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 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 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 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 Human Angiogenesis Array, 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
Human Angiogenesis Array	893313	4 nitrocellulose membranes each containing 55 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 be fore and during use.*	May be stored for up to 3 months at 2-8 °C.*
Array Buffer 5	895876	21 mL of a buffered protein base with preservatives.	
Array Buffer 7	895924	21 mL 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, Human Angiogenisis Array	893312	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	607586	1 transparency overlay template for coordinate reference.	

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

PRECAUTIONS

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.

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.

OTHER SUPPLIES REQUIRED

- Aprotinin (Sigma, Catalog # A6279)
- Leupeptin (Sigma, Catalog # L8511)
- Pepstatin (Sigma, Catalog # P4265)
- 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

OTHER SUPPLIES REQUIRED FOR CELL LYSATE SAMPLES

- 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)

OTHER 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)

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 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 µL for cell culture supernates, 100-300 µg for cell and tissue lysates, and 50-200 µL for serum, plasma, 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. Solubilize cells at 1 x 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 concentrations 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 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.

Serum - Allow blood samples to clot for 30 minutes 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.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 2000 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 using a collection device such as a Salivette® or equivalent. Assay immediately or aliquot and store samples at ≤ -20 °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.**

Detection Antibody Cocktail - One vial of lyophilized biotinylated antibodies. Before use, reconstitute the Detection Antibody Cocktail with 100 µL of deionized or distilled water.

1X Wash Buffer - If crystals have formed in the concentrate, warm bottles to room temperature and mix gently until crystals have completely dissolved. Add 40 mL of 25X Wash Buffer Concentrate to 960 mL of deionized or distilled water to prepare 1000 mL of 1X Wash Buffer.

Chemi Reagent Mix - Chemi Reagent 1 and 2 should be mixed in equal volumes within 15 minutes of use. **Protect from light. 1 mL of resultant mixture is required per membrane.**

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 for the affected analytes.

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

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

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 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 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 5 as necessary.
6. Add 15 µL 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 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 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. 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 the 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 a 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 at room temperature.

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 in an autoradiography film cassette with the identification numbers facing up.

    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

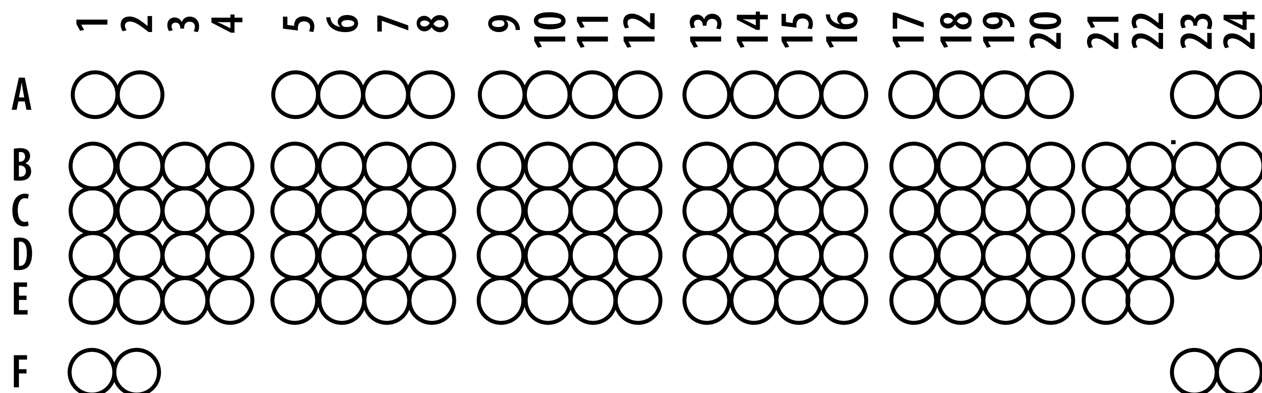
The positive signals seen on developed film can be quickly identified by placing the transparency overlay on the array image and aligning it with the pairs of reference spots in three corners of each array. The stamped identification number on the array should be placed on the left hand side. The location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

Human Angiogenesis Array Coordinates



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

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PROFILING ANGIOGENESIS-RELATED PROTEINS IN CELL CULTURE SUPERNATES

The Human Angiogenesis Array detects multiple analytes in various tissue culture supernates. Cells were treated as indicated below. 500 µL of cell culture supernate was run on each array. Data shown are from a 10 minute exposure to X-ray film.

