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R&D SYSTEMS™

Saliva Sensitive

Proteome Profiler™ Array

Human Adipokine Array Kit

Catalog Number ARY024

For the parallel determination of the relative levels of selected human Adipokines.

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

Obesity is a major public health concern. Analyzing the expression profile of obesity-related proteins is essential for understanding their roles in obesity and associated illnesses such as diabetes and cardiovascular disease. The Proteome Profiler™ Human Adipokine Array is a rapid, sensitive, and economical tool to detect differences in adipokines between samples. The relative expression levels of 58 human adipokines can be determined simultaneously without performing numerous immunoprecipitations, Western blots, or ELISAs. Each capture and detection antibody was carefully selected using 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, tissue lysates, serum, plasma, saliva, urine, or human milk samples are diluted, mixed with a cocktail of biotinylated detection antibodies, and incubated overnight with the Proteome Profiler Human Adipokine Array. 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.**
- If using a LI-COR, additional reagents and protocol modifications are required. Refer to https://www.rndsystems.com/resources/technical/use-proteome-profiler-arrays-li-cor-detection for more details.
- 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.
- 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 Proteome Profiler Array, the possibility of interference cannot be excluded.
- For a procedure demonstration video, 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 #	AMOUNT PROVIDED	STORAGE OF OPENED/RECONSTITUTED MATERIAL
Human Adipokine Array	894728	4 membranes	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	1 vial (21 mL); *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)	
Wash Buffer Concentrate	895003	2 vials (21 mL/vial); *May turn yellow over time.*	
Detection Antibody Cocktail, Human Adipokine Array	894729	1 vial; lyophilized	
Streptavidin-HRP	893019	1 vial (200 µL)	
Chemi Reagent 1	894287	1 vial (2.5 mL)	
Chemi Reagent 2	894288	1 vial (2.5 mL)	
4-Well Rectangular Multi-dish	607544	1 dish	Store at room temperature.
Transparency Overlay Template	607886	1 template	

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

Note: *Additional wash buffer is available for purchase (R&D Systems®, # WA126).*

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 a preservative which may cause an allergic skin reaction. Avoid breathing mist.

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

OTHER SUPPLIES REQUIRED

- Aprotinin (Tocris™, # 4139)
- Leupeptin hemisulfate (Tocris, # 1167)
- Pepstatin A (Tocris, # 1190)
- Igepal® CA-630 (Sigma™, # I3021)
- Pipettes and pipette tips
- Gloves
- Phosphate-Buffered Saline (PBS) (R&D Systems®, # RB01)
- 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
- Paper towels
- Absorbent lab wipes
- Autoradiography cassette
- Film developer
- X-ray film
- Flat-tipped tweezers
- Flatbed scanner with transparency adapter capable of transmission mode
- Computer capable of running image analysis software and Microsoft® Excel

If using cell lysate samples, the following buffers are also required:

- Phosphate-Buffered Saline (PBS) (R&D Systems®, # RB01)
- 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 hemisulfate, and 10 µg/mL Pepstatin A)

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

- PBS with protease inhibitors (10 µg/mL Aprotinin, 10 µg/mL Leupeptin hemisulfate, and 10 µg/mL Pepstatin A)
- Triton™ X-100 (Sigma, # 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 Adipokine 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, plasma, human milk, urine, and saliva 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 supplemented with 10 µg/mL Aprotinin, 10 µg/mL Leupeptin hemisulfate, and 10 µg/mL Pepstatin A. 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 concentration using a total protein assay is recommended. Assay immediately or aliquot and store at ≤ -70 °C. Avoid repeated freeze-thaw cycles.

Tissue Lysates - Excise tissue and homogenize in PBS supplemented with 10 µg/mL Aprotinin, 10 µg/mL Leupeptin hemisulfate, and 10 µg/mL Pepstatin A. 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.

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.

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.

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

Human Milk - Centrifuge for 15 minutes at 1000 x g at 2-8 °C. Collect the aqueous fraction and repeat this process a total of 3 times. 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 Adipokine Array - Four nitrocellulose membranes each containing 58 different capture antibodies printed in duplicate. **Handle membranes only with gloved hands and flat-tipped tweezers.**

Detection Antibody Cocktail - Before use, reconstitute the Detection Antibody Cocktail in 200 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.**

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 array analytes 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 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 30 µL of reconstituted Human Adipokine 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 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 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 by blotting the lower edge onto absorbent paper. 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

