

# Proteome Profiler™ Array

## Human Angiogenesis Array Kit

Catalog Number ARY007

**For the parallel determination of the relative levels of human 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 profiles of angiogenesis-related proteins is helpful in understanding the roles these molecules play in mechanisms related to the physiological process of developing new blood vessels. The Human Angiogenesis Array kit is a rapid, sensitive, and economical tool to simultaneously detect the relative levels of expression of 55 angiogenesis related proteins without performing individual assays for each protein.

## PRINCIPLE OF THE ASSAY

Carefully selected capture antibodies have been spotted in duplicate on nitrocellulose membranes. Samples are diluted and mixed with a cocktail of biotinylated detection antibodies. The sample/antibody mixture is then incubated with the Human Angiogenesis Array. Any protein/detection antibody complex present is bound by its cognate immobilized capture antibody on the membrane. Following a wash to remove unbound material, Streptavidin-HRP and chemiluminescent detection reagents are added sequentially. Light is produced at each spot in proportion to the amount of analyte bound. Refer to the Appendix on page 15 for a list and coordinates of target capture antibodies.

## TECHNICAL HINTS AND LIMITATIONS

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- **For recommended chemiluminescent detection reagents, see the Other Materials Required section. The use of other chemiluminescent reagents may cause either increased background or diminished signal, depending on the reagent. For optimal results, use the recommended chemiluminescent reagents.**
- This kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- Any variation in buffers, operator, pipetting technique, washing technique, and incubation time or temperature can alter the performance of the kit.
- The Human Angiogenesis Array membranes are validated for single use only.
- Always use gloved hands and flat-tipped tweezers to handle the membranes.
- Pick up the membrane from the edge on the side with the identification number.
- A thorough and consistent wash technique is essential for proper assay performance. Individual arrays should be washed in separate containers to prevent high 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 factors have been tested with the Proteome Profiler Array, the possibility of interference cannot be excluded.

## MATERIALS PROVIDED

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

Component	Part #	Storage Conditions	Amount Provided
Human Angiogenesis Array	893313	2-8° C	4 membranes
Array Buffer 4	895022	2-8° C	1 vial (21 mL)
Array Buffer 5	895876	2-8° C	1 vial (21 mL)
Array Buffer 7	895924	2-8° C	1 vial (21 mL)
Wash Buffer Concentrate, 25X	895003	2-8° C	2 vials (21 mL each)
Detection Antibody Cocktail	893312	2-8° C	1 vial
Streptavidin-HRP	890803	2-8° C	1 vial
4-Well Rectangular Multi-dish	607544	Room Temperature	1 dish
Transparency Overlay Template	607586	Room Temperature	1 template

## OTHER MATERIALS REQUIRED

- **Chemiluminescent detection reagent (Pierce, Catalog # 32106 or Amersham, Catalog # RPN2132)**
- Aprotinin (Sigma, Catalog # A6279)
- Leupeptin (Sigma, Catalog # L8511)
- Pepstatin (Sigma, Catalog # P4265)
- Igepal<sup>®</sup> CA-630 (Sigma, Catalog # I3021)
- Pipettes and pipette tips
- Gloves
- Phosphate-Buffered Saline (PBS)
- Deionized or distilled water
- Rocking platform shaker
- Microcentrifuge
- Collection device for saliva samples that has no protein binding or filtering capabilities such as a Salivette<sup>®</sup> or equivalent.
- A plastic container with the capacity to hold 50 mL (for washing the arrays)
- Plastic transparent sheet protector
- Plastic wrap
- Autoradiography cassette
- Film developer
- X-ray film (Kodak<sup>®</sup> BioMax<sup>™</sup> 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<sup>®</sup> Excel

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

- Lysis buffer (1% Igepal, 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)

## SAMPLE COLLECTION AND STORAGE

Since the Human 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  $\mu\text{L}$  for cell culture supernates, 100-300  $\mu\text{g}$  for cell and tissue lysates, and 50-200  $\mu\text{L}$  for serum, plasma, and saliva samples.

**Cell Culture Supernates** - Remove particulates by centrifugation and assay immediately or aliquot and store samples at  $\leq -20^\circ\text{C}$ . 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  $2-8^\circ\text{C}$  for 30 minutes. Microcentrifuge at  $14,000 \times g$  for 5 minutes, and transfer the supernate into a clean test tube. Quantitation of sample protein concentrations using a total protein assay is recommended. For the initial experiment, use a quantity of lysate similar to that used for Western blot. Use the lysates immediately or aliquot and store at  $\leq -70^\circ\text{C}$ . Avoid repeated freeze-thaw cycles.

**Tissue Lysates** - Excise tissue and homogenize in PBS with protease inhibitors. After homogenization, add Triton<sup>®</sup> X-100 to a final concentration of 1%. Freeze samples at  $\leq -70^\circ\text{C}$ , thaw, and centrifuge at  $10,000 \times g$  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  $\leq -70^\circ\text{C}$ . Avoid repeated freeze-thaw cycles.

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

**Plasma** - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 20 minutes at  $2000 \times g$  within 30 minutes of collection. Assay immediately or aliquot and store samples at  $\leq -20^\circ\text{C}$ . Avoid repeated freeze-thaw cycles.

**Saliva** - Collect saliva using a collection device such as a Salivette or equivalent. Assay immediately or aliquot and store samples at  $\leq -20^\circ\text{C}$ . Avoid repeated freeze-thaw cycles.

**Note:** Saliva collector must not have any protein binding or filtering capabilities.

## REAGENT PREPARATION

Bring all reagents to room temperature before use.

