Proteome Profiler™ Array

Human Protease Inhibitor Array Kit

Catalog Number ARY023

For the parallel determination of the relative levels of selected human protease inhibitors.

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 protease inhibitors is essential for understanding their roles in normal cellular function and their dysregulation in diseases such as cancer. The Human Protease Inhibitor Array is a rapid, sensitive, and economical tool to simultaneously detect protease inhibitor differences between samples. The relative expression of 32 human protease inhibitors can be determined without performing numerous immunoprecipitations or Western blots. Each capture and detection antibody was carefully selected using commonly used sample types.

PRINCIPLE OF THE ASSAY

Capture and control antibodies have been spotted in duplicate on nitrocellulose membranes. Cell culture supernates, cell lysates, serum, plasma, human milk, urine, saliva, or tissue lysates are diluted, mixed with a cocktail of biotinylated detection antibodies, and incubated overnight with the Proteome Profiler Human Protease Inhibitor 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 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.
- 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 kit, 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 Protease Inhibitor Array	894581	4 nitrocellulose membranes each containing 32 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 6	893573	2 vials (21 mL/vial) of a buffered protein base with preservatives.	May be stored for up to 3 months at 2-8 °C.*
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 Protease Inhibitor Array	894667	1 vial of a biotinylated antibody cocktail; lyophilized.	
Streptavidin-HRP	890803	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	607391	1 transparency overlay template for coordinate reference.	

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

OTHER SUPPLIES REQUIRED

- 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

SUPPLIES REQUIRED FOR CELL LYSATE SAMPLES

- Phosphate-Buffered Saline (PBS)
- Lysis Buffer 17 (R&D Systems, Catalog # 895943)
- Aprotinin (Sigma, Catalog # A6279)
- Leupeptin (Tocris, Catalog # 1167)
- Pepstatin (Tocris, Catalog # 1190)

SUPPLIES REQUIRED FOR TISSUE LYSATE SAMPLES

- Phosphate-Buffered Saline (PBS)
- Aprotinin (Sigma, Catalog # A6279)
- Leupeptin (Tocris, Catalog # 1167)
- Pepstatin (Tocris, Catalog # 1190)
- 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 Protease Inhibitor 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 50-100 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 17 supplemented with 10 µg/mL Aprotinin, 10 µg/mL Leupeptin, and 10 µg/mL Pepstatin. 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.

Serum - Allow blood samples to clot for 1 hour 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.

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.

Urine - Collect urine and centrifuge to remove particulate matter. Assay immediately or aliquot and store 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.

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

REAGENT PREPARATION

Bring all reagents to room temperature before use.

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.*

Human Protease Inhibitor Array - Immediately before use, remove each membrane being used from between the protective sheets with a flat-tipped tweezers. **Handle the membranes only with gloved hands and flat-tipped tweezers.**

Detection Antibody Cocktail - Reconstitute the Human Protease Inhibitor Detection Antibody Cocktail in 100 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.

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.**

1X Streptavidin-HRP - Immediately before use, dilute the Streptavidin-HRP in Array Buffer 6. See vial label for dilution factor.

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. Wear protective gloves, clothing, eye, and face protection when using these reagents.

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.

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.

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 2.0 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. Place each membrane in a separate well. 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 4-Well Multi-dish so that each membrane rocks end to end in its well.
5. While the membranes are blocking, prepare samples. *Refer to the Sample Collection & Storage section for recommended sample amount to use.* Adjust to a final volume of 1.5 mL with Array Buffer 6 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 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. Pipette 2.0 mL of 1X 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 1X Streptavidin-HRP. Cover the wells with the lid.
13. Incubate for 30 minutes at room temperature on a rocking platform shaker.

ASSAY 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 1.0 mL of the prepared Chemi Reagent Mix evenly onto each membrane.

    Note: *Using less than 1.0 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

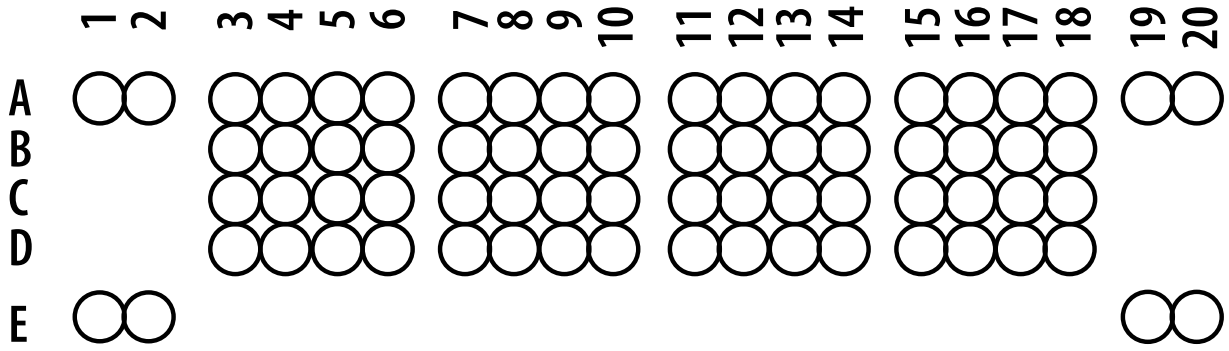
The positive signals seen on developed film can be quickly identified by placing the transparency overlay template on the array image and aligning it with the pairs of reference spots in three corners of each array. The stamped identification number on the array should be placed on the left hand side.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

Human Protease Inhibitor Array Coordinates



This image is not to scale. It is for coordinate reference only.
Please use the Transparency Overlay Template for analyte identification.