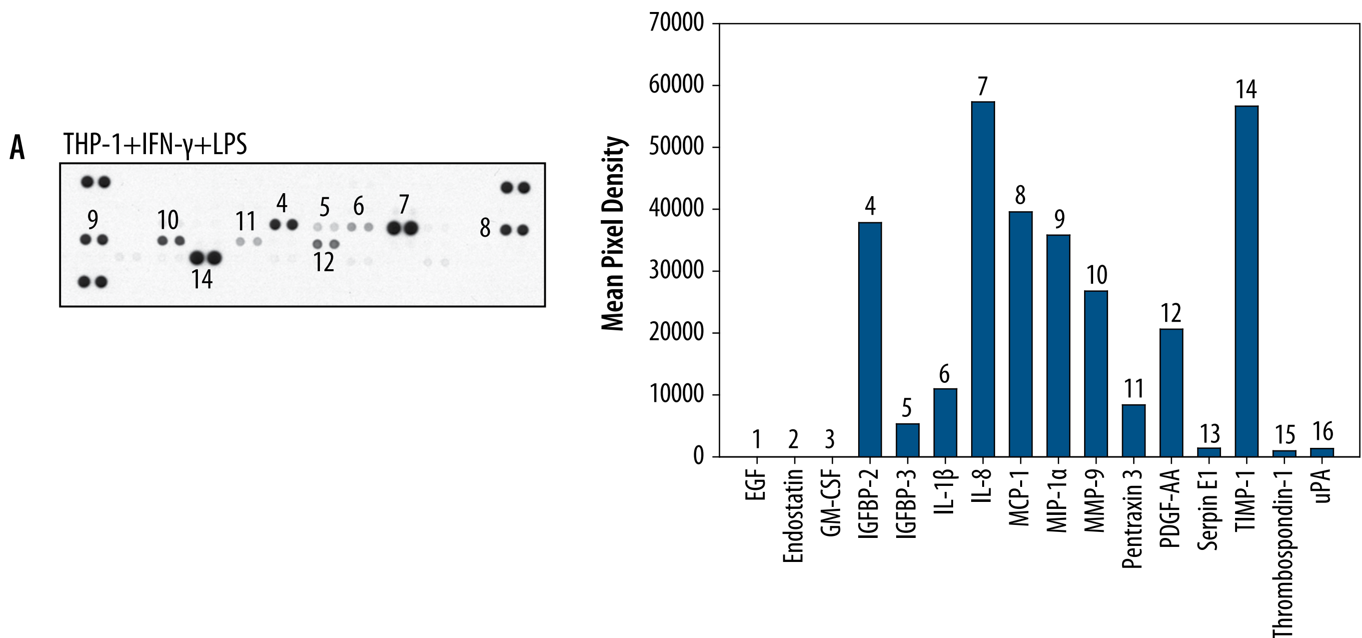


Figure 1A: THP-1 human acute monocytic leukemia cells were treated with 1 µg/mL rhIFN-γ (R&D Systems, Catalog # 285-IF) for 8 hours followed by the addition of 1 µg/mL of lipopolysaccharide (LPS) for 16 hours.