**Human Angiogenesis Array** - Four nitrocellulose membranes each containing 55 different capture antibodies printed in duplicate. **Handle membranes only with gloved hands and flat-tipped tweezers.** After opening, reseal unused membranes in the foil pouch with desiccant and store at  $2-8^\circ\text{C}$  for up to 3 months.\*

**Detection Antibody Cocktail** - One vial of lyophilized biotinylated antibodies. Before use, reconstitute the Detection Antibody Cocktail in 100  $\mu\text{L}$  of deionized or distilled water. Store at  $2-8^\circ\text{C}$  for up to 3 months after reconstitution.\*

**Array Buffer 4** - Ready to use. Store at  $2-8^\circ\text{C}$  for up to 3 months after initial use.\*

**Array Buffer 5** - Ready to use. Store at  $2-8^\circ\text{C}$  for up to 3 months after initial use.\*

**Array Buffer 7** - Ready to use. Store at  $2-8^\circ\text{C}$  for up to 3 months after initial use.\*

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

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

## OPTIONAL PRETREATMENT

Some array targets may be complexed with binding proteins in human serum. A pretreatment step can dissociate the binding proteins and increase the signals seen on the array. See the Appendix on page 15 for the affected analytes.

1. Prepare a solution of 0.025 N hydrochloric acid in deionized water.
2. Mix 50  $\mu$ L of serum with 250  $\mu$ L of acid solution.
3. Incubate for 15 minutes on a rocking platform.

The sample is now ready for use in **step 5** of the Array Protocol.

## ARRAY PROTOCOL

**Bring all reagents to room temperature before use. Keep samples on ice. To avoid contamination, wear gloves while performing the procedures.**

1. Prepare all reagents and samples as directed in the previous sections.
2. Pipette 2.0 mL of Array Buffer 7 into each well of the 4-Well Multi-dish to be used. Array Buffer 7 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 7, the blue dye from the spots will disappear, but the capture antibodies are retained in their specific locations.*

4. Incubate for one hour on a rocking platform. Orient the tray so that each membrane rocks end to end in its well.
5. While the membranes are blocking, prepare samples by adding up to 1 mL of each sample to 0.5 mL of Array Buffer 4 in separate tubes. Adjust to a final volume of 1.5 mL with Array Buffer 5 as necessary.
6. Add 15  $\mu$ L of reconstituted Detection Antibody Cocktail to each prepared sample. Mix and incubate at room temperature for one hour.
7. Aspirate Array Buffer 7 from the wells of the 4-Well Multi-dish and add sample/antibody mixtures prepared in step 6. Place the lid on the 4-Well Multi-dish.
8. Incubate overnight at 2-8° C on a rocking platform.

**Note:** *A shorter incubation time may be used if optimal sensitivity is not required.*

9. Carefully remove each membrane and place into individual plastic containers with 20 mL of 1X Wash Buffer. Rinse the 4-Well Multi-dish with deionized or distilled water and dry thoroughly.
10. Wash each membrane with 1X Wash Buffer for 10 minutes on a rocking platform shaker. Repeat two times for a total of three washes.

11. Dilute the Streptavidin-HRP in Array Buffer 5 using the dilution factor on the vial label. Pipette 2.0 mL of diluted Streptavidin-HRP into each well of the 4-Well Multi-dish.
12. Carefully remove each membrane from its wash container. Allow excess buffer to drain from the membrane 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.
14. Wash each array as described in steps 9 and 10.
15. Carefully remove each membrane from the wash container. Allow excess Wash Buffer to drain from the membrane. Place each membrane on a transparent sheet protector.
16. Expose membranes to chemiluminescent reagents as directed by the manufacturer. Use 0.5 mL of chemiluminescent reagent per array.
17. Cover the membranes with plastic wrap taking care to smooth out any air bubbles between the plastic wrap and the membranes. Place membranes, with the identification number up, in an X-ray film cassette.
18. Expose to X-ray film for 1-10 minutes. Multiple exposures are recommended.