PROFILING PROTEINS IN CELL CULTURE SUPERNATES

The Human Protease Inhibitor Array detects multiple protease inhibitors in cell culture supernates. Cells were either untreated or treated as indicated below. 200 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.

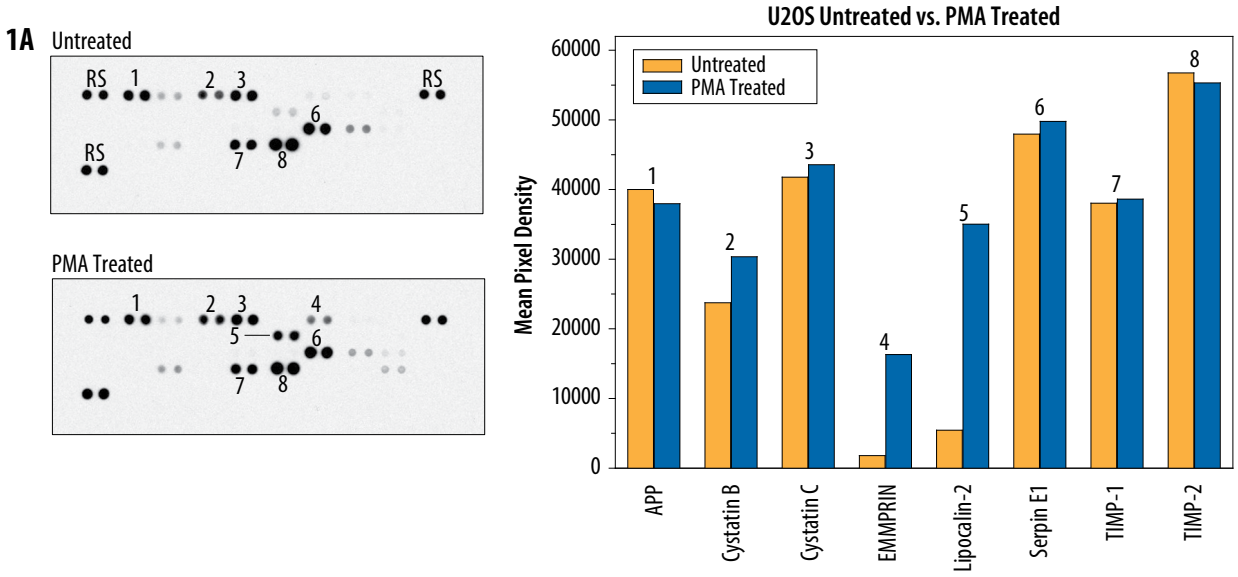


Figure 1A: U2OS human osteosarcoma cells were untreated or treated with 50 nM PMA (Tocris, Catalog # 1201) for 24 hours. RS=Reference Spots.