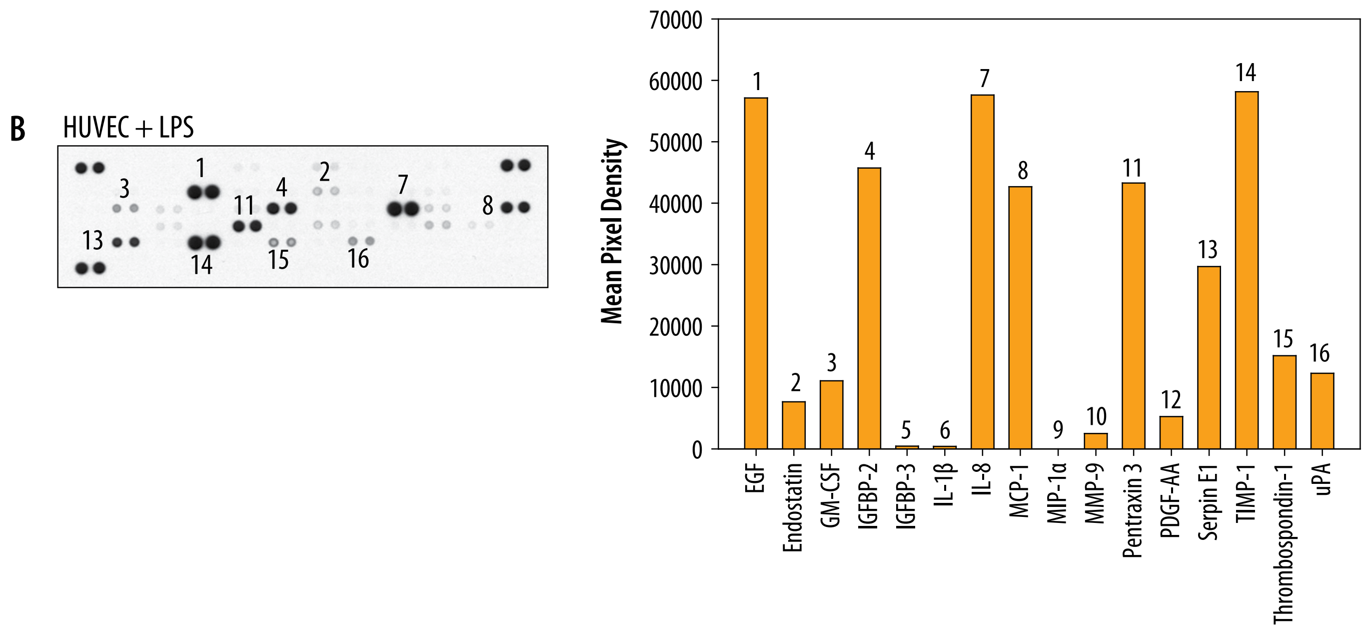
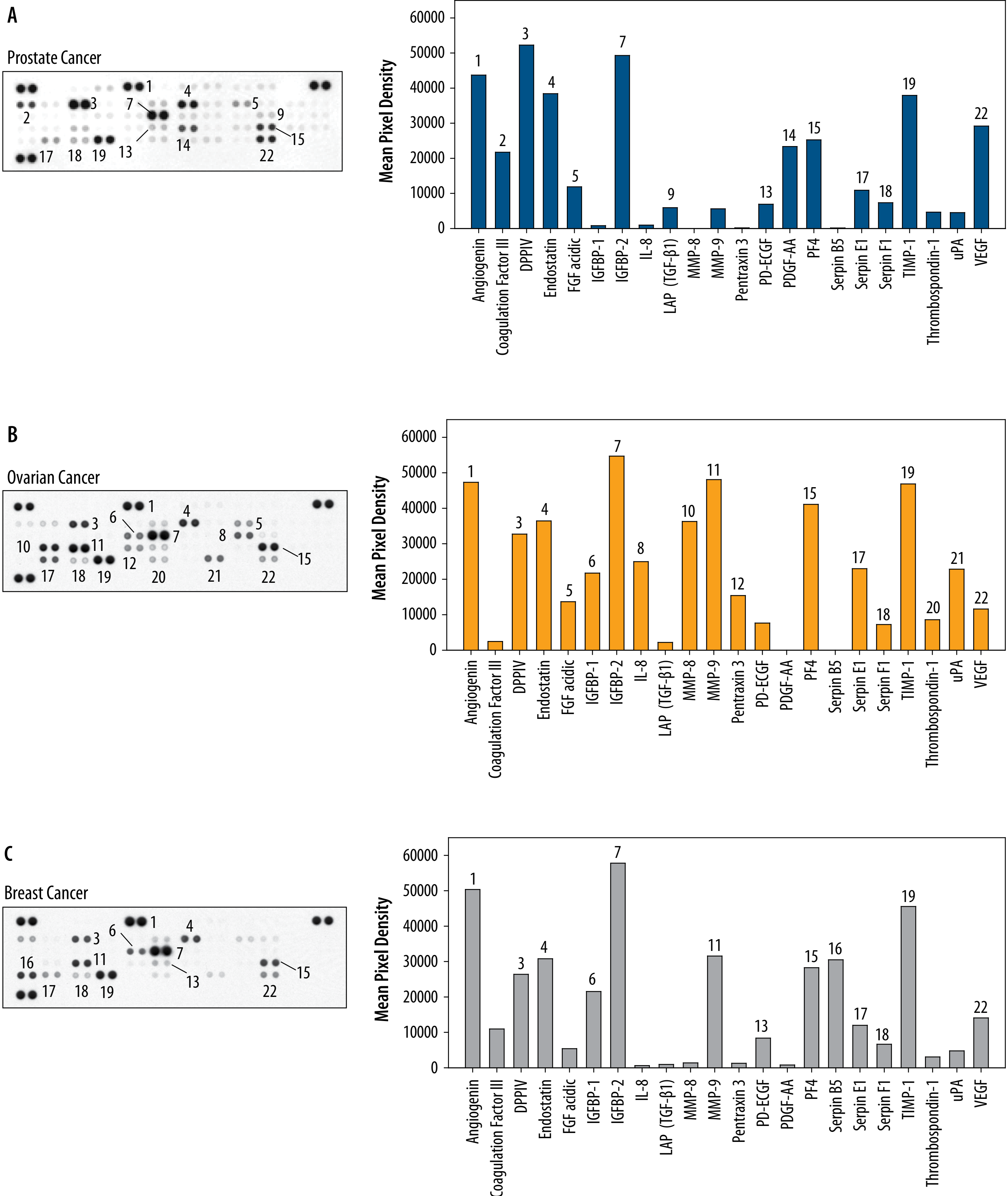