## **DATA ANALYSIS**

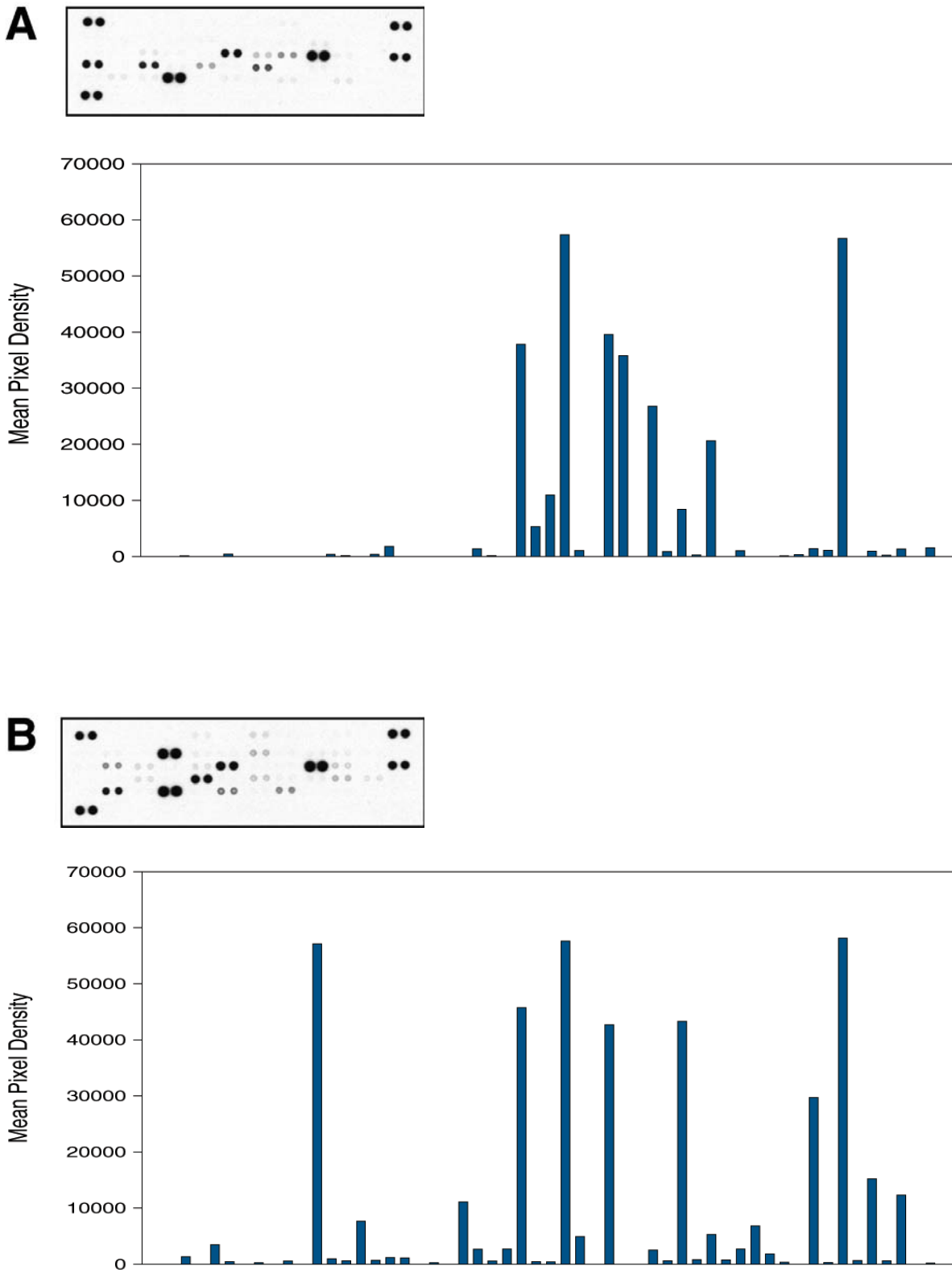
The positive signals seen on developed film can be quickly identified by placing the transparency overlay on the array image and aligning it with the three pairs of positive control spots in the corners of each array. The stamped identification number on the array should be placed on the left hand side. The location of controls and angiogenesis capture antibodies is listed in the Appendix on page 15.

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

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

# PROFILING ANGIOGENESIS-RELATED PROTEINS: CELL CULTURE SUPERNATATES

Figure 1



Analyte	Mean Pixel Density	
	A	B
EGF	100	57,100
Endostatin/Collagen XVIII	100	7700
GM-CSF	0	11,100
IGFBP-2	37,800	45,700
IGFBP-3	5300	400
IL-1 $\beta$	11,000	400
IL-8	57,300	57,600
MCP-1	39,600	42,700
MIP-1 $\alpha$	35,800	0
MMP-9	26,800	2500
Pentraxin 3	8400	43,300
PDGF-AA	20,600	5300
Serpin E1	1400	29,700
TIMP-1	56,700	58,200
Thrombospondin-1	1000	15,200
uPA	1300	12,300

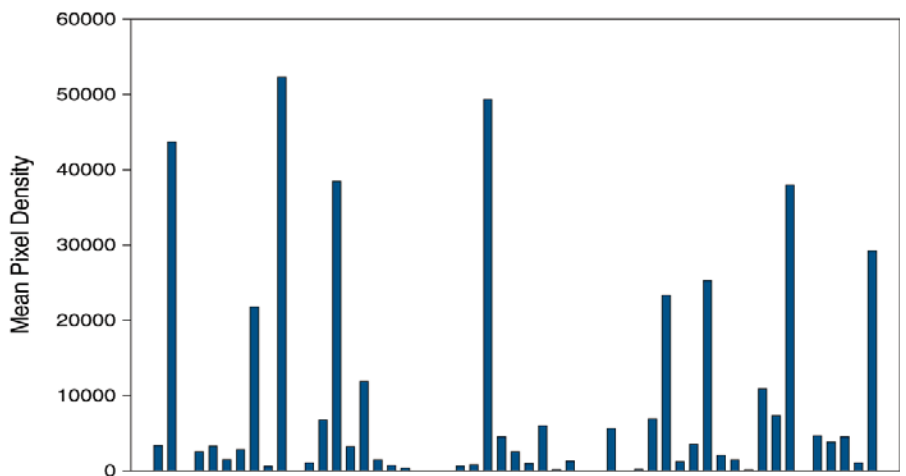
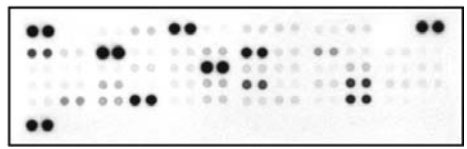
**Figure 1:** The Human Angiogenesis Array detects multiple analytes in various tissue culture supernates. 500  $\mu$ L of supernate was tested from A) THP-1 human acute monocytic leukemia cells treated with 1  $\mu$ g/mL rhIFN- $\gamma$  for 24 hours and 1  $\mu$ g/mL of lipopolysaccharide (LPS) for 16 hours and B) HUVEC cells treated with 1  $\mu$ g/mL of LPS for 24 hours.

Array images are shown on the left and profiles created by quantifying the mean spot pixel densities are shown on the right. Array signals from scanned X-ray film images were analyzed using image analysis software. Array images are from 10 minute exposures to X-ray film.