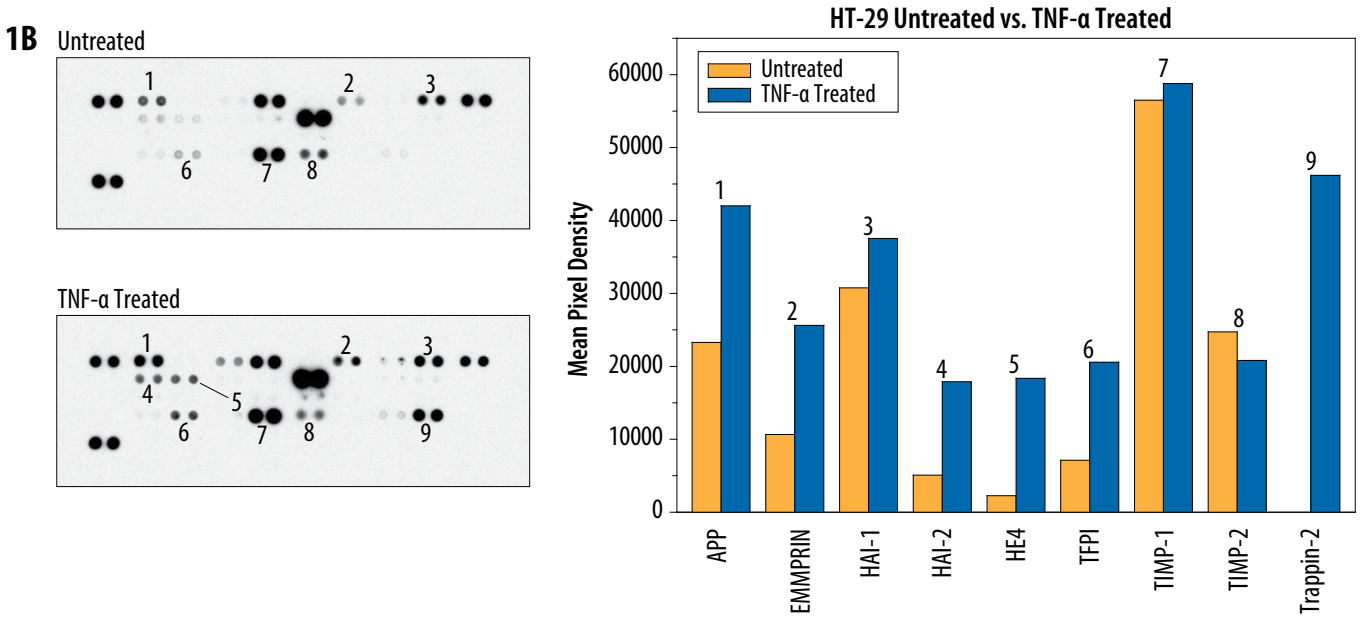


Figure 1B: HT-29 human colon adenocarcinoma cells were untreated or treated with 10 ng/mL recombinant human TNF-α (R&D Systems, Catalog # 210-TA) for 24 hours.

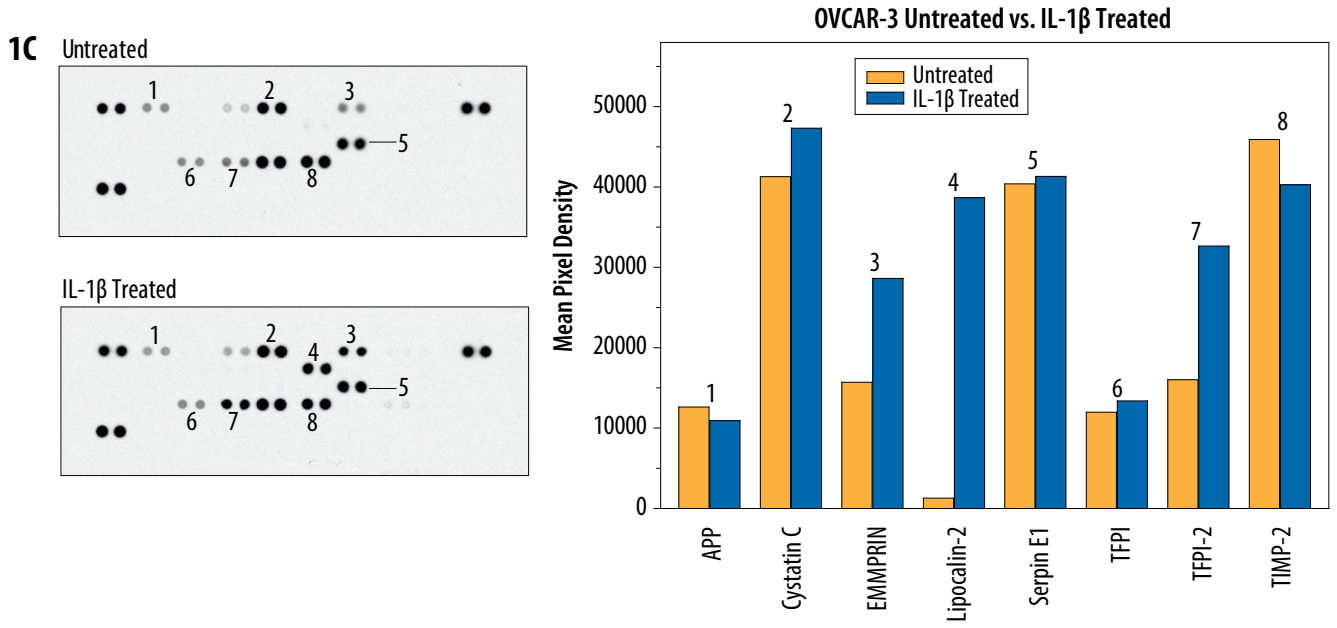


Figure 1C: OVCAR-3 human ovarian carcinoma cells were untreated or treated with 10 ng/mL recombinant human IL-1β /IL-1F2 (R&D Systems, Catalog # 201-LB) for 18 hours.