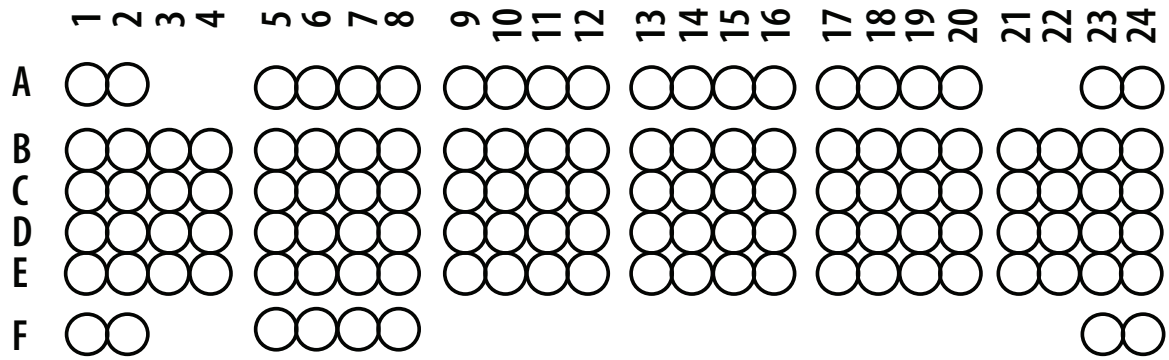
The positive signals seen on developed film can be quickly identified by placing the transparency overlay template on the array image and aligning it with the pairs of reference spots in three corners of each array. The stamped identification number on the array should be placed on the left hand side.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

Human Adipokine 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 Adipokine Array detects multiple adipokines 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 5 minute exposure to X-ray film. Profiles of mean spot pixel density were created using a transmission-mode scanner and image analysis software.

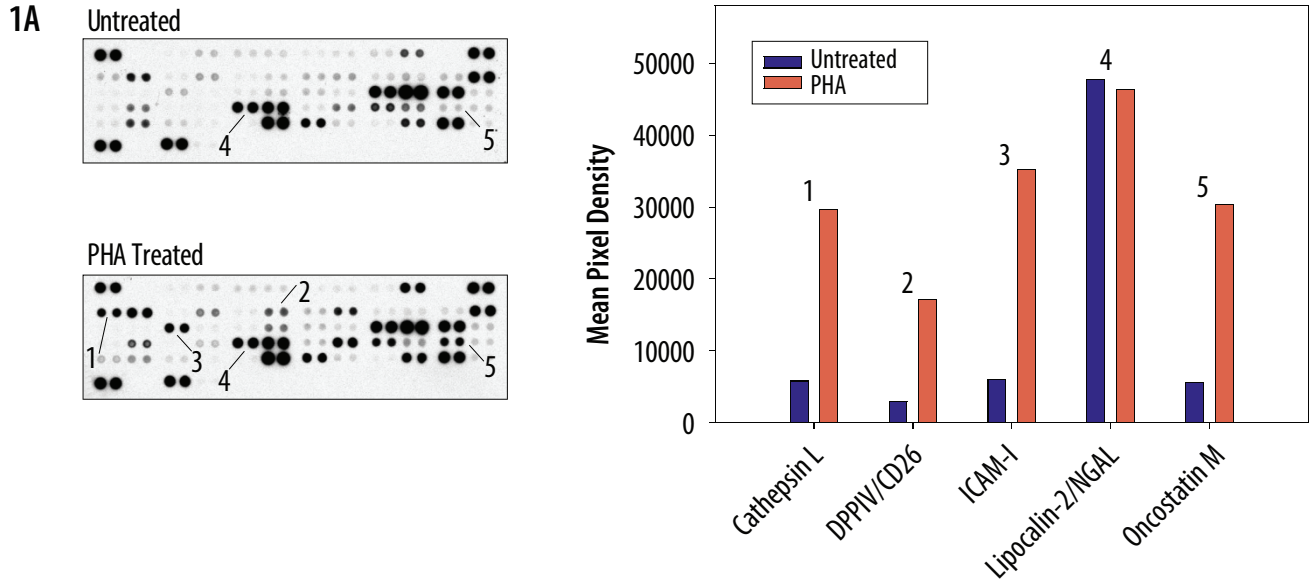


Figure 1A: PBMCs were untreated or treated with 10 µg/mL PHA for 5 days.