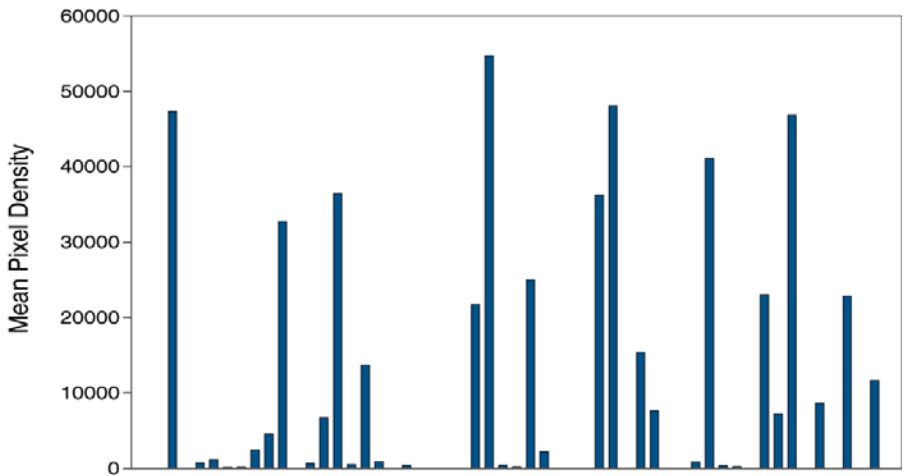
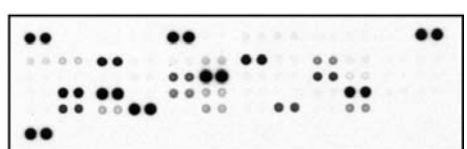
# PROFILING ANGIOGENESIS-RELATED PROTEINS: TUMOR TISSUE LYSATES

