

Proteome Profiler™ Array

Mouse Angiogenesis Array Kit

Catalog Number ARY015

For the parallel determination of the relative levels of selected mouse angiogenesis-related proteins.

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 angiogenesis-related proteins is essential for understanding the roles these molecules play in mechanisms related to the physiological process of developing new blood vessels. The Mouse Angiogenesis Array is a rapid, sensitive, and economical tool to simultaneously detect changes between samples. The relative expression levels of 53 mouse angiogenesis related proteins can be determined 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 Angiogenesis 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 Angiogenesis 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 Angiogenesis Array, the possibility of interference cannot be excluded.

PRECAUTION

Chemi Reagents 1 and 2 contain Boric Acid which is suspected of damaging fertility or the unborn child. Do not handle until all safety precautions in the MSDS have been read and understood. Wear protective gloves, clothing, eye, and face protection when using these reagents.

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
Mouse Angiogenesis Array	893761	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 be stored for up to 3 months at 2-8 °C.* <i>May contain a precipitate. Mix well before and during use.</i>
Array Buffer 6	893573	2 vials (21 mL/vial)	May be stored for up to 3 months at 2-8 °C.*
Wash Buffer Concentrate, 25X	895003	2 vials (21 mL/vial)	
Detection Antibody Cocktail, Mouse Angiogenesis Array	893760	1 vial	
Streptavidin-HRP	890803	1 vial	
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	1 dish	
Transparency Overlay Template	607682	1 template	

* 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
- 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 (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

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

- 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)

If using tissue lysate samples, the following are 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 & STORAGE

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

Since the Mouse Angiogenesis 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 μ L for cell culture supernates, 100-300 μ g for cell and tissue lysates, and 50-200 μ L for serum 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 1×10^7 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 supernatant into a clean test tube. Quantitation of sample protein concentrations using a total protein assay is recommended. Assay 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 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 Angiogenesis Array - Four nitrocellulose membranes each containing 53 different capture antibodies 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 in 100 μ 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. Dilute 40 mL of 25X Wash Buffer Concentrate into 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.**

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 μ L 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.

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

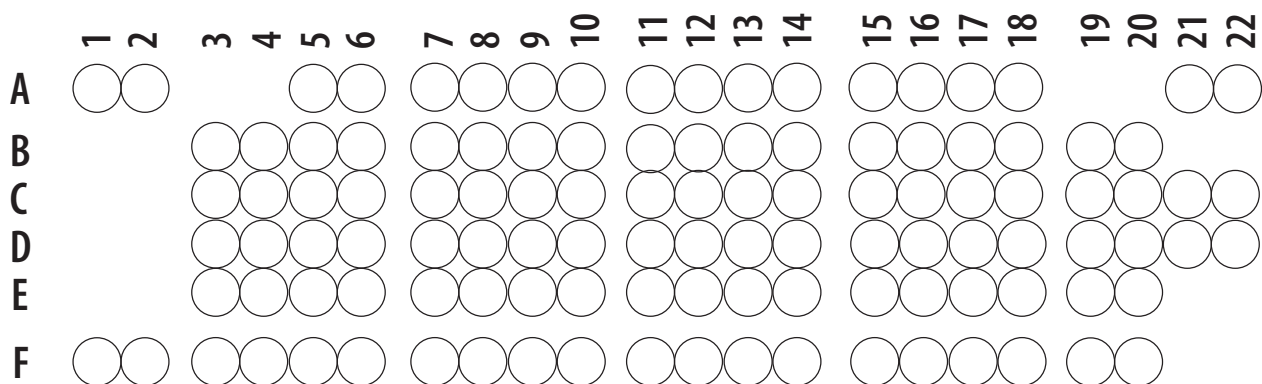
The positive signals seen on developed film can be quickly identified by placing the transparency overlay template on the array image and aligning it with the pairs of reference spots in three corners of each array. The stamped identification number on the array should be placed on the left hand side.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

