

# **Proteome Profiler™ Array**

## **Mouse Adipokine Array Kit**

Catalog Number ARY013

For the parallel determination of the relative levels of selected mouse 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 now a major public health concern. Analyzing the expression profile of obesity related proteins is essential for understanding the roles these molecules play in obesity per se and associated illnesses such as diabetes and cardiovascular disease. The Proteome Profiler™ Mouse Adipokine Array Kit is a rapid, sensitive, and economical tool to detect changes between samples. The relative expression levels of 38 mouse Adipokines can be determined simultaneously without performing numerous immunoprecipitations or Western blots. Each capture and detection antibody was carefully selected using both natural and recombinant proteins.

## PRINCIPLE OF THE ASSAY

Capture and control antibodies have been spotted in duplicate on nitrocellulose membranes. Cell culture supernates, cell lysates, serum samples, or tissue lysates are diluted, mixed with a cocktail of biotinylated detection antibodies, and incubated overnight with the Proteome Profiler Mouse 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.**
- 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 Adipokine 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. However, until these proteins have been tested with the Mouse Adipokine Array, the possibility of interference cannot be excluded.
- For a procedure demonstration video, please visit:  
[www.RnDSystems.com/ProteomeProfilerVideo](http://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 Adipokine Array	893371	4 nitrocellulose membranes each containing 38 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. <i>May contain a precipitate. Mix well before and during use.</i>	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. <i>May turn yellow over time.</i>	
Detection Antibody Cocktail, Mouse Adipokine Array	893370	1 vial of a biotinylated antibody cocktail; lyophilized	
Streptavidin-HRP	893019	200 µL of streptavidin conjugated to horseradish-peroxidase.	
Chemi Reagent 1	894287	1 vial (2.5 mL)	
Chemi Reagent 2	894288	1 vial (2.5 mL)	Store at room temperature.
4-Well Rectangular Multi-dish	607544	Clear 4-well rectangular multi-dish.	
Transparency Overlay Template	607680	1 transparency overlay template for coordinate reference.	

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

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

## 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 Mouse 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  $\mu$ L for cell culture supernates, 100-500  $\mu$ g for cell and tissue lysates, and 50-200  $\mu$ L for serum samples. Adiponectin, CRP, Fetuin A, PTX2/SAP, and RBP4 are highly abundant circulating proteins in serum and exceed the optimal working range of this kit. If relative values of these five analytes are to be obtained, a separate array membrane should be used with 1-20  $\mu$ L of serum sample.

**Cell Culture Supernates** - Remove particulates by centrifugation. Assay immediately or aliquot and store samples at  $\leq -20$  °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  $1 \times 10^7$  cells/mL in lysis buffer. Pipette up and down to resuspend and rock the lysates gently at 2-8 °C for 30 minutes. Microcentrifuge at 14,000 x g for 5 minutes, and transfer the supernate into a clean test tube. Quantitation of sample protein concentration using a total protein assay is recommended. Assay immediately or aliquot and store at  $\leq -70$  °C. Avoid repeated freeze-thaw cycles.

**Serum** - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 x g. Remove serum and assay immediately or aliquot and store samples at  $\leq -20$  °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  $\leq -70$  °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  $\leq -70$  °C. Avoid repeated freeze-thaw cycles.

## 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 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.

## REAGENT PREPARATION

**Bring all reagents to room temperature before use.**

**Mouse Adipokine Array** - Four nitrocellulose membranes each containing 38 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 100  $\mu$ L 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.**

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 15  $\mu$ L of reconstituted Mouse 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