Figure 2

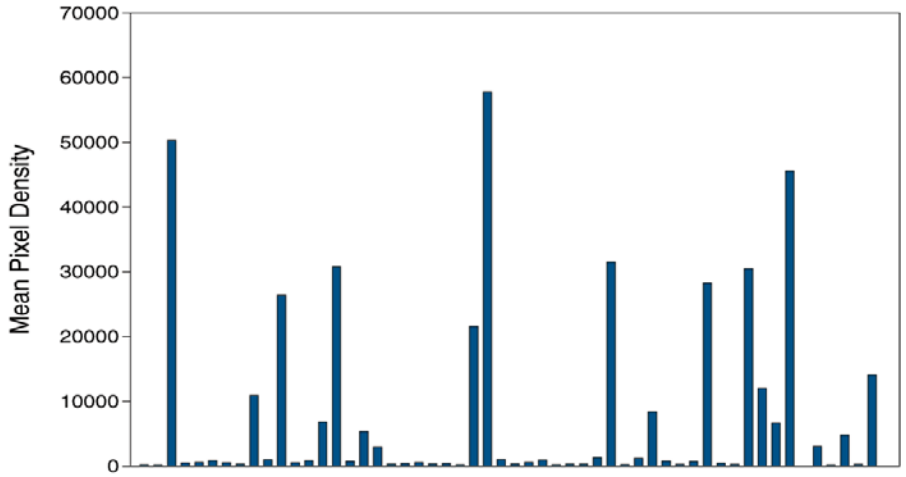
**A**



**B**



**C**



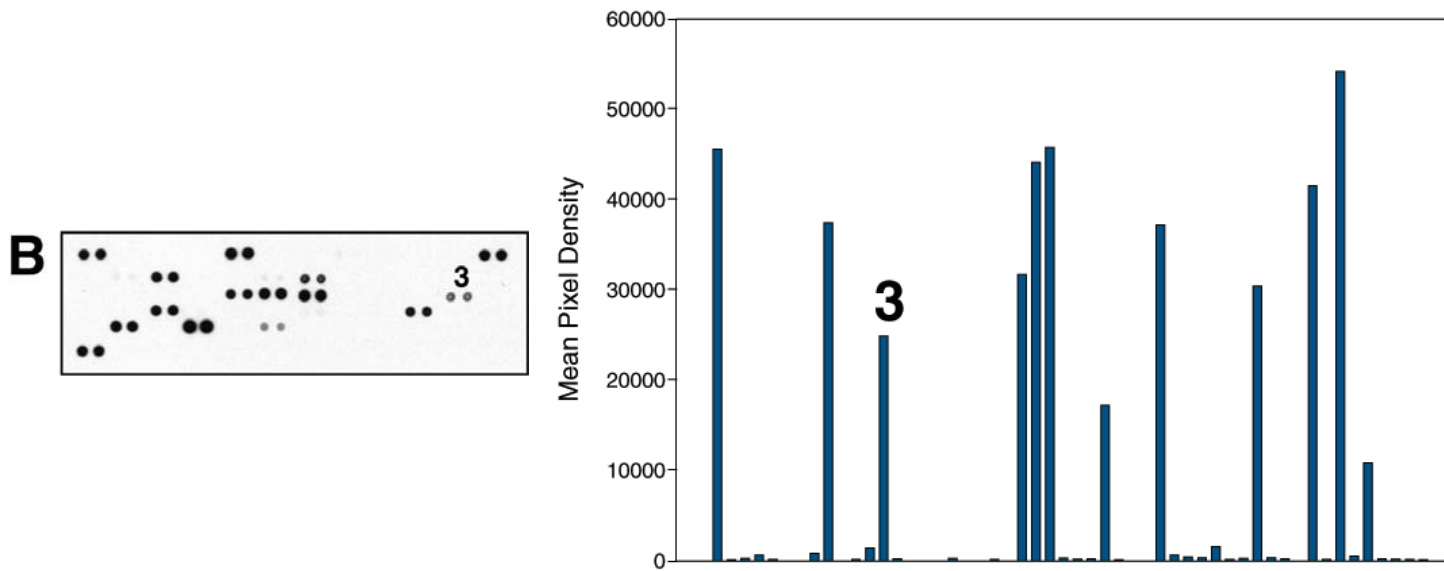
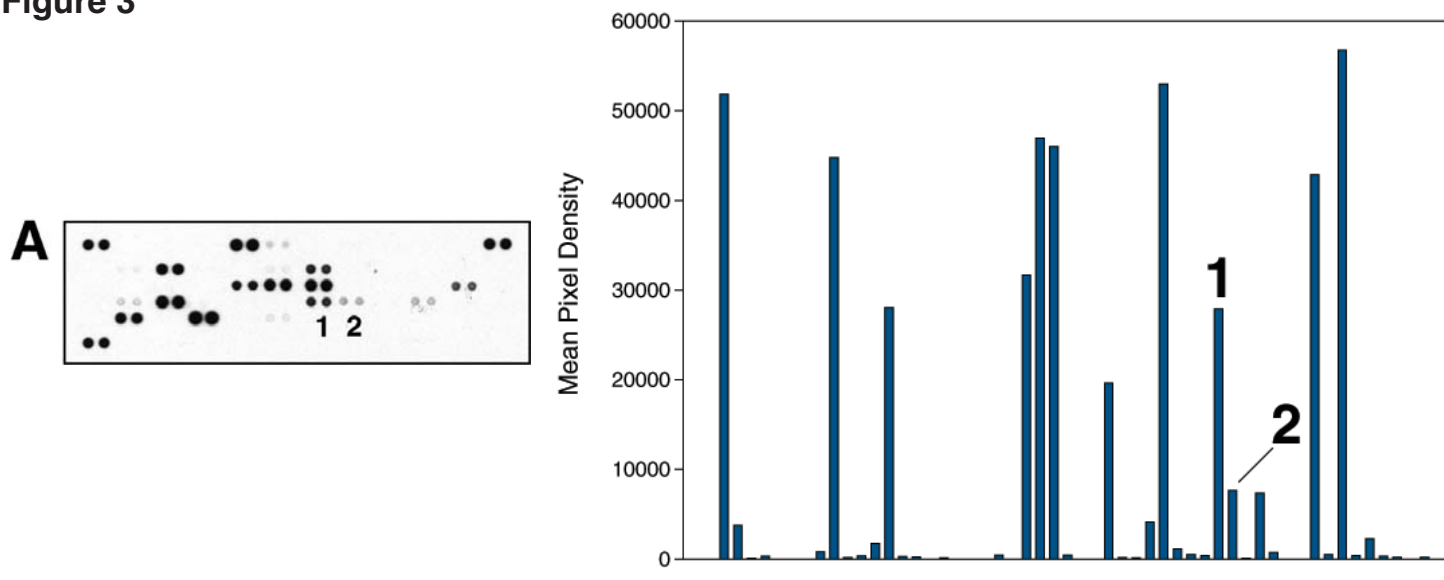
Analyte	Mean Pixel Density		
	A	B	C
Angiogenin	43,700	47,300	50,400
Coagulation Factor III	21,700	2500	11,000
DPPIV	52,300	32,700	26,400
Endostatin/Collagen XVIII	38,400	36,400	30,800
Endothelin-1	3200	500	800
FGF acidic	11,900	13,700	5400
FGF basic	1500	900	3000
IGFBP-1	800	21,700	21,500
IGFBP-2	49,300	54,700	57,800
IL-8	1000	25,000	600
LAP	5900	2200	900
MMP-8	0	36,300	1400
MMP-9	5600	48,100	31,500
Pentraxin 3	200	15,400	1300
PD-ECGF	6900	7700	8400
PDGF-AA	23,300	100	800
Platelet Factor 4	25,300	41,100	28,300
Serpin B5	100	0	30,500
Serpin E1	10,900	23,000	12,000
Serpin F1	7300	7200	6600
TIMP-1	37,900	46,900	45,600
Thrombospondin-1	4600	8600	3100
Thrombospondin-2	3800	0	200
uPA	4500	22,800	4800
VEGF	29,200	11,600	14,100

**Figure 2:** The Human Angiogenesis Array detects multiple analytes in various tumor tissue lysates. 200  $\mu$ g of lysate from A) Prostate Cancer, B) Ovarian Cancer, and C) Breast Cancer was run in this array.