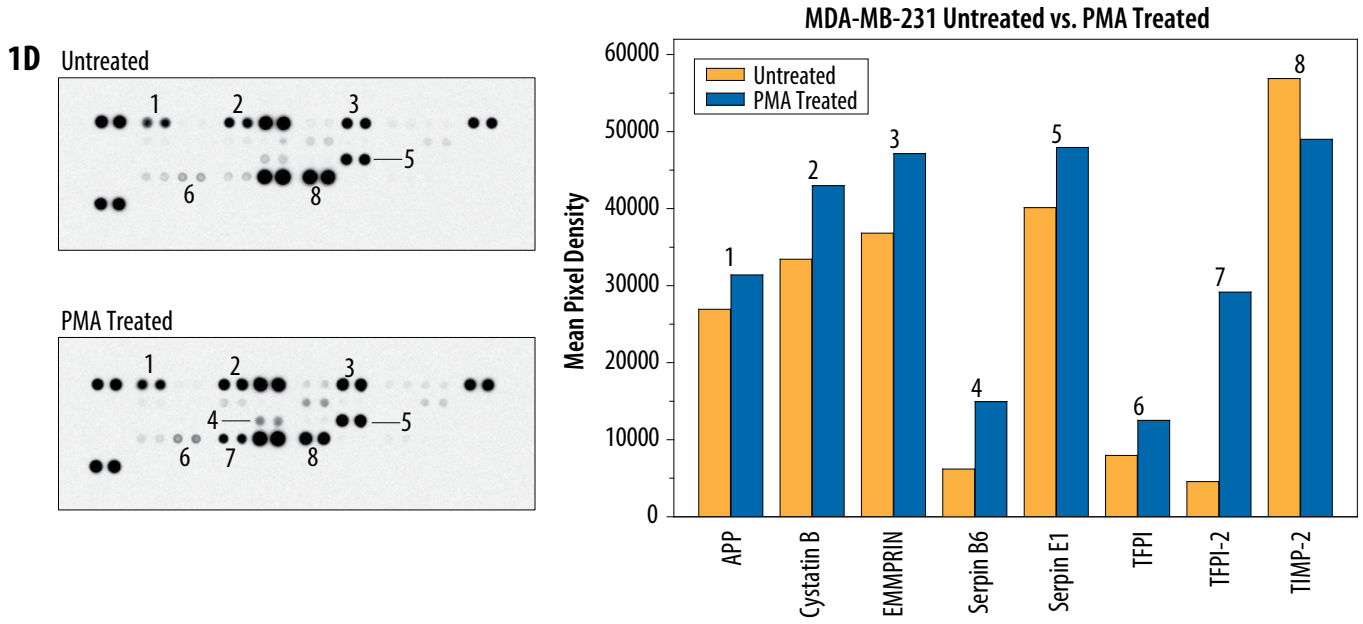


Figure 1D: MDA-MB-231 human breast cancer cells were untreated or treated with 50 nM PMA (Tocris, Catalog # 1201) for 24 hours.

PROFILING PROTEINS IN CELL LYSATES

The Human Protease Inhibitor Array detects multiple analytes in cell lysates. Cells were either untreated or treated as indicated below. 200 µg of cell lysate was run on each array. The duration of exposure to X-ray film is listed below. Profiles of mean spot pixel density were created using a transmission-mode scanner and image analysis software.

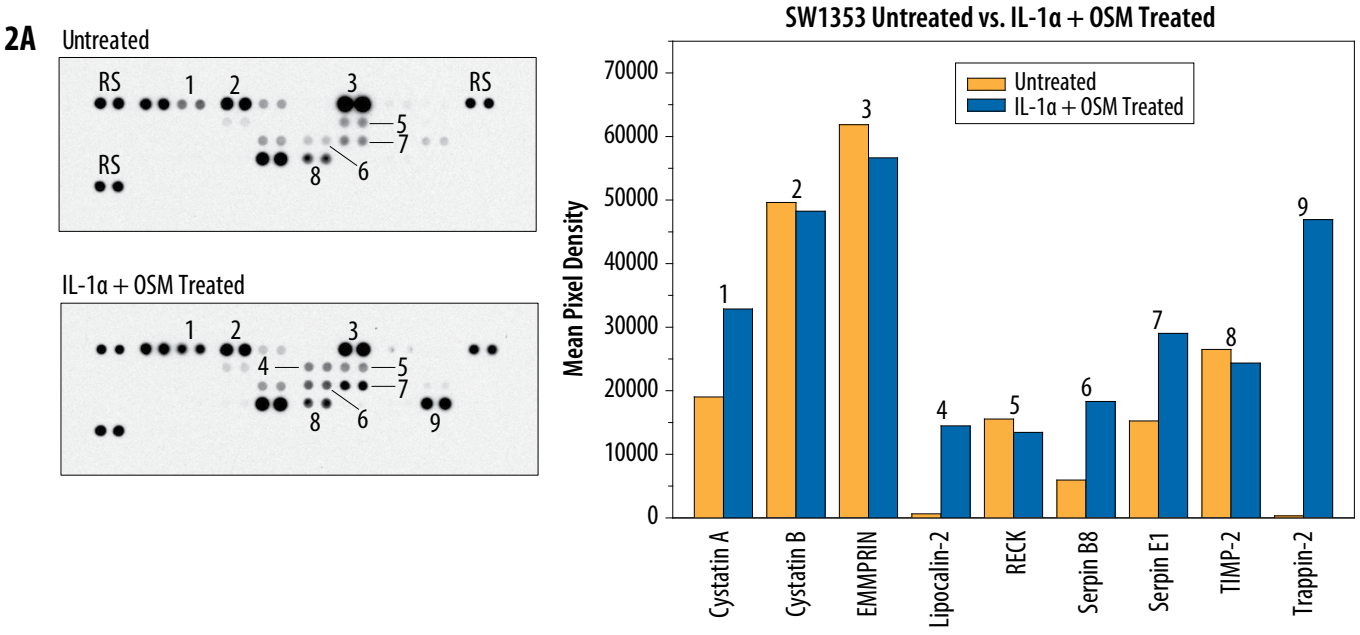


Figure 2A: SW1353 human chondrosarcoma cells were untreated or treated with 5 ng/mL recombinant human IL-1α (R&D Systems, Catalog # 200-LA) and 10 ng/mL recombinant human Oncostatin M (OSM) (R&D Systems, Catalog # 295-OM) for 24 hours (5 minute exposure). RS=Reference Spots.