Figure 1B: HUVEC human umbilical vein endothelial cells were treated with 1 µg/mL of LPS for 24 hours.

PROFILING ANGIOGENESIS-RELATED PROTEINS IN TUMOR TISSUE LYSATES

The Human Angiogenesis Array detects multiple analytes in various tumor tissue lysates. 200 µg of lysate was run on each array. Data shown are from a 10 minute exposure to X-ray film.



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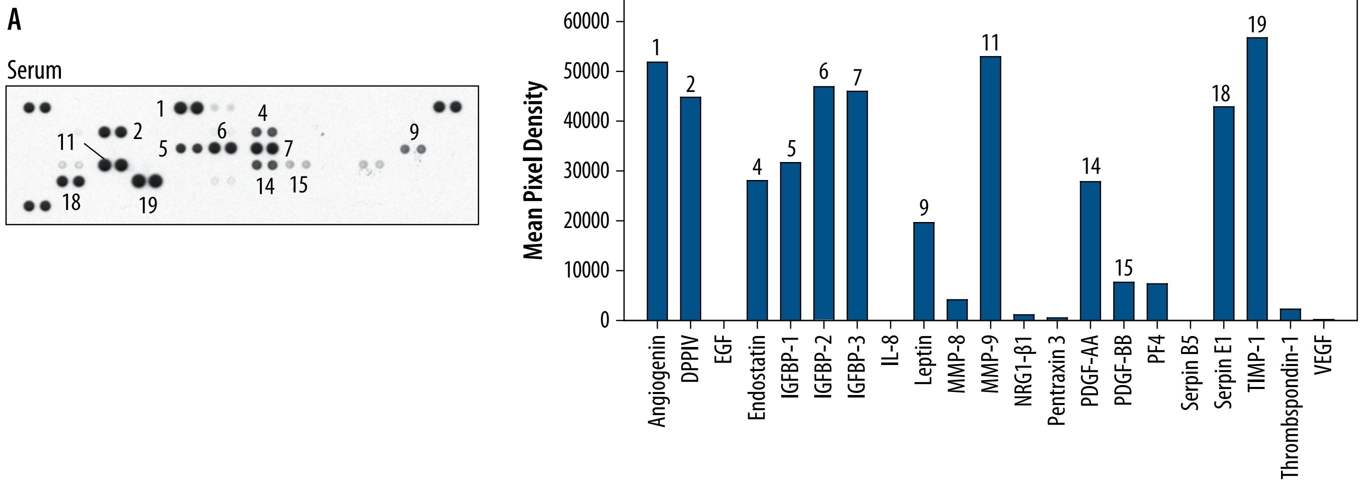
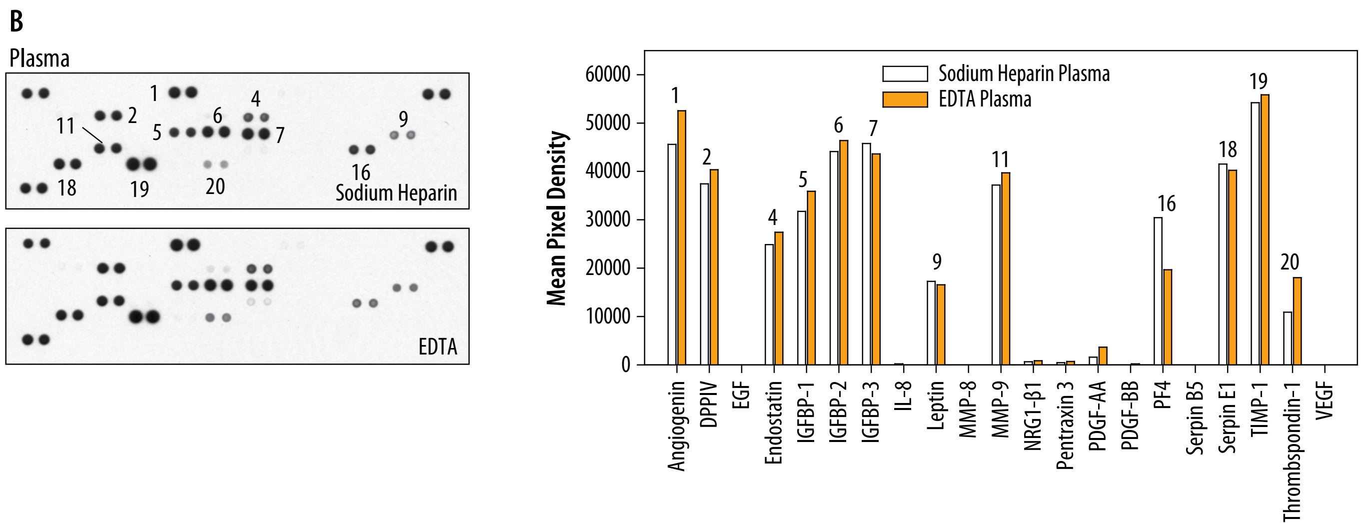
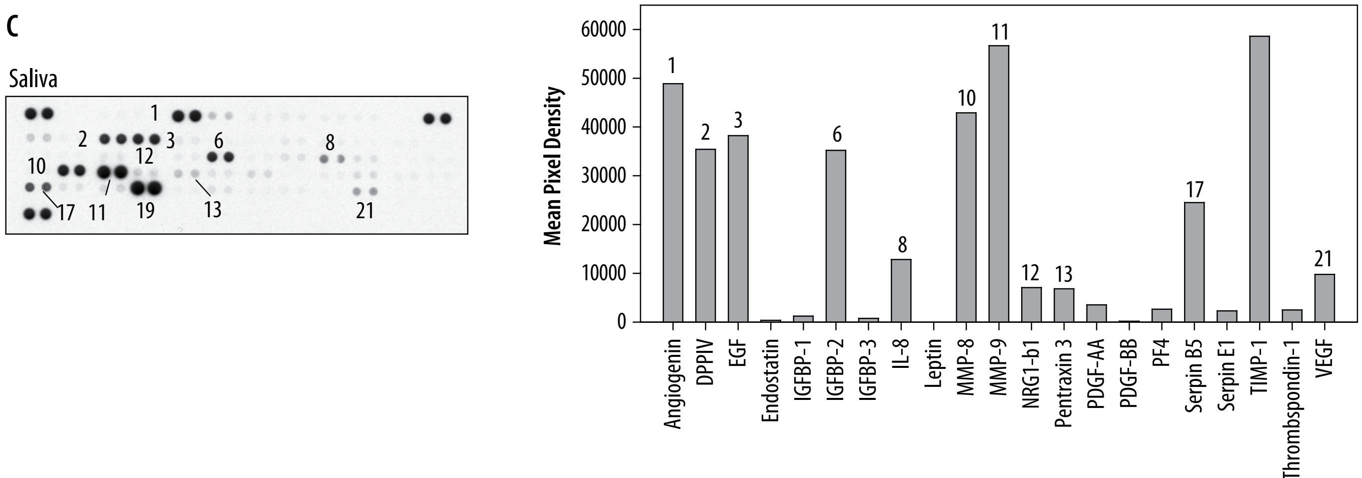
PROFILING ANGIOGENESIS-RELATED PROTEINS IN SERUM, PLASMA, AND SALIVA

