXCL5, GCP-2, ENA-78
H9, H10	M-CSF	12977	CSF-1
H11, H12	MMP-2	17390	Gelatinase A
H13, H14	MMP-3	17392	Stromelysin-1
H15, H16	MMP-9	17395	Clg4b, Gelatinase B, GELB
H17, H18	Myeloperoxidase	17523	MPO
H19, H20	Osteopontin (OPN)	20750	Eta-1, Spp1
H21, H22	Osteoprotegerin/TNFRSF11B	18383	OPG, Ocif
H23, H24	PD-ECGF/Thymidine phosphorylase	72962	dThdPase, ECGF1, Gliostatin, MEDPS1, MNGIE
I1, I2	PDGF-BB	18591	———
I3, I4	Pentraxin 2/SAP	20219	PTX2
I5, I6	Pentraxin 3/TSG-14	19288	PTX3
I7, I8	Periostin/OSF-2	50706	Fasciclin I-like, POSTN, TRIF52
I9, I10	Pref-1/DLK-1/FA1	13386	DLK1, pG2, ZOG
I11, I12	Proliferin	18811	MRP
I13, I14	Proprotein Convertase 9/PCSK9	100102	NARC-1
I15, I16	RAGE	11596	AGER
I17, I18	RBP4	19662	Retinol-Binding Protein 4
I19, I20	Reg3G	19695	PAP3
I21, I22	Resistin	57264	ADSF, FIZZ3
J1, J2	Reference Spots	N/A	———
J3, J4	E-Selectin/CD62E	20339	ELAM1, LECAM2, Sele
J5, J6	P-Selectin/CD62P	20344	GMP-140, LECAM3, Selep
J7, J8	Serpin E1/PAI-1	18787	Nexin, PLANH1
J9, J10	Serpin F1/PEDF	20317	EPC-1
J11, J12	Thrombopoietin	21832	Tpo, MGDF
J13, J14	TIM-1/KIM-1/HAVCR	171283	———
J15, J16	TNF-α	21926	TNFSF1A
J17, J18	VCAM-1/CD106	22329	———
J19, J20	VEGF	22339	VEGF-A, VPF
J21, J22	WISP-1/CCN4	22402	———
J23, J24	Negative Control	N/A	———

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