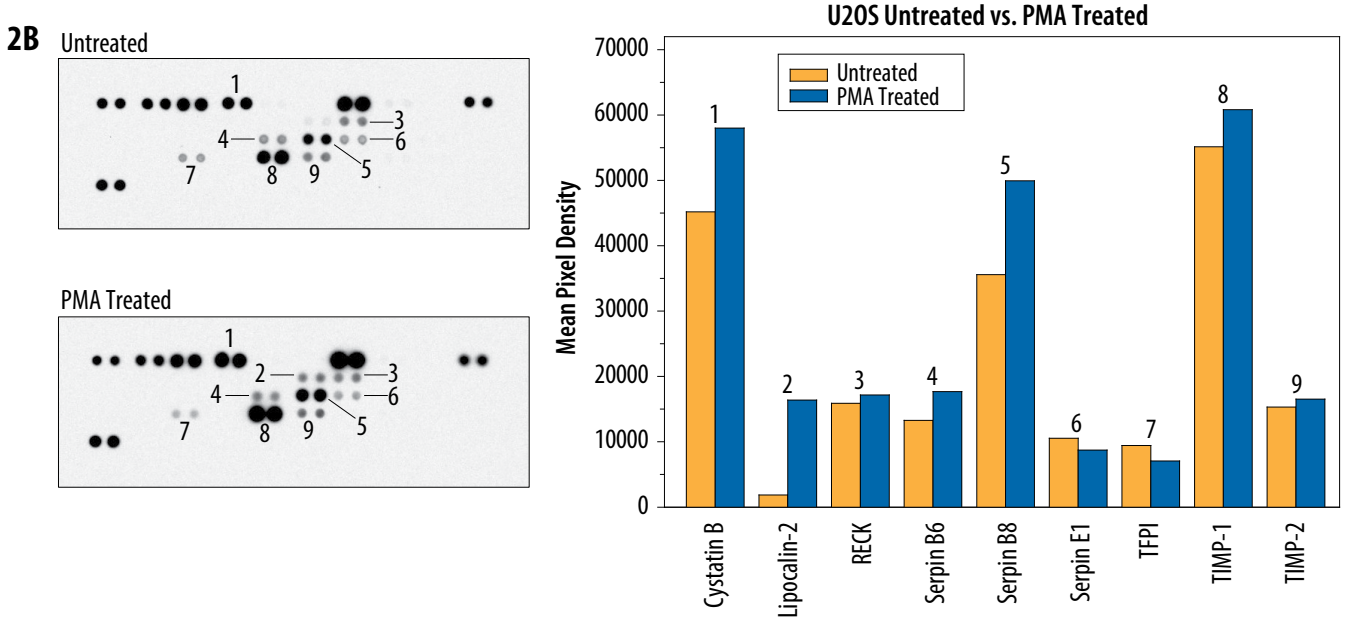


Figure 2B: U2OS human osteosarcoma cells were untreated or treated with 50 nM PMA (Tocris, Catalog # 1201) for 24 hours (5 minute exposure).