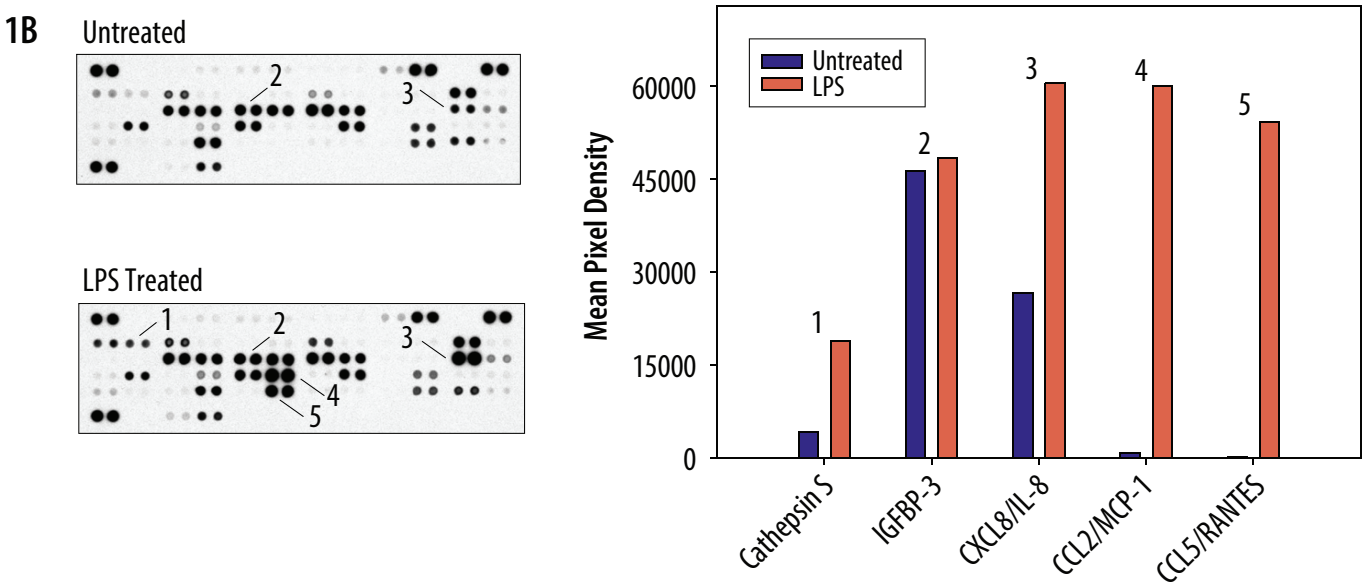


Figure 1B: SW480 human colorectal adenocarcinoma cells were untreated or treated with 100 ng/mL of LPS for 24 hours.

PROFILING PROTEINS IN CELL LYSATES

The Human Adipokine Array detects multiple adipokines 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. Profiles of mean spot pixel density were created using a transmission-mode scanner and image analysis software.