Mouse Angiogenesis 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

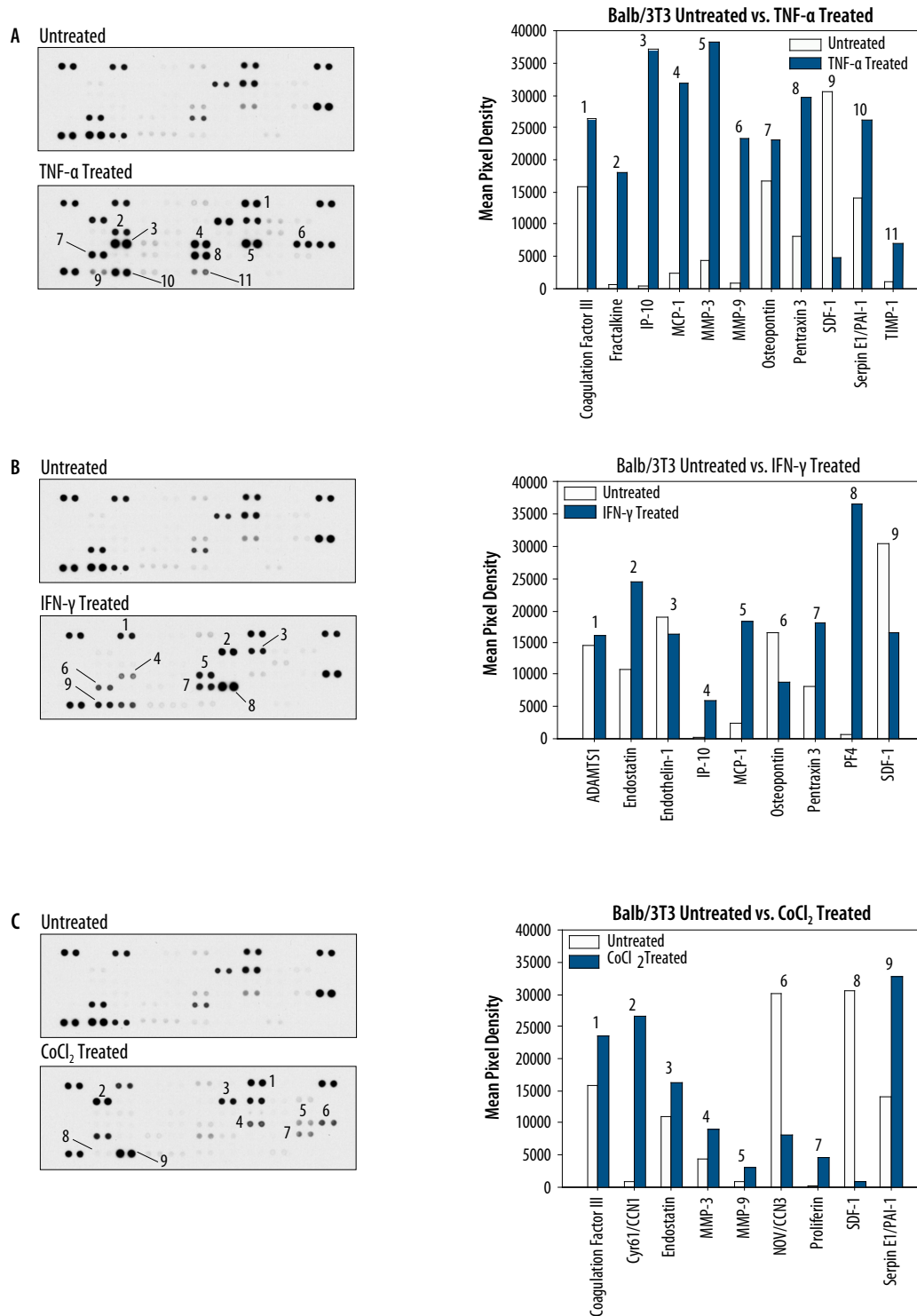


Figure 1: The Mouse Angiogenesis Array detects multiple analytes in cell lysates.