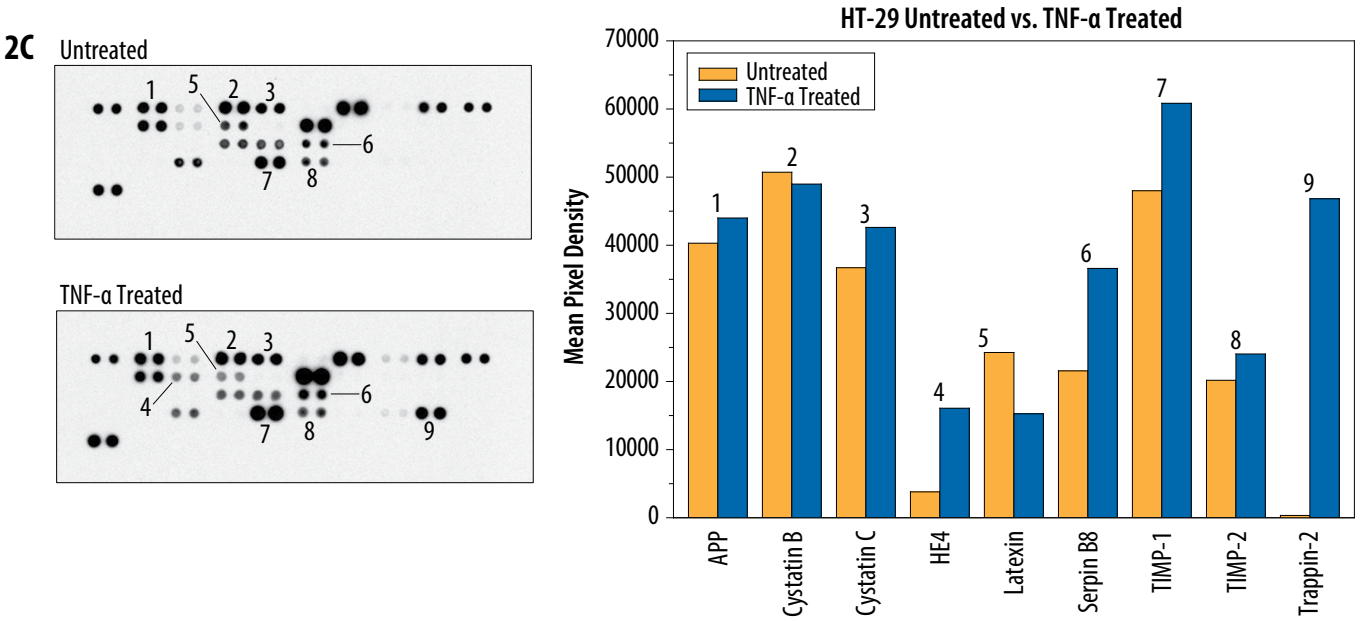


Figure 2C: HT-29 human colon adenocarcinoma cells were untreated or treated with 10 ng/mL recombinant human TNF-α (R&D Systems, Catalog # 210-TA) for 24 hours (2 minute exposure).

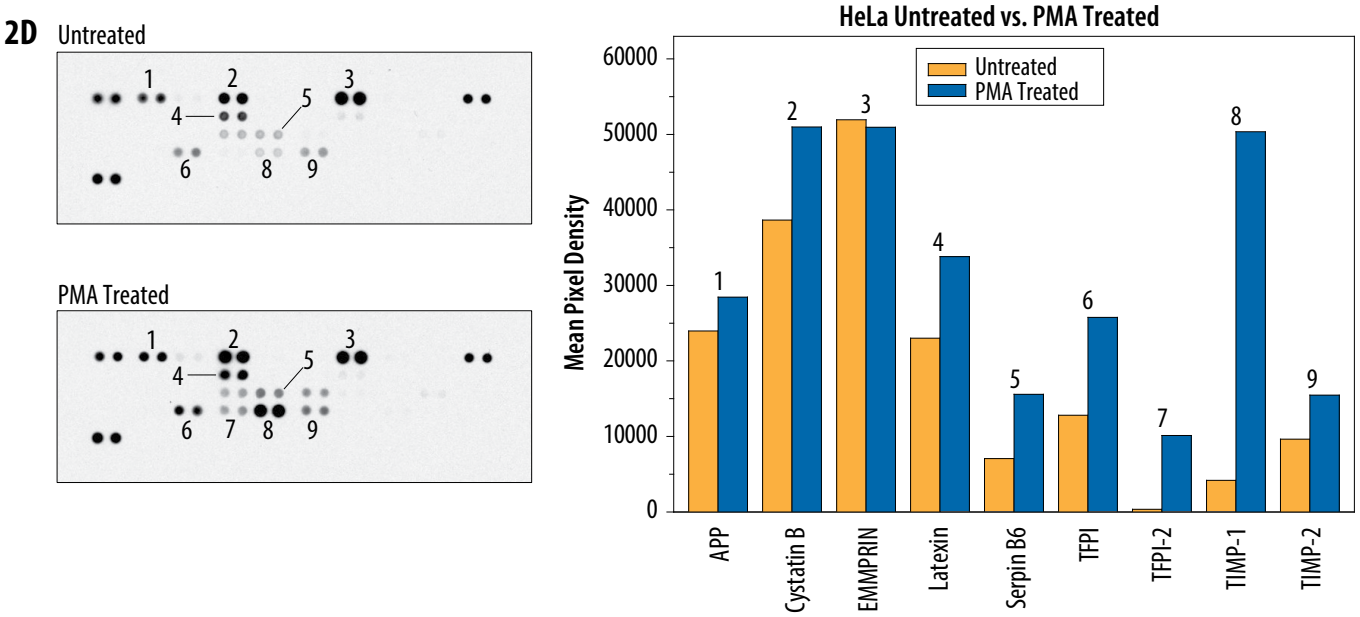
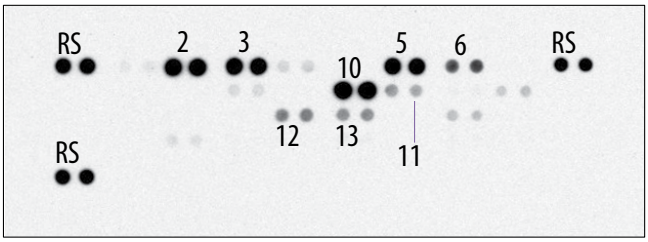


Figure 2D: HeLa human cervical adenocarcinoma cells were untreated or treated with 200 nM PMA (Tocris, Catalog # 1201) for 24 hours (2 minute exposure).

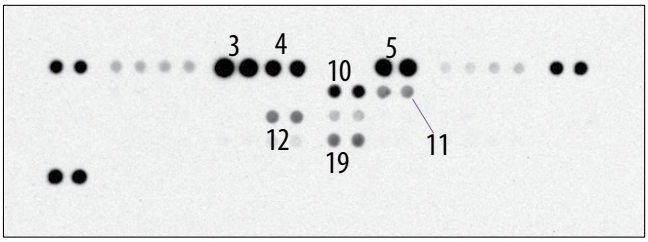
PROFILING PROTEINS IN TISSUE LYSATES

The Human Protease Inhibitor Array detects multiple analytes 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. RS = Reference Spots.