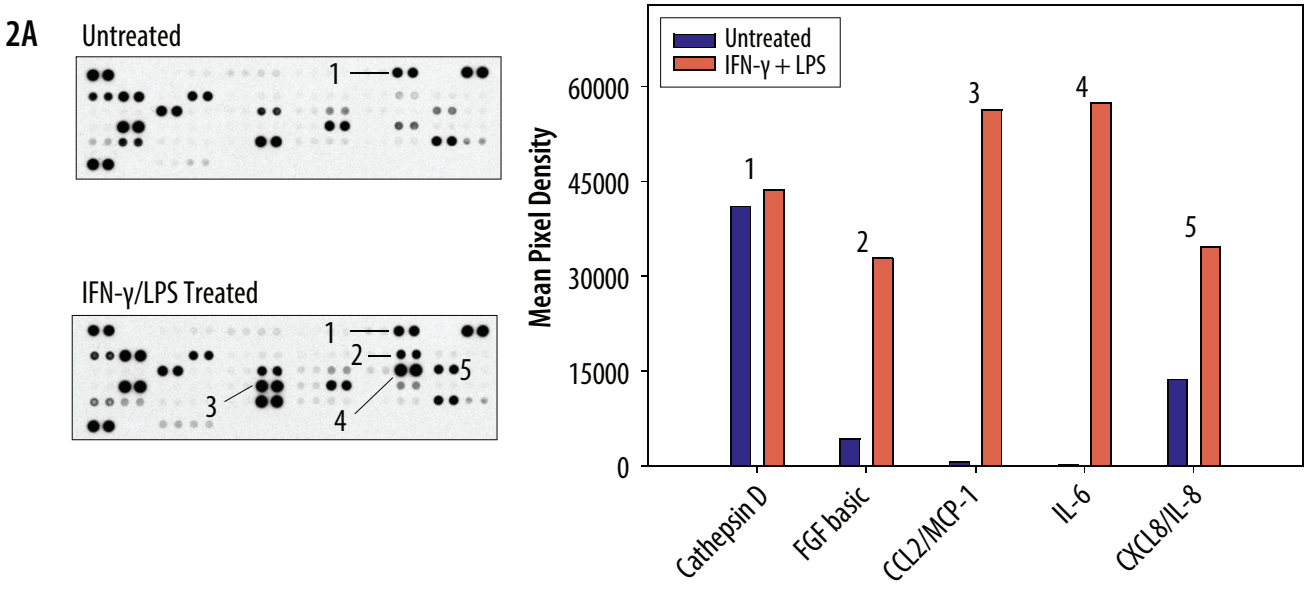


Figure 2A: THP-1 human acute monocytic leukemia cells were untreated or treated with 1 µg/mL of recombinant human IFN-γ (R&D Systems®, # 285-IF) for 8 hours, followed by 1 µg/mL of LPS for 16 hours.

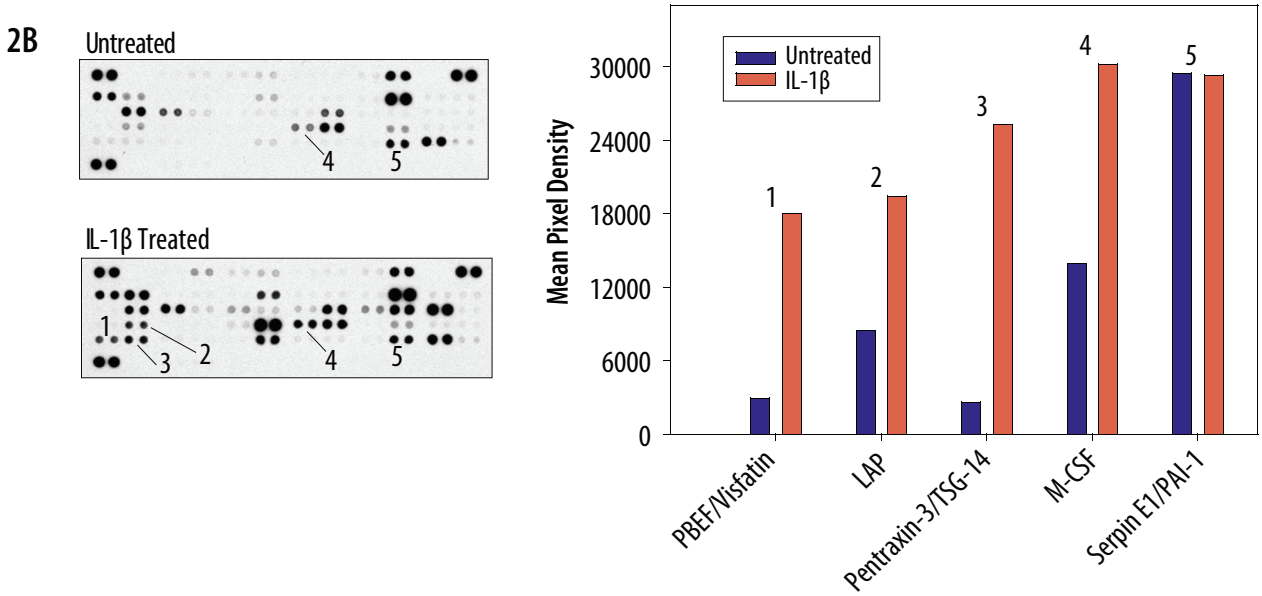
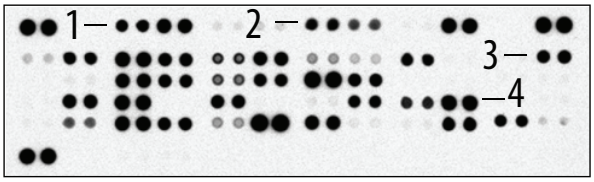


Figure 2B: MG63 human osteosarcoma cells were untreated or treated with 1 ng/mL of Recombinant Human IL-1β (R&D Systems, # 201-LB) for 48 hours.

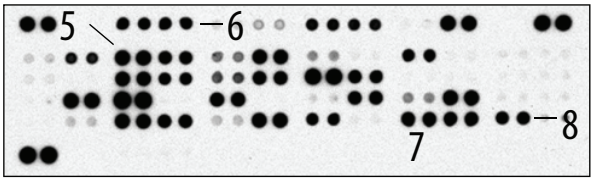
PROFILING PROTEINS IN BODY FLUIDS

The Human Adipokine Array detects multiple adipokines in serum, plasma, human milk, urine, and saliva samples. The sample type and quantity used are listed below. Data shown are from a five minute and exposure to X-ray film.