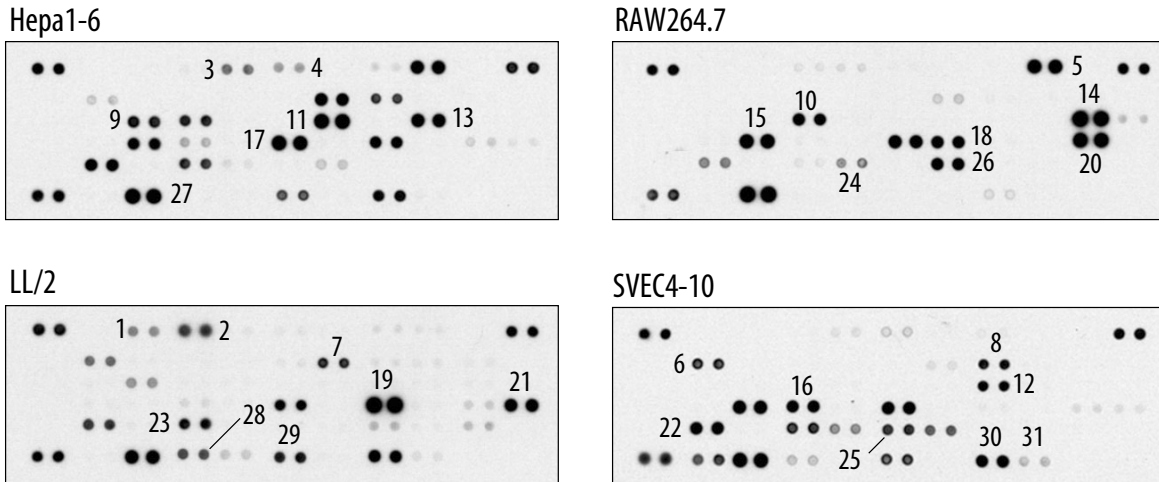
Balb/3T3 mouse embryonic fibroblast cells were either untreated or treated as indicated below. 200 µg of cell lysate was run on each array. Data shown are from a 2 minute exposure to X-ray film.

A. Recombinant mouse TNF-α (R&D Systems, Catalog # 410-MT); 100 ng/mL for 24 hours.

B. Recombinant mouse IFN-γ (R&D Systems, Catalog # 485-MI); 100 ng/mL for 24 hours.

C. CoCl₂; 200 µM for 24 hours.

PROFILING PROTEINS IN CELL CULTURE SUPERNATES



1	ADAMTS1	12	IGFBP-2	23	PDGF-AA
2	Amphiregulin	13	IGFBP-3	24	PDGF-AB/PDGF-BB
3	Angiogenin	14	IL-1 α	25	Pentraxin 3
4	Angiopoietin-1	15	IP-10	26	PF4
5	CXCL16	16	KC	27	Serpin E1/PAI-1
6	Cyr61/CCN1	17	MCP-1	28	Serpin F1/PEDF
7	Endostatin	18	MIP-1 α	29	TIMP-1
8	Endothelin-1	19	MMP-3	30	VEGF
9	Fractalkine	20	MMP-9	31	VEGF-B
10	GM-CSF	21	NOV/CCN3		
11	IGFBP-1	22	Osteopontin		

Figure 2: The Mouse Angiogenesis Array detects multiple analytes in cell culture supernates. Conditioned media (400-750 μ L) from various cell types was run on each array. Data shown are from untreated cells with the exception of the RAW 264.7 mouse monocyte/macrophage cells, which were treated with 100 ng/mL of LPS for 24 hours.