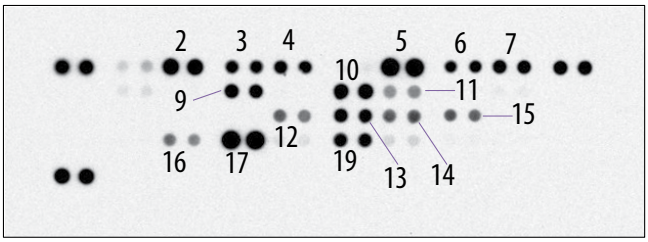
Liver



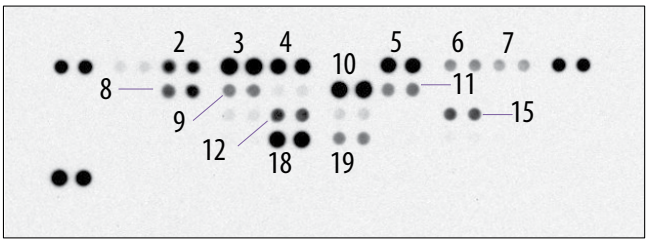
Pancreas



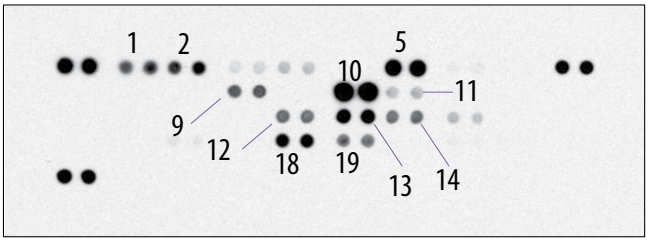
Placenta



Prostate



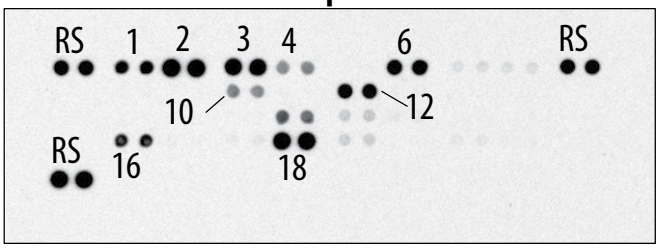
Spleen



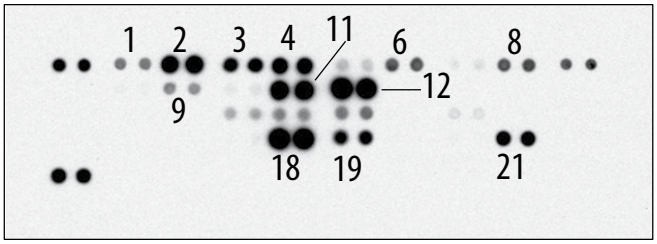
1	APP/Protease Nexin II
2	Cystatin A
3	Cystatin B
4	Cystatin C
5	EMMPRIN/CD147
6	Fetuin B
7	HAI-1
8	HE4/WFDC2
9	Latexin
10	Lipocalin-2 /NGAL
11	RECK
12	Serpin B6
13	Serpin B8/Angiotensinogen
14	Serpin E1/PAI-1
15	Serpin F1/PEDF
16	TFPI
17	TFPI-2
18	TIMP-1
19	TIMP-2

PROFILING PROTEINS IN PBMC SUPERNATES & BODY FLUIDS

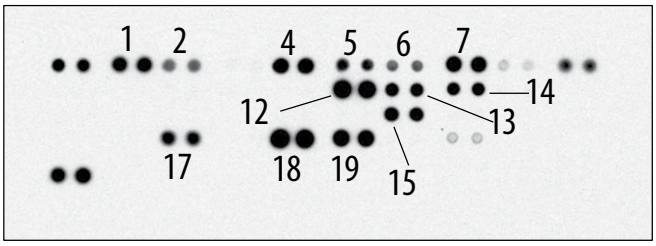
PHA Treated PBMC Supernates



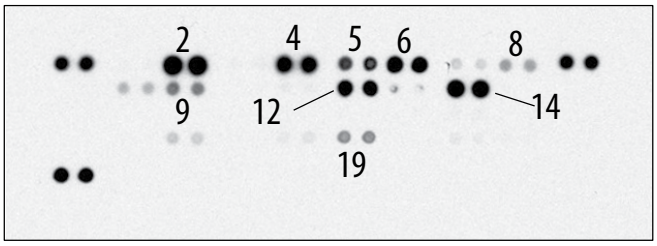
Saliva



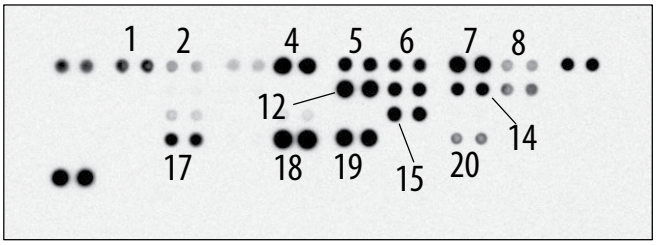
Serum



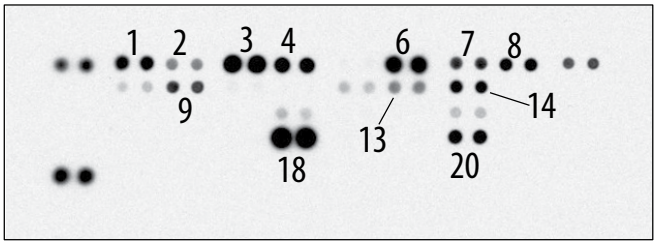
Urine



Plasma



Human Milk



The Human Protease Inhibitor Array detects multiple protease inhibitors in PBMC supernates, serum, plasma, saliva, urine, and human milk samples. The sample type and quantity used per array are listed below. Data shown are from a 5 minute exposure to X-ray film. RS=Reference Spots.