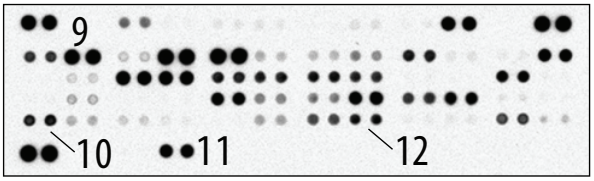
A. Serum, 200 µL per array



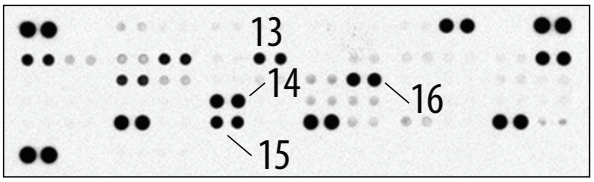
B. Plasma, 200 µL per array



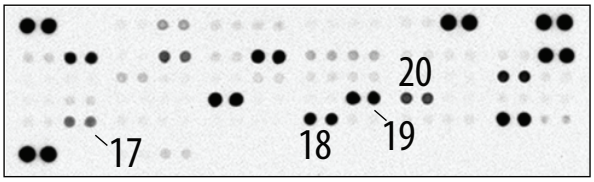
C. Human Milk, 200 µL per array



D. Urine, 200 µL per array



E. Saliva, 100 µL per array



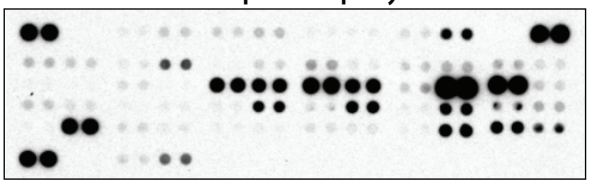
PROFILING PROTEINS IN BODY FLUIDS *CONTINUED*

		MEAN PIXEL DENSITY				
		Serum	Plasma	Human Milk	Urine	Saliva
1	Adiponectin/Acrp30	31,758	33,920	14,125	2704	926
2	Angiopoietin-like 3	30,119	29,921	209	450	1042
3	Fibrinogen	36,852	1326	34,886	40,357	46,775
4	Nidogen-1/Entactin	49,745	49,834	31,895	445	559
5	Chemerin	48,250	50,624	4553	5600	515
6	Angiopoietin-1	43,337	34,122	523	586	8594
7	Serpin A12	863	43,821	845	4043	1123
8	TIMP-1	29,870	36,714	19,157	45,524	39,114
9	Cathepsin S	32,749	26,739	49,608	4152	28,369
10	PBEF/Visfatin	601	485	25,075	504	1278
11	VEGF	300	657	37,835	237	3062
12	Serpin A8/AGT	1125	1038	21,487	3256	1229
13	DPPIV/CD26	39,312	47,095	5342	26,544	37,764
14	Lipocalin-2/NGAL	37,264	44,037	34,835	40,447	40,955
15	RAGE	10,686	5381	318	32,455	531
16	IGFBP-7/IGFBP-rp1	35,789	38,950	20,380	38,860	2130
17	Pentraxin-3/TSG-14	16,803	4944	5765	542	14,345
18	Resistin	43,166	32,493	17,963	46,224	30,675
19	MIF	31,265	40,881	37,993	4003	36,456
20	Myeloperoxidase	24,353	11,446	22,204	299	20,811

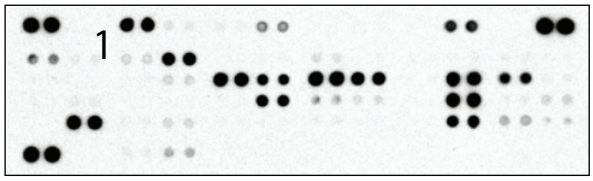
PROFILING PROTEINS DURING ADIPOCYTE DIFFERENTIATION

The Human Adipokine Array detects multiple adipokines secreted during the differentiation of pre-adipocytes to adipocytes. 500 µL of cell culture supernate was run on each array. Data shown are from a 5 minute exposure to X-ray film. Profiles of mean spot pixel density were created using a transmission-mode scanner and image analysis software.