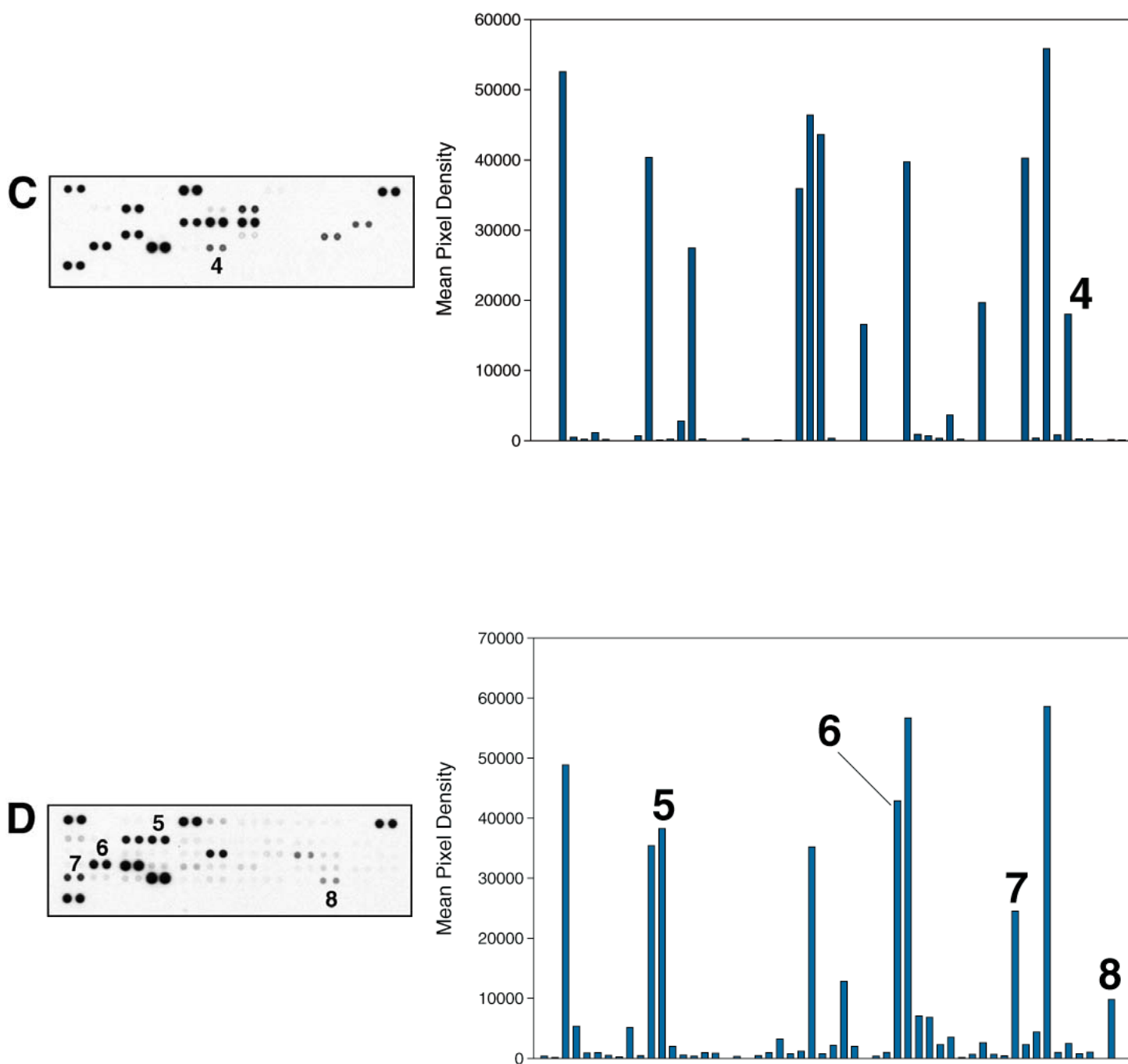
Array images are shown on the left and profiles created by quantifying the mean spot pixel densities are shown on the right. Array signals from scanned X-ray film images were analyzed using image analysis software. Array images are from 10 minute exposures to X-ray film.