The Human Angiogenesis Array detects multiple analytes in serum, plasma, and saliva. All samples were obtained from the same donor. 100 µL of sample was run on each array. Data shown are from a 10 minute exposure to X-ray film.

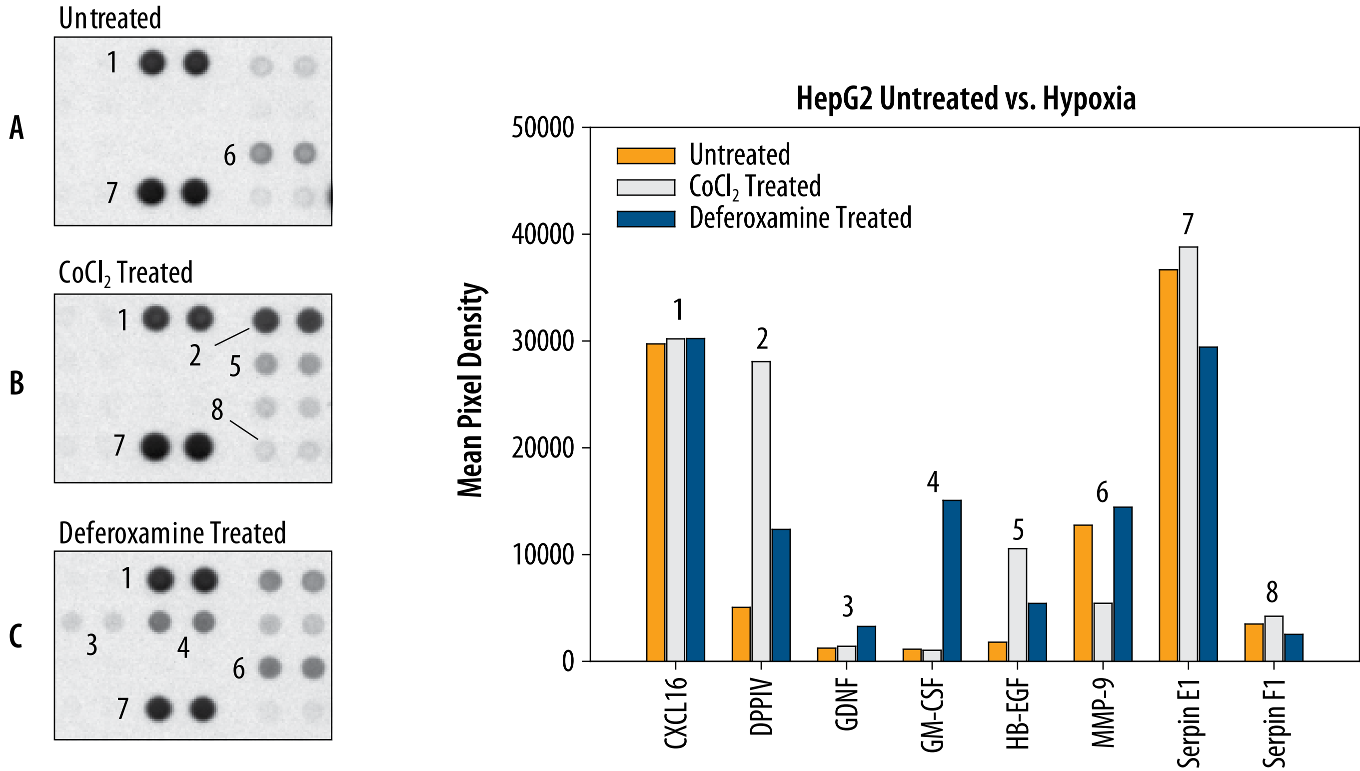






EFFECTS OF HYPOXIA ON SOLUBLE FACTORS

The Human Angiogenesis Array shows the effects of hypoxia on soluble factors. HepG2 human hepatocellular carcinoma cells were untreated or treated as below. 500 µL of cell culture supernate was run on each array. Data shown are from a 10 minute exposure to X-ray film.



A. Untreated

B. Treated with 300 µM $CoCl_2$ for 24 hours.

C. Treated with 300 µM Deferoxamine for 24 hours.

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APPENDIX

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

Coordinate	Target/Control	Gene ID	Alternate Nomenclature	Effects of Pretreatment
A1, A2	Reference Spots	N/A		
A5, A6	Activin A	3624		
A7, A8	ADAMTS-1	9510		
A9, A10	Angiogenin	283	ANG	
A11, A12	Angiopoietin-1	284	Ang-1	–
A13, A14	Angiopoietin-2	285	Ang-2	
A15, A16	Angiostatin/Plasminogen	5340		
A17, A18	Amphiregulin	374	AR	
A19, A20	Artemin	9048		
A23, A24	Reference Spots	N/A		
B1, B2	Coagulation Factor III	2152	TF	
B3, B4	CXCL16	58191		
B5, B6	DPPIV	1803	CD26	
B7, B8	EGF	1950		
B9, B10	EG-VEGF	84432	PK1	
B11, B12	Endoglin	2022	CD105	