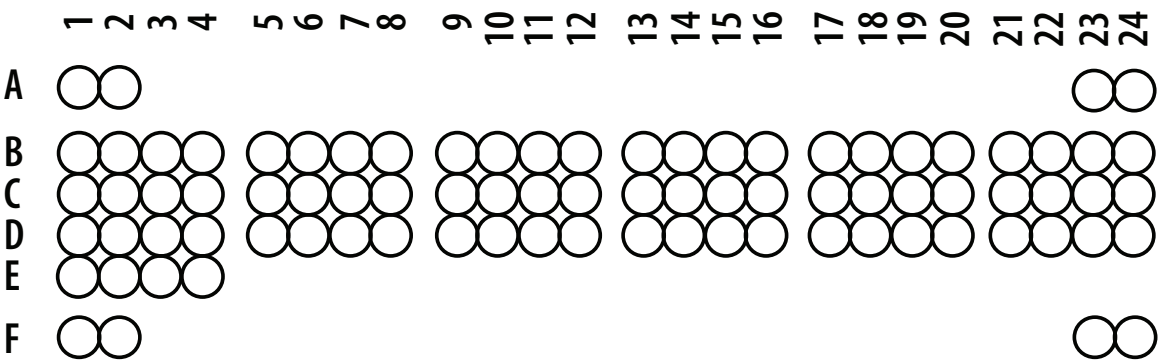
The positive signals seen on developed film can be quickly identified by placing the transparency overlay template on the array image and aligning it with the pairs of reference spots in three corners of each array. The stamped identification number on the array should be placed on the left hand side.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

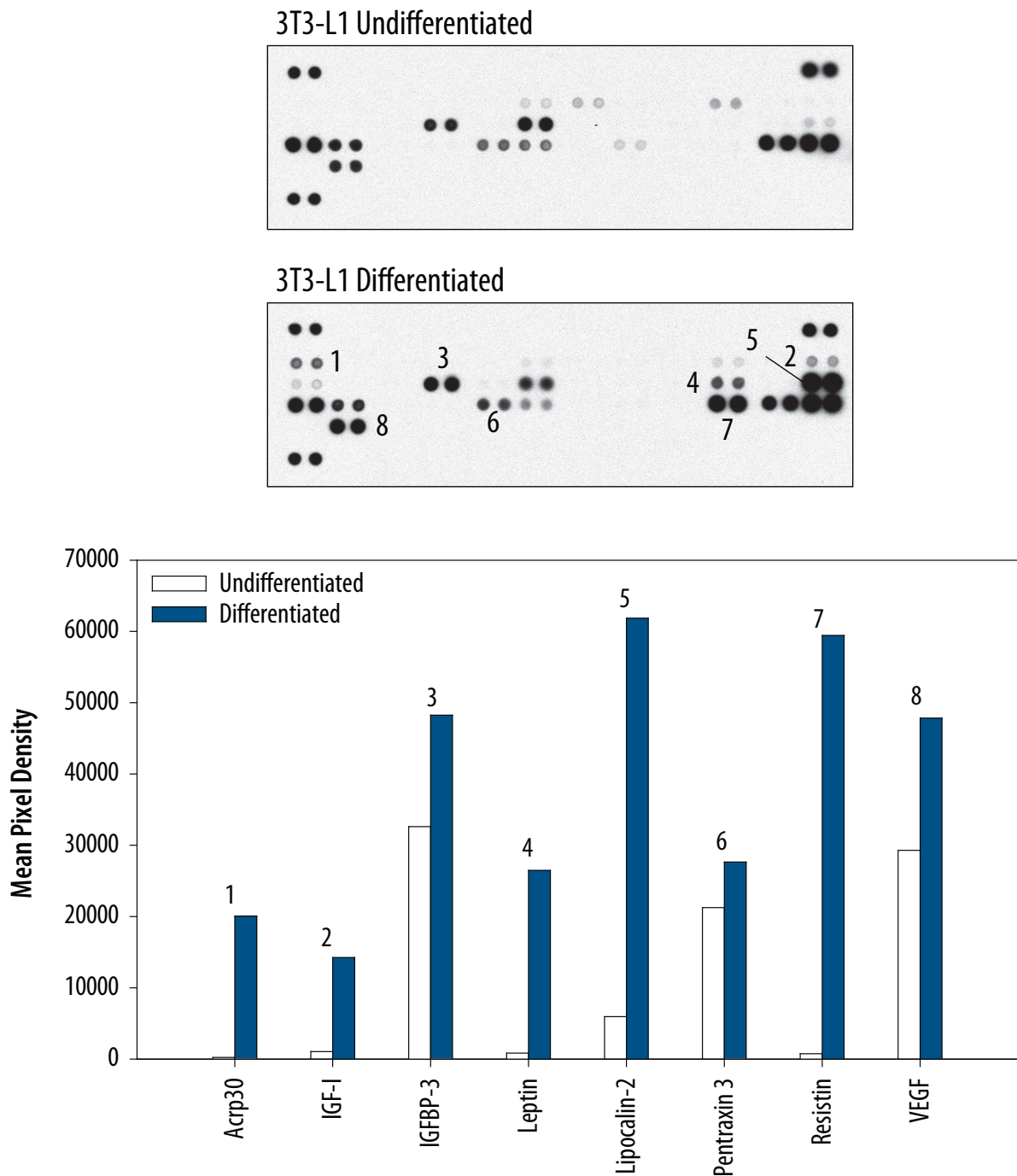
- 1. Create a template to analyze pixel density in each spot of the array.
- 2. Export signal values to a spreadsheet file for manipulation in a program such as Microsoft Excel.
- 3. Determine the average signal (pixel density) of the pair of duplicate spots representing each adipokine.
- 4. Subtract 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.