PROFILING PROTEINS IN SERUM

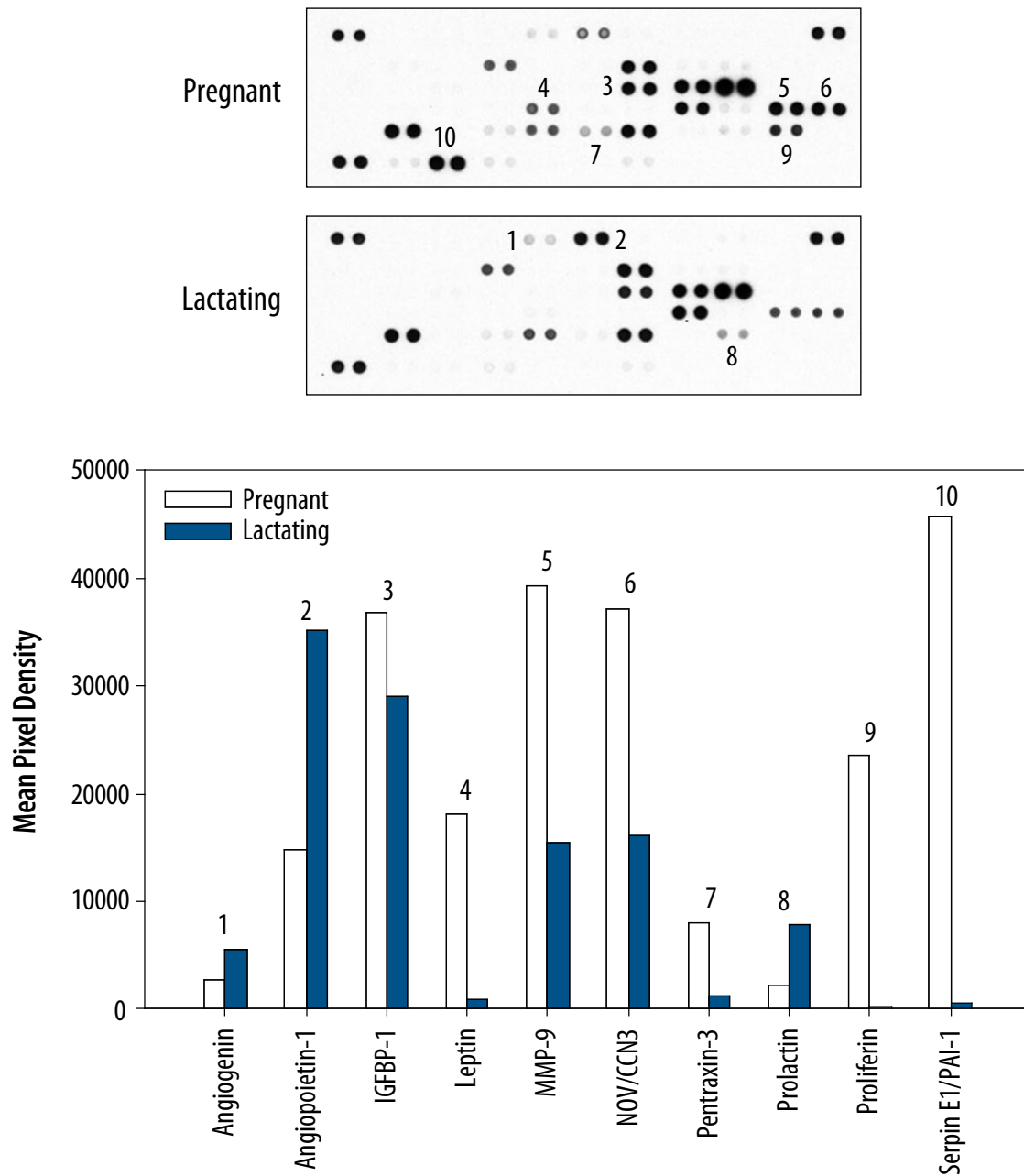


Figure 3: The Mouse Angiogenesis Array detects multiple analytes in serum samples.