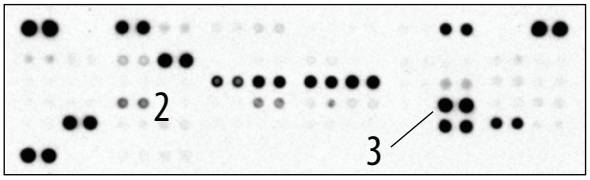
Undifferentiated pre-adipocytes



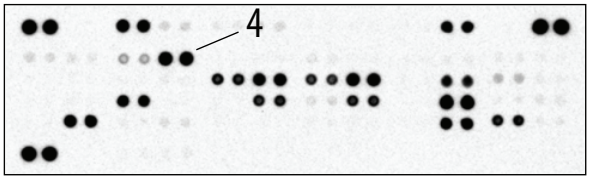
1 week old adipocytes

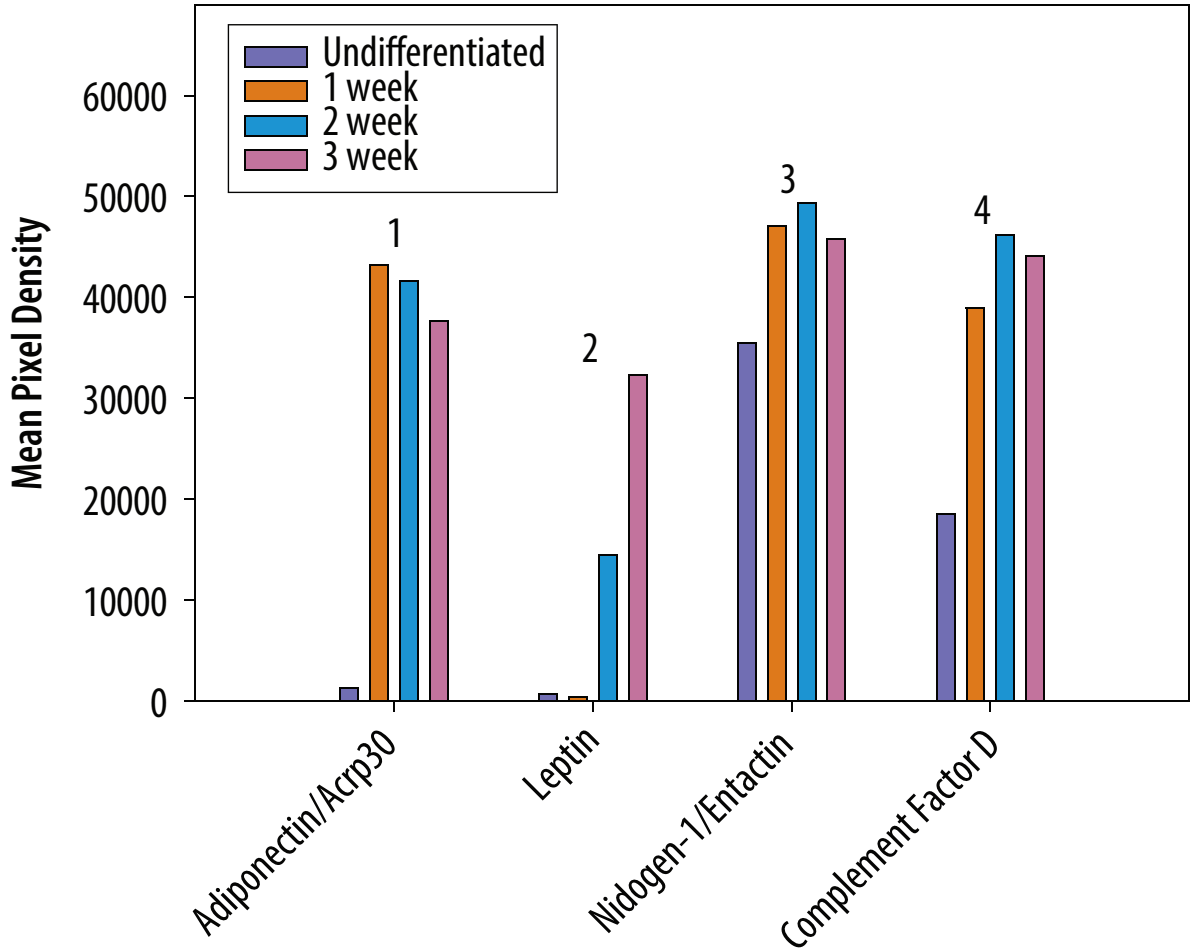


2 week old adipocytes

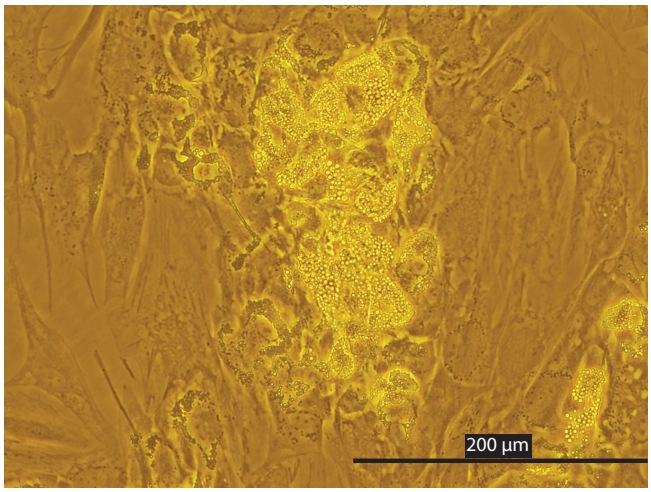


3 week old adipocytes

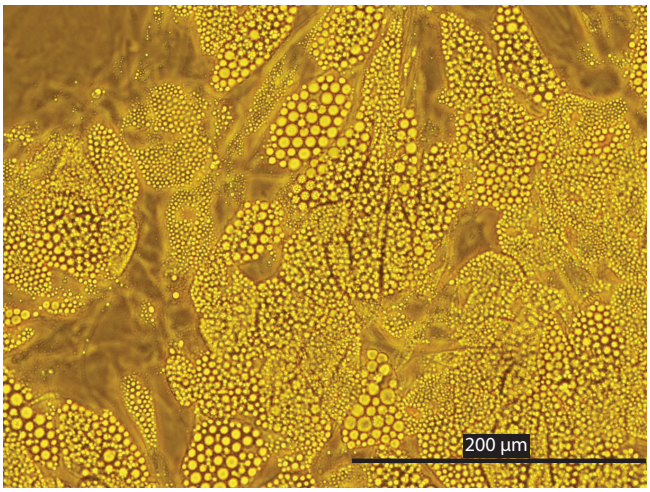




1 week old adipocytes



2 week old adipocytes

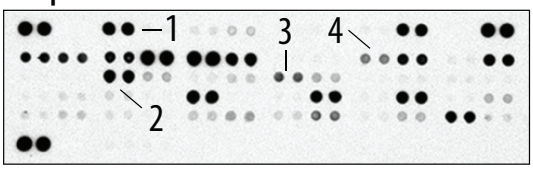


Phase-contrast microscopy images of 1 week old cultured adipocytes and 2 week old cultured adipocytes. Lipid droplets are more apparent at two weeks.

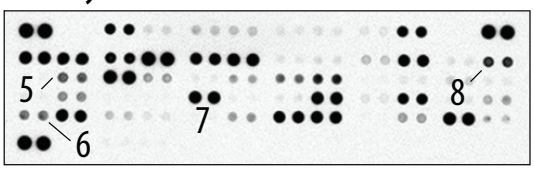