Mouse Adipokine Array Coordinates



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

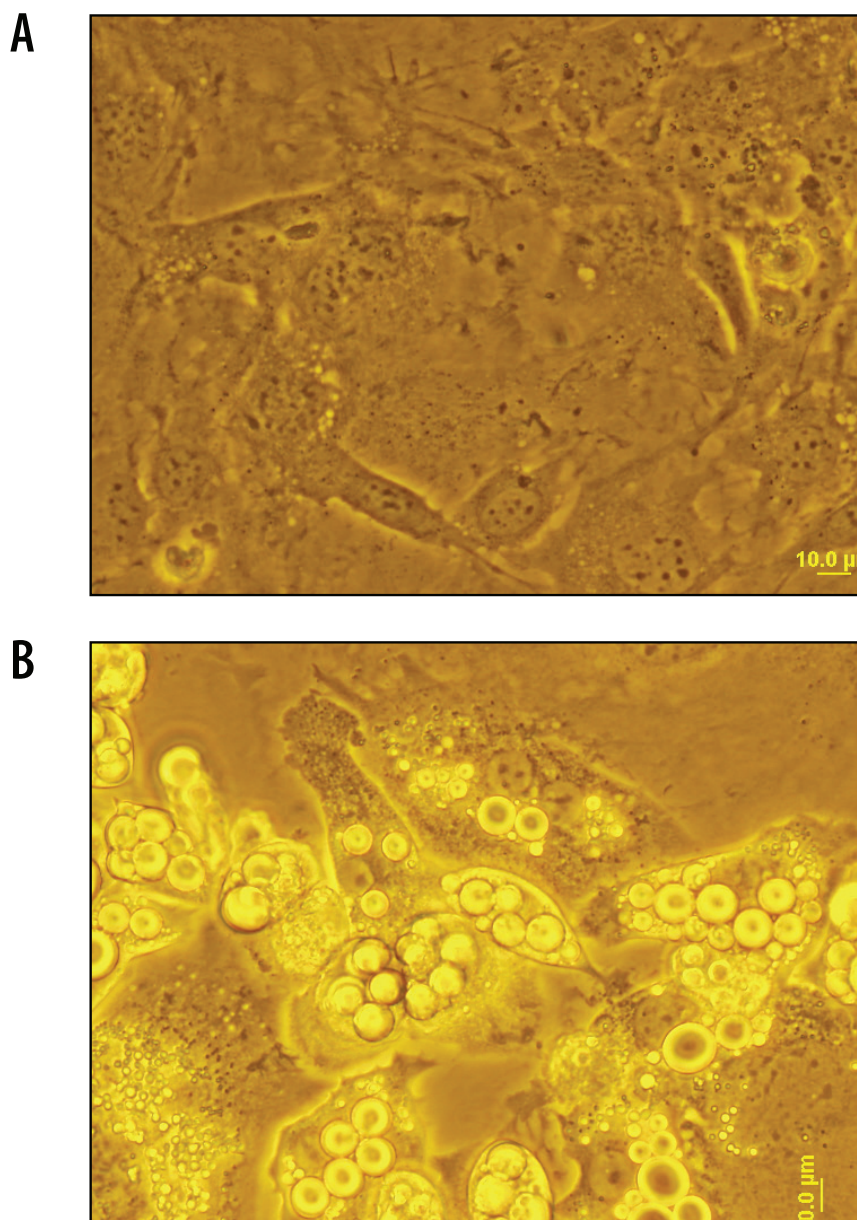
# PROFILING PROTEINS IN CELL CULTURE SUPERNATES