25 μ L of serum from pregnant or lactating mice was run on each array. Data shown are from a 4 minute exposure to X-ray film.

PROFILING PROTEINS IN TISSUE LYSATES

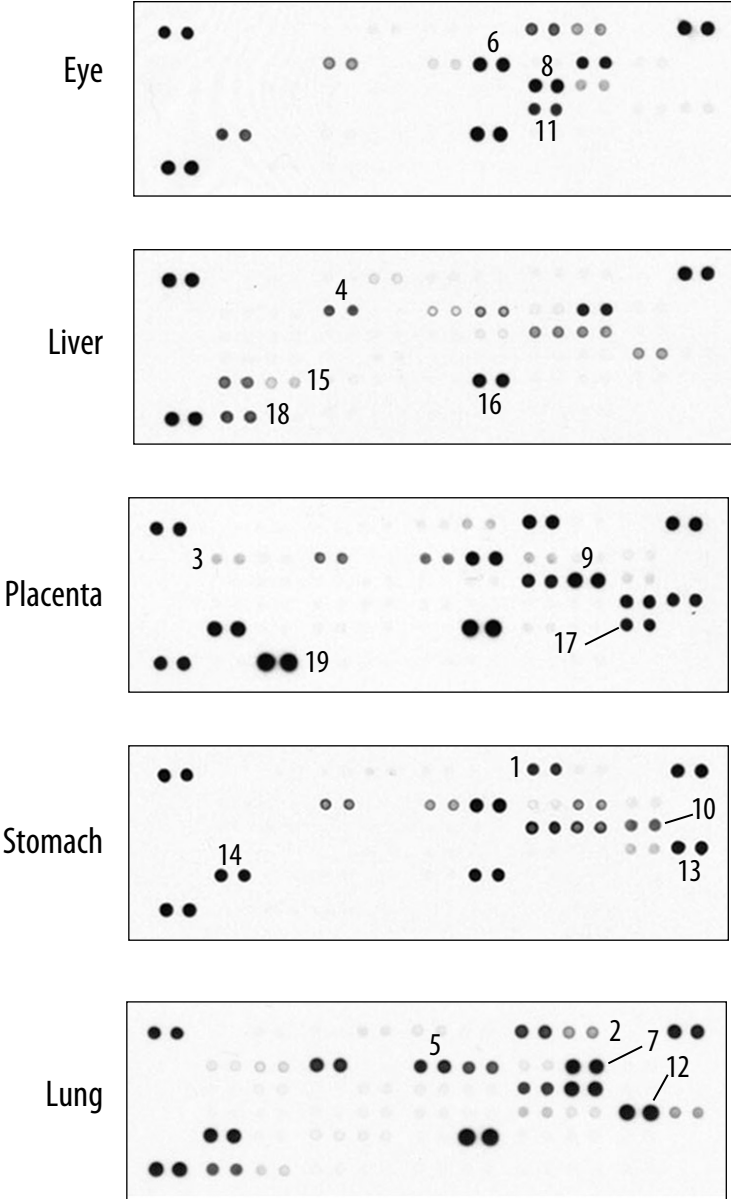


Figure 4: The Mouse Angiogenesis Array detects multiple analytes in tissue lysates. 200 µg of tissue lysate was run on each array. Data shown are from a 4 minute exposure to X-ray film.