B13, B14	Endostatin/Collagen XVIII	80781		
B15, B16	Endothelin-1	1906	ET-1	+
B17, B18	FGF acidic	2246	FGF-1	
B19, B20	FGF basic	2263	FGF-2	
B21, B22	FGF-4	2249		
B23, B24	FGF-7	2252	KGF	
C1, C2	GDNF	2668		
C3, C4	GM-CSF	1437		
C5, C6	HB-EGF	1839		
C7, C8	HGF	3082		
C9, C10	IGFBP-1	3484		
C11, C12	IGFBP-2	3485		
C13, C14	IGFBP-3	3486		
C15, C16	IL-1β	3553	IL-1F2	
C17, C18	IL-8	3576	CXCL8	
C19, C20	LAP (TGF-β1)	7040		+
C21, C22	Leptin	3952		
C23, C24	MCP-1	6347	CCL2	

continued on next page...

APPENDIX *CONTINUED*

Coordinate	Target/Control	Gene ID	Alternate Nomenclature	Effects of Pretreatment
D1, D2	MIP-1α	6348	CCL3	
D3, D4	MMP-8	4317		+
D5, D6	MMP-9	4318		
D7, D8	NRG1-β1	3084	HRG1-β1	
D9, D10	Pentraxin 3 (PTX3)	5806	TSG-14	–
D11, D12	PD-ECGF	1890		
D13, D14	PDGF-AA	5154		
D15, D16	PDGF-AB/PDGF-BB	5155		
D17, D18	Persephin	5623		
D19, D20	Platelet Factor 4 (PF4)	5196	CXCL4	
D21, D22	PlGF	5228		
D23, D24	Prolactin	5617		
E1, E2	Serpin B5	5268	Maspin	+
E3, E4	Serpin E1	5054	PAI-1	
E5, E6	Serpin F1	5176	PEDF	
E7, E8	TIMP-1	7076		
E9, E10	TIMP-4	7079		
E11, E12	Thrombospondin-1	7057	TSP-1	
E13, E14	Thrombospondin-2	7058	TSP-2	
E15, E16	uPA	5328		
E17, E18	Vasohibin	22846		
E19, E20	VEGF	7422		
E21, E22	VEGF-C	7424		
F1, F2	Reference Spots	N/A		
F23, F24	Negative Control	N/A	Control (-)	

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