**Figure 1: The Mouse Adipokine Array detects multiple analytes in cell culture supernates.**

3T3-L1 mouse embryonic fibroblast adipose-like cells were either untreated or treated (differentiated) with 115 µg/mL of methylisobutylxanthine, 10 µg/mL of insulin, and 390 ng/mL of dexamethasone for 48 hours followed by the addition of 10 µg/mL insulin for the next 48 hours. The cells were then grown in standard media with media changes every 48 hours for an additional 96 hours.

500 µL of cell culture supernate was run on each array. Data shown are from a 2 minute exposure to X-ray film.

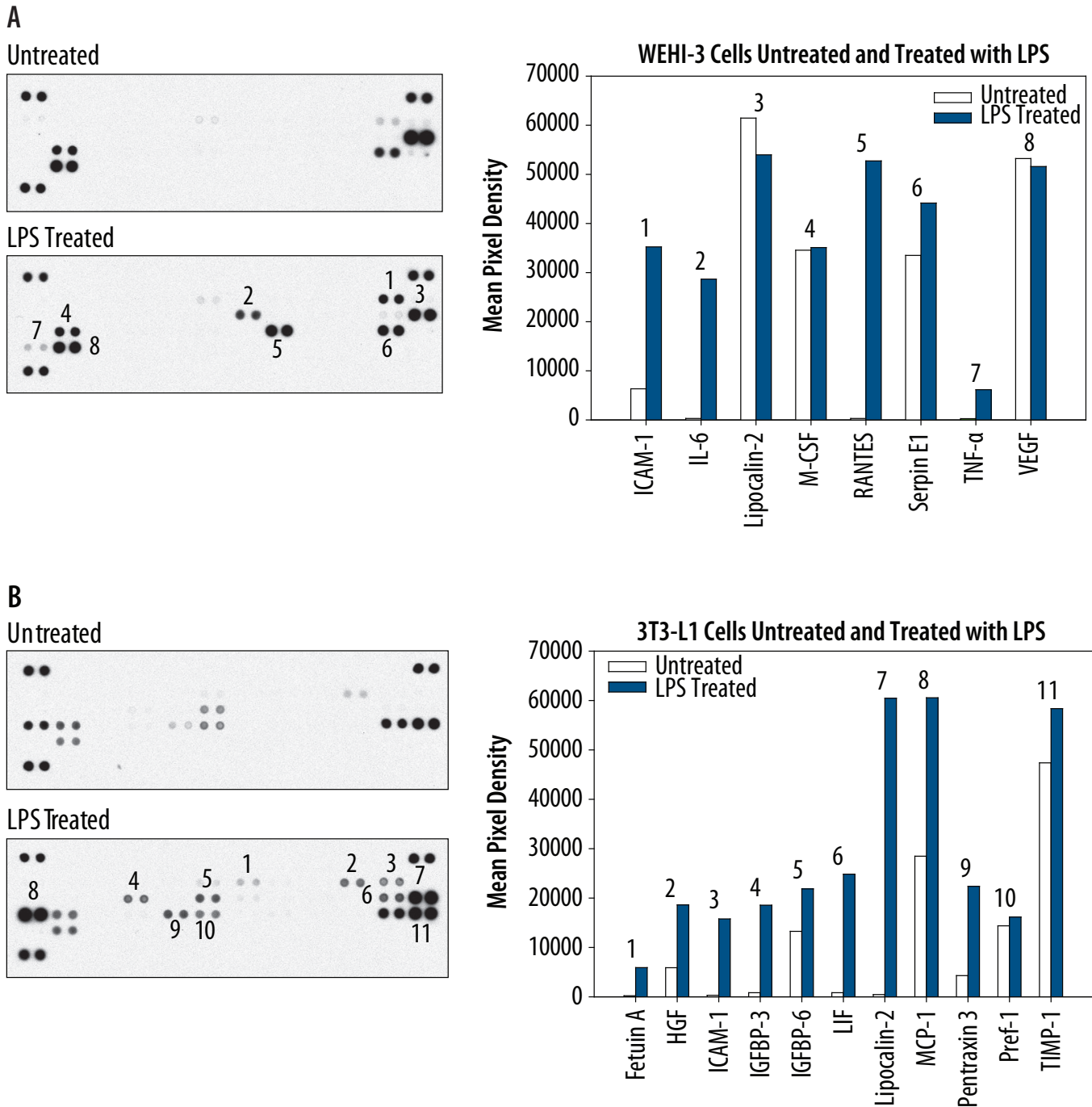