# PROFILING ANGIOGENESIS-RELATED PROTEINS: SERUM, PLASMA, AND SALIVA

Figure 3



1	PDGF-AA	5	EGF
2	PDGF-AB/PDGF-BB	6	MMP-8
3	Endostatin/Collagen XVIII	7	Serpin B5
4	Thrombospondin-1	8	VEGF

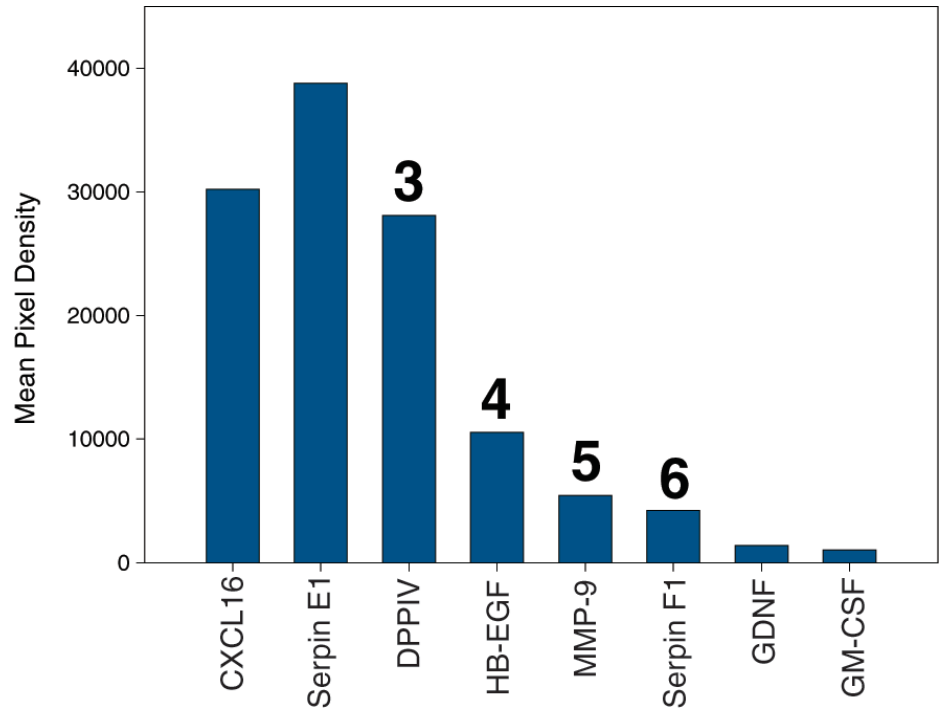
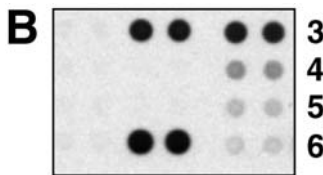
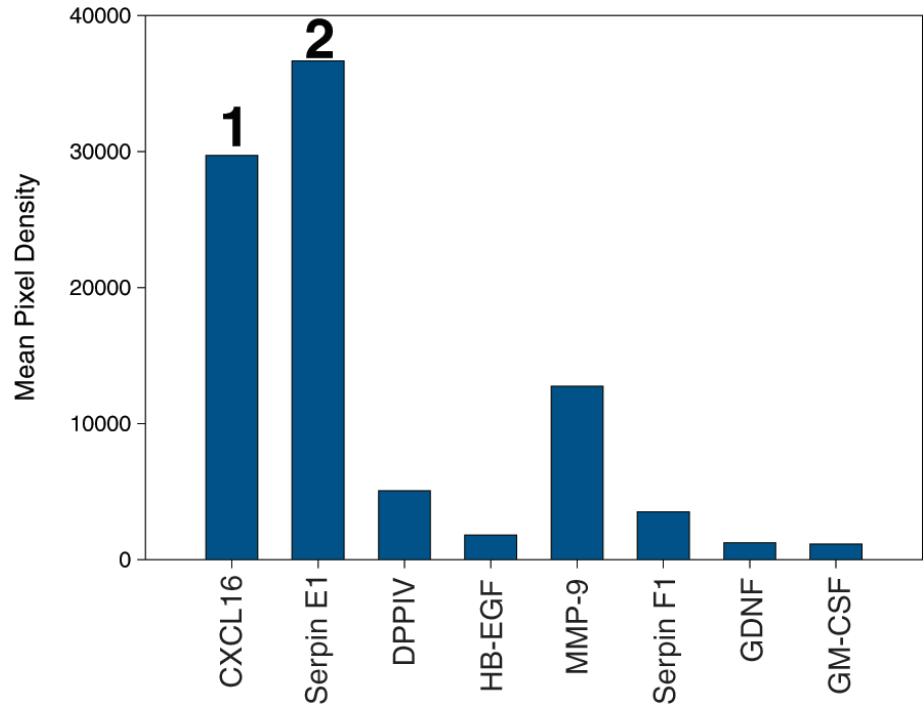
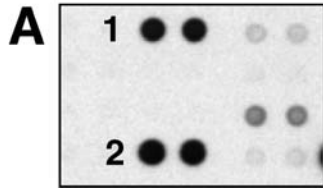


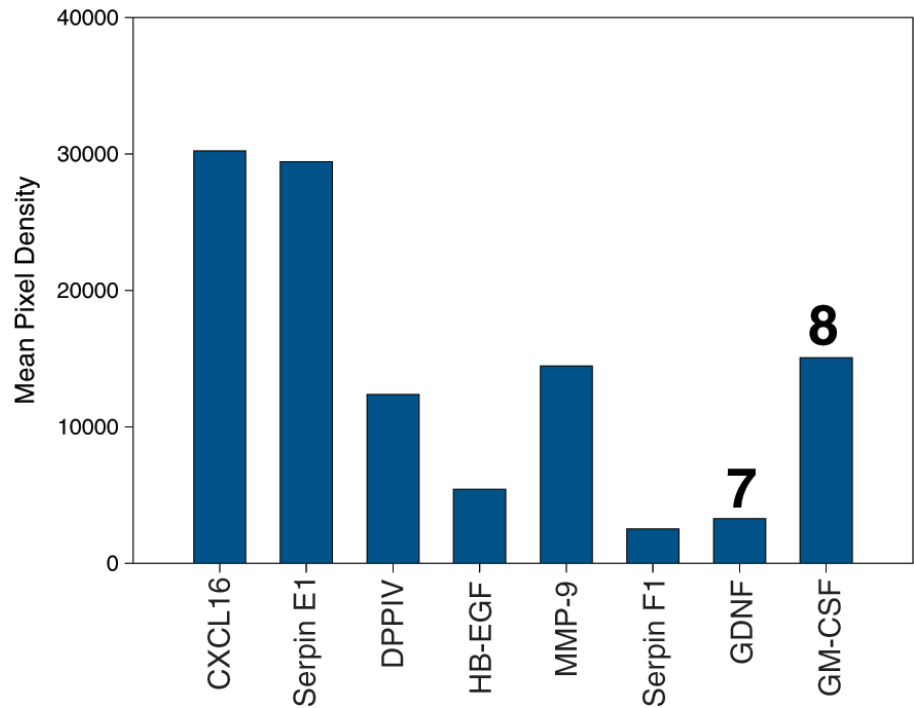
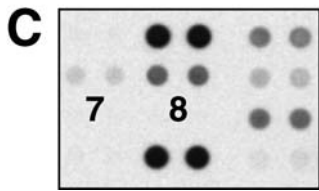
**Figure 3:** The Human Angiogenesis Array detects multiple analytes in various sample types. 100  $\mu$ L of A) serum, B) sodium heparin plasma, C) EDTA plasma, and D) saliva from the same donor was run in this array.

Array images are shown on the left and profiles created by quantifying the mean spot pixel densities are shown on the right. Array signals from scanned X-ray film images were analyzed using image analysis software. Array images are from 10 minute exposures to X-ray film.