PROFILING PROTEINS IN TISSUE LYSATES

The Human Adipokine Array detects multiple adipokines in tissue lysates. 200 µg of tissue lysate was run on each array. Data shown are from a five minute exposure to X-ray film.

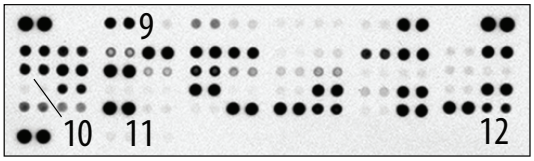
Adipose



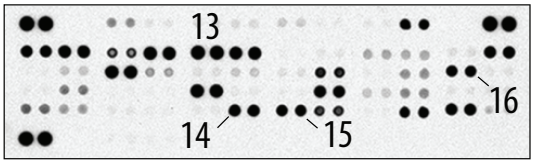
Kidney



Placenta



Liver



1	Adiponectin/Acrp30
2	ICAM-1
3	IGFBP-6
4	Fetuin B
5	HGF
6	PBEF/Visfatin
7	Lipocalin-2/NGAL
8	Fibrinogen
9	Angiopoietin-2
10	Growth Hormone
11	Pref-1/DLK-1/FA1
12	TIMP-3
13	C-Reactive Protein/CRP
14	CCL5/RANTES
15	Resistin
16	CXCL8/IL-8