PROFILING PROTEINS IN TISSUE LYSATES *CONTINUED*

		MEAN PIXEL DENSITY				
		Eye	Liver	Placenta	Stomach	Lung
1	Coagulation Factor III/TF	17,609	560	32,836	20,244	27,388
2	CXCL16	10,119	484	156	855	12,680
3	Cyr61/CCN1	169	169	4188	183	1875
4	DPPIV/CD26	12,299	14,351	12,858	12,479	30,329
5	Endoglin	2528	4678	12,178	9188	29,366
6	Endostatin/Collagen XVIII	32,669	11,251	32,440	31,922	21,224
7	FGF acidic	23,930	23,540	821	10,657	37,705
8	IGFBP-2	31,066	12,357	28,492	23,145	23,720
9	IGFBP-3	6676	11,315	38,082	18,605	38,587
10	IL-1 α	92	234	2357	16,083	286
11	MMP-3	21,462	248	137	447	5171
12	MMP-9	495	9896	28,360	4301	44,580
13	NOV/CCN3	966	378	26,768	28,062	9749
14	Osteopontin	18,442	15,862	36,708	26,194	34,038
15	PD-ECGF	183	4458	6	105	191
16	PF4	35,661	31,115	45,519	27,189	49,021
17	Proliferin	51	213	25,055	131	168
18	SDF-1	59	19,019	284	150	20,177
19	Serpin E1/PAI-1	343	278	51,974	205	3202

APPENDIX

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

Coordinate	Analyte/Control	Alternate Nomenclature
A1, A2	Reference Spots	—
A5, A6	ADAMTS1	METH1
A7, A8	Amphiregulin	AR
A9, A10	Angiogenin	ANG
A11, A12	Angiopoietin-1	Ang-1
A13, A14	Angiopoietin-3	Ang-3
A15, A16	Coagulation Factor III	Tissue Factor, TF
A17, A18	CXCL16	—
A21, A22	Reference Spots	—
B3, B4	Cyr61	CCN1, IGFBP-10
B5, B6	DLL4	—
B7, B8	DPPIV	CD26
B9, B10	EGF	—
B11, B12	Endoglin	CD105
B13, B14	Endostatin/Collagen XVIII	—
B15, B16	Endothelin-1	ET-1
B17, B18	FGF acidic	FGF-1, ECGF, HBGF-1
B19, B20	FGF basic	FGF-2
C3, C4	KGF	FGF-7
C5, C6	Fractalkine	CX3CL1
C7, C8	GM-CSF	—
C9, C10	HB-EGF	—
C11, C12	HGF	Hepatopoietin A
C13, C14	IGFBP-1	—
C15, C16	IGFBP-2	—
C17, C18	IGFBP-3	—
C19, C20	IL-1 α	IL-1F1
C21, C22	IL-1 β	IL-1F2
D3, D4	IL-10	CSIF
D5, D6	IP-10	CXCL10, CRG-2
D7, D8	KC	CXCL1, CINC-1, GRO α
D9, D10	Leptin	OB
D11, D12	MCP-1	CCL2/JE
D13, D14	MIP-1 α	CCL3
D15, D16	MMP-3 (pro and mature form)	—

continued on next page....

APPENDIX CONTINUED

Coordinate	Analyte/Control	Alternate Nomenclature
D17, D18	MMP-8 (pro form)	—
D19, D20	MMP-9 (pro and active form)	—
D21, D22	NOV	CCN3, IGFBP-9
E3, E4	Osteopontin	OPN
E5, E6	PD-ECGF	—
E7, E8	PDGF-AA	—
E9, E10	PDGF-AB/PDGF-BB	—
E11, E12	Pentraxin-3	PTX3, TSG-14
E13, E14	Platelet Factor 4	CXCL4, PF4
E15, E16	PIGF-2	—
E17, E18	Prolactin	PRL
E19, E20	Proliferin	—
F1, F2	Reference Spots	—
F3, F4	SDF-1	CXCL12
F5, F6	Serpin E1	PAI-1
F7, F8	Serpin F1	PEDF
F9, F10	Thrombospondin-2	TSP-2
F11, F12	TIMP-1	—
F13, F14	TIMP-4	—
F15, F16	VEGF	VPF
F17, F18	VEGF-B	VRF
F19, F20	Negative Control	Control (-)

NOTES

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