- PBMC supernates were treated with 10 µg/mL PHA for five days; 500 µL of cell culture supernate per array.
- Serum; 100 µL per array.
- Heparin plasma; 100 µL per array.
- Saliva; 100 µL per array.
- Urine; 200 µL per array.
- Human milk; 100 µL per array.

PROFILING PROTEINS IN PBMC SUPERNATES & BODY FLUIDS *CONTINUED*

		MEAN PIXEL DENSITY					
		PBMCs	Serum	Plasma	Saliva	Urine	Human Milk
1	APP/Protease Nexin II	29,687	37,561	23,769	13,589	544	31,121
2	Cystatin A	51,589	18,254	7497	52,392	57,525	11,424
3	Cystatin B	46,789	375	6304	37,671	651	53,165
4	Cystatin C	13,340	42,385	47,886	47,532	47,227	40,427
5	Cystatin E/M	112	23,585	32,295	7401	27,260	861
6	EMMPRIN/CD147	38,158	14,361	28,047	21,792	44,410	48,307
7	Fetuin B	2004	42,030	46,242	1556	3255	21,231
8	HAI-1	1913	3379	8191	21,415	9422	27,072
9	HE4/WFDC2	0	159	593	12,449	17,597	20,328
10	Latexin	13,371	0	452	2157	2	1050
11	Lipocalin-1	0	107	209	58,561	1032	461
12	Lipocalin-2 /NGAL	35,545	52,377	48,153	61,724	40,407	5739
13	RECK	456	30,383	32,037	303	3006	14,234
14	Serpin A5/Proteinase C Inhibitor	81	28,316	29,496	0	50,178	25,722
15	Serpin E1/PAI-1	1060	34,477	33,883	237	4	398
16	Testican 2	24,240	0	0	0	0	184
17	TFPI	0	33,553	28,911	13	4266	19
18	TIMP-1	49,536	54,830	56,096	61,041	1416	60,827
19	TIMP-2	3332	46,974	48,293	34,727	12,316	563
20	TIMP-4	929	4,725	11,929	442	733	32,168
21	Trappin-2/Elafin	333	0	191	37,670	318	294

APPENDIX

Refer to the table below for the Human Protease Inhibitor Array coordinates.

Coordinate	Analyte/Control	Entrez Gene ID	Alternate Nomenclature
A1, A2	Reference Spots	N/A	RS
A3, A4	APP/Protease Nexin II (pan)	351	———
A5, A6	Cystatin A	1475	Stefin A, Cystatin SA, Keratolinin, CSTA
A7, A8	Cystatin B	1476	Stefin B, CSTB
A9, A10	Cystatin C	1471	CST3
A11, A12	Cystatin E/M	1474	CST6
A13, A14	EMMPRIN/CD147	682	Basigin
A15, A16	Fetuin B	26998	FETUB
A17, A18	HAI-1	6692	SPINT1, MANSC2
A19, A20	Reference Spots	N/A	RS
B3, B4	HAI-2	10653	SPINT2
B5, B6	HE4/WFDC2	10406	WAP5, EDDM4
B7, B8	Latexin	56925	LXN, ECI, TCI
B9, B10	Lipocalin-1	3933	VEGP, LCN1, TLC
B11, B12	Lipocalin-2/NGAL	3934	LCN2, MSFI
B13, B14	RECK	8434	ST15
B15, B16	Serpin A5/Protein C Inhibitor	5104	PCI, PAI-3
B17, B18	Serpin A8/Angiotensinogen	183	AGT, ANHU
C3, C4	Serpin A9/Centerin	327657	GCET1
C5, C6	Serpin A12	145264	Vaspin
C7, C8	Serpin B5/Maspin	5268	PI5
C9, C10	Serpin B6	5269	CAP, PI6, PTI
C11, C12	Serpin B8/Proteinase Inhibitor 8	5271	PI8, CAP2
C13, C14	Serpin E1/PAI-1	5054	———
C15, C16	Serpin F1/PEDF	5176	EPC-1, PIG35
C17, C18	Testican 1/SPOCK1	6695	TIC1
D3, D4	Testican 2/SPOCK2	9806	———
D5, D6	TFPI	7035	EPI, LACI, TFI
D7, D8	TFPI-2	7980	PP5, REF-1
D9, D10	TIMP-1	7076	———
D11, D12	TIMP-2	7077	———
D13, D14	TIMP-3	7078	———
D15, D16	TIMP-4	7079	———
D17, D18	Trappin-2/Elafin	5266	WFDC14, ESI, SKALP
E1, E2	Reference Spots	N/A	RS
E19, E20	Negative Control	N/A	Control (-)

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