APPENDIX

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

Coordinate	Analyte/Control	Entrez Gene ID	Alternate Nomenclature
A1, A2	Reference Spots	N/A	———
A5, A6	Adiponectin/Acrp30	9370	AdipoQ, ApM1, GBP28
A7, A8	Angiopoietin-1	284	ANGPT1
A9, A10	Angiopoietin-2	285	ANGPT2
A11, A12	Angiopoietin-like 2	23452	ANGPTL2, ANGRP2
A13, A14	Angiopoietin-like 3	27329	ANGPT5, Ang-5, Angiopoietin-5, ANGPTL3
A15, A16	BAFF/BLyS/TNFSF13B	10673	CD257, TALL1, THANK, ZTNF4
A17, A18	BMP-4	652	———
A19, A20	Cathepsin D	1509	CTSD
A23, A24	Reference Spots	N/A	———
B1, B2	Cathepsin L	1514	CTSL, CTSL1
B3, B4	Cathepsin S	1520	CTSS
B5, B6	Chemerin	5919	RARRES2, TIG-2
B7, B8	Complement Factor D	1675	Adipsin, AND, AMBP-1, CFD, PFD
B9, B10	C-Reactive Protein/CRP	1401	———
B11, B12	DPPIV/CD26	1803	DPP4
B13, B14	Endocan	11082	ESM1, ESM-1, IGFBP-rp6
B15, B16	EN-RAGE	6283	CAAF1, CAAFI, CAGC, CAGCS100, Calgranulin C, CGRP, MRP6, S100A12
B17, B18	Fetuin B	26998	FETUB
B19, B20	FGF basic	2247	FGF-2, FGF2AS, GFG1, HBGH-2, NUDT6, Prostatropin
B21, B22	FGF-19	9965	———
B23, B24	Fibrinogen	2243	———
C1, C2	Growth Hormone	2688	GH1, Somatotropin
C3, C4	HGF	3082	Hepatopoietin A, SF
C5, C6	ICAM-I/CD54	3383	———
C7, C8	IGFBP-2	3485	———
C9, C10	IGFBP-3	3486	———
C11, C12	IGFBP-4	3487	———
C13, C14	IGFBP-6	3489	———
C15, C16	IGFBP-rp1/IGFBP-7	3490	IGFBP7, Mac25, PSF
C17, C18	IL-1β/IL-1F2	3553	IL1B
C19, C20	IL-6	3569	BSF-2, IFN-β2, MGI-2A
C21, C22	CXCL8/IL-8	3576	GCP1, LAI, MDNCF, NAP1, NCF, TCF, TSG1
C23, C24	IL-10	3586	CSIF
D1, D2	IL-11	3589	AGIF
D3, D4	LAP (TGF-β1)	7040	———

continued on next page...

APPENDIX *CONTINUED*

Coordinate	Analyte/Control	Entrez Gene ID	Alternate Nomenclature
D5, D6	Leptin	3952	LEP, OB
D7, D8	LIF	3976	———
D9, D10	Lipocalin-2/NGAL	3934	LCN2, Siderocalin
D11, D12	CCL2/MCP-1	6347	MCAF
D13, D14	M-CSF	1435	CSF1, CSF-1
D15, D16	MIF	4282	———
D17, D18	Myeloperoxidase	4353	Lactoperoxidase, MPO
D19, D20	Nidogen-1/Entactin	4811	NID1
D21, D22	Oncostatin M (OSM)	5008	———
D23, D24	Pappalysin-1/PAPP-A	5069	ASBABP2, DIPLA1, IGFBP-4ase, PAPA, PAPPA, PAPP-A1
E1, E2	PBEF/Visfatin	10135	NAmPRTase, NAMPT, PBEF1
E3, E4	Pentraxin-3/TSG-14	5806	PTX3
E5, E6	Pref-1/DLK-1/FA1	8788	DLK1, DLK-1, FA1, pG2, ZOG
E7, E8	Proprotein Convertase 9/PCSK9	255738	FH3, HCHOLA3, NARC-1, PC9
E9, E10	RAGE	177	AGER
E11, E12	CCL5/RANTES	6352	SISd
E13, E14	Resistin	56729	ADSF; FIZZ3; RETN
E15, E16	Serpin A8/AGT	183	Angiotensin II, Angiotensinogen, Aogen, PAT
E17, E18	Serpin A12	145264	OL-64, Vaspin
E19, E20	Serpin E1/PAI-1	5054	Nexin, PLANH1
E21, E22	TIMP-1	7076	———
E23, E24	TIMP-3	7078	———
F1, F2	Reference Spots	N/A	———
F5, F6	TNF-α	7124	Cachetin, DIF, TNF, TNFA, TNFSF1A, TNFSF2
F7, F8	VEGF	7422	VAS, Vasculotropin, VEGFA, VPF
F23, F24	Negative Controls	N/A	———

NOTES