# EFFECTS OF HYPOXIA ON SOLUBLE FACTORS

Figure 4





1	CXCL16
2	Serpin E1/PAI-1
3	DPPIV/C26
4	HB-EGF
5	MMP-9
6	Serpin F1/PEDF
7	GDNF
8	GM-CSF

**Figure 4:** 500  $\mu$ L of supernates from HepG2 human hepatocellular carcinoma cells A) untreated, B) treated with 300  $\mu$ M  $\text{CoCl}_2$  for 24 hours, or C) treated with 300  $\mu$ M Deferoxamine for 24 hours were run in this array.

Array images are shown on the left and profiles created by quantifying the mean spot pixel densities are shown on the right. Array signals from scanned X-ray film images were analyzed using image analysis software. Array images are from 10 minute exposures to X-ray film.

## APPENDIX

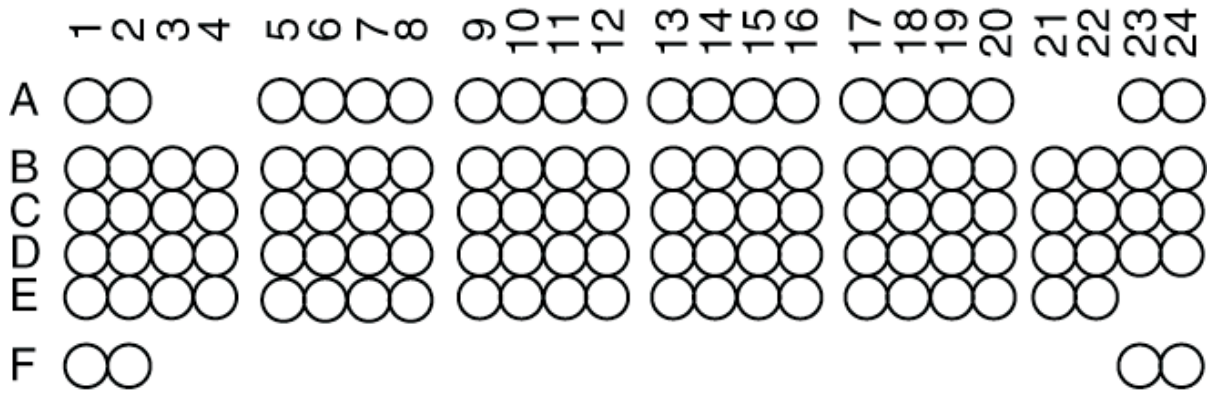
Refer to the table below for the Cytokine Array coordinates.

Coordinate	Target/Control	Alternate Nomenclature	Effects of Pretreatment
A1, A2	Positive Control	Control (+)	
A5, A6	Activin A		
A7, A8	ADAMTS-1		
A9, A10	Angiogenin	ANG	
A11, A12	Angiopoietin-1	Ang-1	-
A13, A14	Angiopoietin-2	Ang-2	
A15, A16	Angiostatin/Plasminogen		
A17, A18	Amphiregulin	AR	
A19, A20	Artemin		
A23, A24	Positive Control	Control (+)	
B1, B2	Coagulation Factor III	TF	
B3, B4	CXCL16		
B5, B6	DPPIV	CD26	
B7, B8	EGF		
B9, B10	EG-VEGF	PK1	
B11, B12	Endoglin	CD105	
B13, B14	Endostatin/Collagen XVIII		
B15, B16	Endothelin-1	ET-1	+
B17, B18	FGF acidic	FGF-1	
B19, B20	FGF basic	FGF-2	
B21, B22	FGF-4		
B23, B24	FGF-7	KGF	
C1, C2	GDNF		
C3, C4	GM-CSF		
C5, C6	HB-EGF		
C7, C8	HGF		
C9, C10	IGFBP-1		
C11, C12	IGFBP-2		
C13, C14	IGFBP-3		
C15, C16	IL-1 $\beta$	IL-1F2	
C17, C18	IL-8	CXCL8	

continued on next page...

<b>Coordinate</b>	<b>Target/Control</b>	<b>Alternate Nomenclature</b>	<b>Effects of Pretreatment</b>
C19, C20	LAP (TGF- $\beta$ 1)		+
C21, C22	Leptin		
C23, C24	MCP-1	CCL2	
D1, D2	MIP-1 $\alpha$	CCL3	
D3, D4	MMP-8		+
D5, D6	MMP-9		
D7, D8	NRG1- $\beta$ 1	HRG1- $\beta$ 1	
D9, D10	Pentraxin 3 (PTX3)	TSG-14	-
D11, D12	PD-ECGF		
D13, D14	PDGF-AA		
D15, D16	PDGF-AB/PDGF-BB		
D17, D18	Persephin		
D19, D20	Platelet Factor 4 (PF4)	CXCL4	
D21, D22	P/IGF		
D23, D24	Prolactin		
E1, E2	Serpin B5	Maspin	+
E3, E4	Serpin E1	PAI-1	
E5, E6	Serpin F1	PEDF	
E7, E8	TIMP-1		
E9, E10	TIMP-4		
E11, E12	Thrombospondin-1	TSP-1	
E13, E14	Thrombospondin-2	TSP-2	
E15, E16	uPA		
E17, E18	Vasohibin		
E19, E20	VEGF		
E21, E22	VEGF-C		
F1, F2	Positive Control	Control (+)	
F23, F24	Negative Control	Control (-)	

# Human Angiogenesis Array Coordinates



This image is not to scale; it is for coordinate reference only.  
Please use the transparency overlay for analyte identification.  
See the Appendix on page 15.

# NOTES

# NOTES

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