**Figure 2:** Phase-contrast microscopy images of the live 3T3-L1 mouse embryonic fibroblast adipose-like cells used to obtain the cell culture supernates in Figure 1.

**A.** Undifferentiated 3T3-L1 cells

**B.** Differentiated 3T3-L1 cells (visible lipid droplets)

## PROFILING PROTEINS IN CELL LYSATES



**Figure 3: The Mouse Adipokine Array detects multiple analytes in untreated and treated cell lysates.**

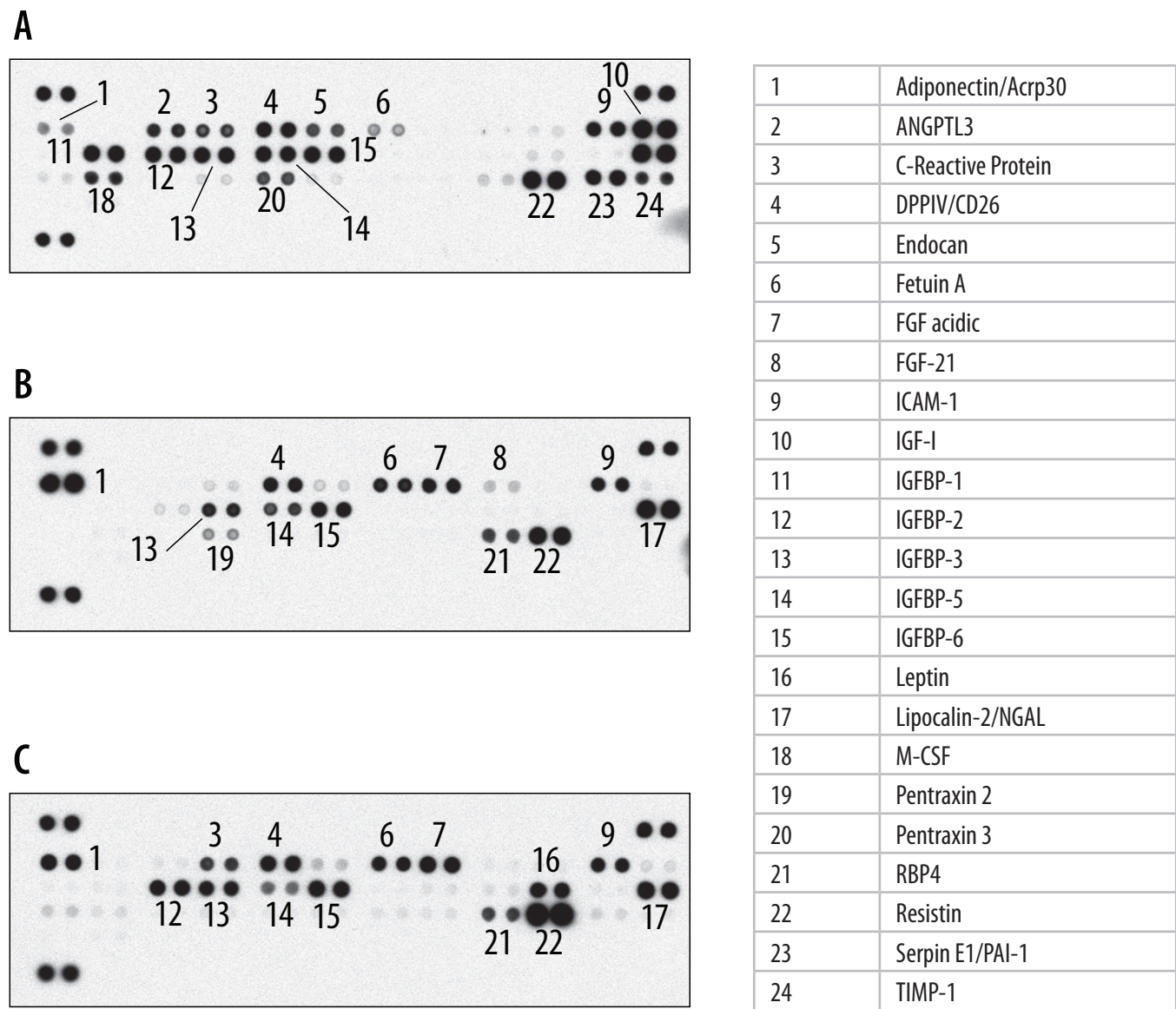
**A.** WEHI-3 mouse myelomonocytic leukemia cells were either untreated or treated with 100 ng/mL LPS for 24 hours. 300  $\mu$ g of cell lysate was run on each array.

**B.** 3T3-L1 mouse embryonic fibroblast adipose-like cells were either untreated or treated with 100 ng/mL LPS for 24 hours. 400  $\mu$ g of cell lysate was run on each array.

Data shown are from a 2 minute exposure to X-ray film.



PROFILING PROTEINS IN SERUM AND TISSUE LYSATES



**Figure 4: The Mouse Adipokine Array detects multiple analytes in serum and tissue lysates.**

- A.** 100  $\mu$ L of normal mouse serum was run on the array.
- B.** Adipose tissue was excised from a normal mouse and prepared as described in the Sample Collection and Storage section. 400  $\mu$ g of lysate was run on the array.
- C.** White adipose tissue from an 8 week old female mouse homozygous for the Db allele (a mutation in the Leptin receptor and common model of Type II Diabetes) was excised and prepared as described in the Sample Collection and Storage section. This sample was collected independently of the sample in B. 400  $\mu$ g of lysate was run on the array.

Data shown are from a 2 minute exposure to X-ray film.

## APPENDIX

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

Coordinate	Analyte/Control	Alternate Nomenclature
A1, A2	Reference Spots	—
A23, A24	Reference Spots	—
B1, B2	Adiponectin	Acrp30/AdipoQ
B3, B4	AgRP	ART
B5, B6	Angiopoietin-like 3	ANGPTL3
B7, B8	C-Reactive Protein	CRP
B9, B10	DPPIV	CD26/DPP4
B11, B12	Endocan	ESM-1
B13, B14	Fetuin A	AHSG
B15, B16	FGF acidic	FGF-1
B17, B18	FGF-21	—
B19, B20	HGF	—
B21, B22	ICAM-1	CD54
B23, B24	IGF-I	Somatomedin C
C1, C2	IGF-II	Somatomedin A
C3, C4	IGFBP-1	—
C5, C6	IGFBP-2	—
C7, C8	IGFBP-3	—
C9, C10	IGFBP-5	—
C11, C12	IGFBP-6	—
C13, C14	IL-6	—
C15, C16	IL-10	—
C17, C18	IL-11	—
C19, C20	Leptin	OB
C21, C22	LIF	—
C23, C24	Lipocalin-2	NGAL

continued on next page...

APPENDIX CONTINUED

Coordinate	Analyte/Control	Alternate Nomenclature
D1, D2	MCP-1	CCL2/JE
D3, D4	M-CSF	CSF-1
D5, D6	Oncostatin M	OSM
D7, D8	Pentraxin 2	PTX2/SAP
D9, D10	Pentraxin 3	PTX3/TSG-14
D11, D12	Pref-1	DLK-1/FA1
D13, D14	RAGE	—
D15, D16	RANTES	CCL5
D17, D18	RBP4	—
D19, D20	Resistin	ADSF/FIZZ3
D21, D22	Serpin E1	PAI-1
D23, D24	TIMP-1	—
E1, E2	TNF- $\alpha$	TNFSF1A
E3, E4	VEGF	VEGF-A
F1, F2	Reference Spots	—
F23, F24	PBS (Negative Control)	Control (-)

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