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R&D SYSTEMS™

Saliva Sensitive

Proteome Profiler™ Array

Human Protease Array Kit

Catalog Number ARY021B

For the parallel determination of the relative levels of selected human proteases.

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 proteases is essential for understanding their roles in normal cellular function and their dysregulation in diseases such as cancer. The Proteome Profiler™ Human Protease Array is a rapid, sensitive, and economical tool to detect protease differences between samples. The relative expression of 35 human proteases 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 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, 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 Array	894359	4 nitrocellulose membranes each containing 35 different protease 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 Array	894780	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/vial)	
Chemi Reagent 2	894288	1 vial (2.5 mL/vial)	
4-Well Multi-dish	607544	Clear 4-well rectangular multi-dish.	Store at room temperature.
Transparency Overlay Template	607921	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).*

PRECAUTIONS

Chemi Reagents 1 and 2 contain Boric Acid which is suspected of damaging fertility or the unborn child.

High levels of some proteins are found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.

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.

OTHER SUPPLIES REQUIRED

- 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 17 (1% Igepal CA-630, 20 mM Tris-HCl (pH 8.0), 137 mM NaCl, 2 mM EDTA, 200 mM Sodium Orthovanadate, 5 mM NaF) (R&D Systems, # 895943)
- Aprotinin (Tocris™, # 4139)
- Leupeptin hemisulfate (Tocris, # 1167)
- Pepstatin A (Tocris, # 1190)

If using tissue lysate samples, the following are also required:

- Phosphate-Buffered Saline (PBS) (R&D Systems, # RB01)
- Aprotinin (Tocris, # 4139)
- Leupeptin hemisulfate (Tocris, # 1167)
- Pepstatin A (Tocris, # 1190)
- 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 Human Protease 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 hemisulfate, and 10 µg/mL Pepstatin A. 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 30 minutes 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 hemisulfate, and 10 µg/mL Pepstatin A. 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 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.

Protease Detection Antibody Cocktail - Reconstitute the Human Protease 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.

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 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 Protease 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 with the corresponding membrane. 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 mL of 1X Streptavidin-HRP into each well of the 4-Well Multi-dish.

ARRAY PROCEDURE *CONTINUED*

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.

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

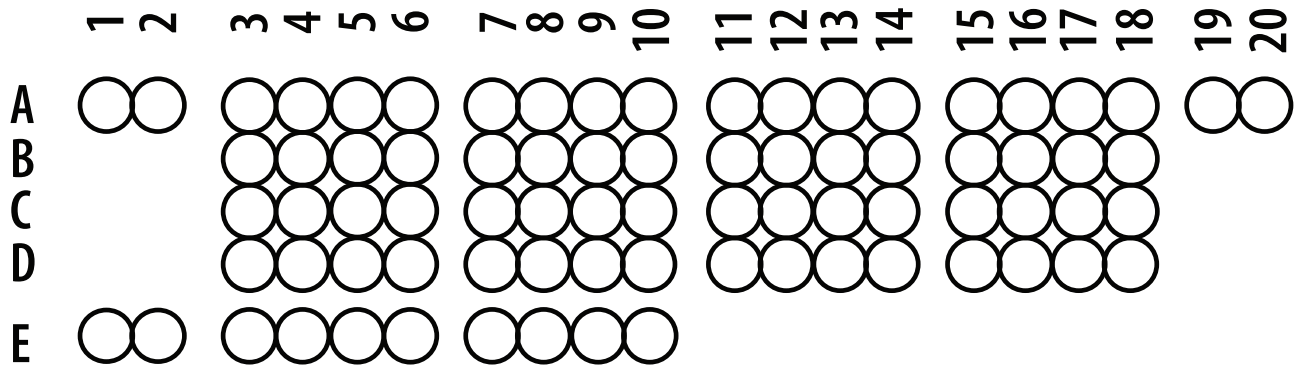
The positive signals seen on developed film can be quickly identified by placing the transparency overlay template on the array image and aligning it with the pairs of reference spots in three corners of each array. The stamped identification number on the array should be placed on the left hand side.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



These images are not to scale. They are for coordinate reference only.
Please use the Transparency Overlay Template for analyte identification.

PROFILING PROTEINS IN CELL CULTURE SUPERNATES

The Human Protease Array detects multiple proteins 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.

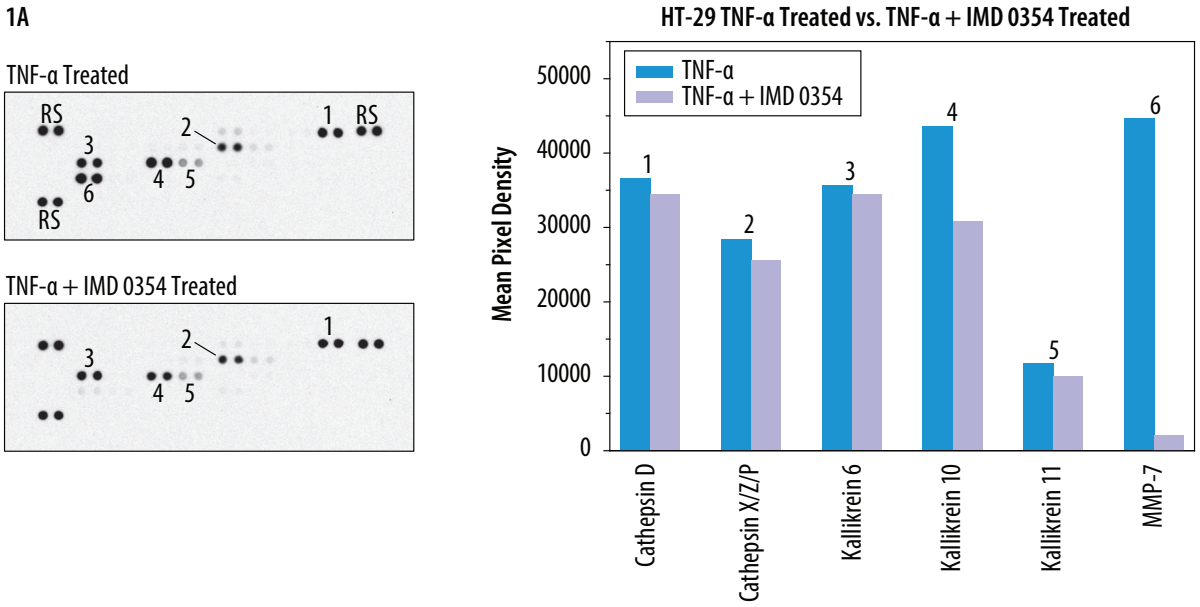


Figure 1A: HT-29 human colon adenocarcinoma cells were untreated or treated with 20 µM of IMD 0354 Inhibitor of IKKβ (Tocris™, # 2611) for 30 minutes followed by treatment with 10 ng/mL of Recombinant Human TNF-α (R&D Systems®, # 210-TA) for 6 hours. RS=Reference Spots.

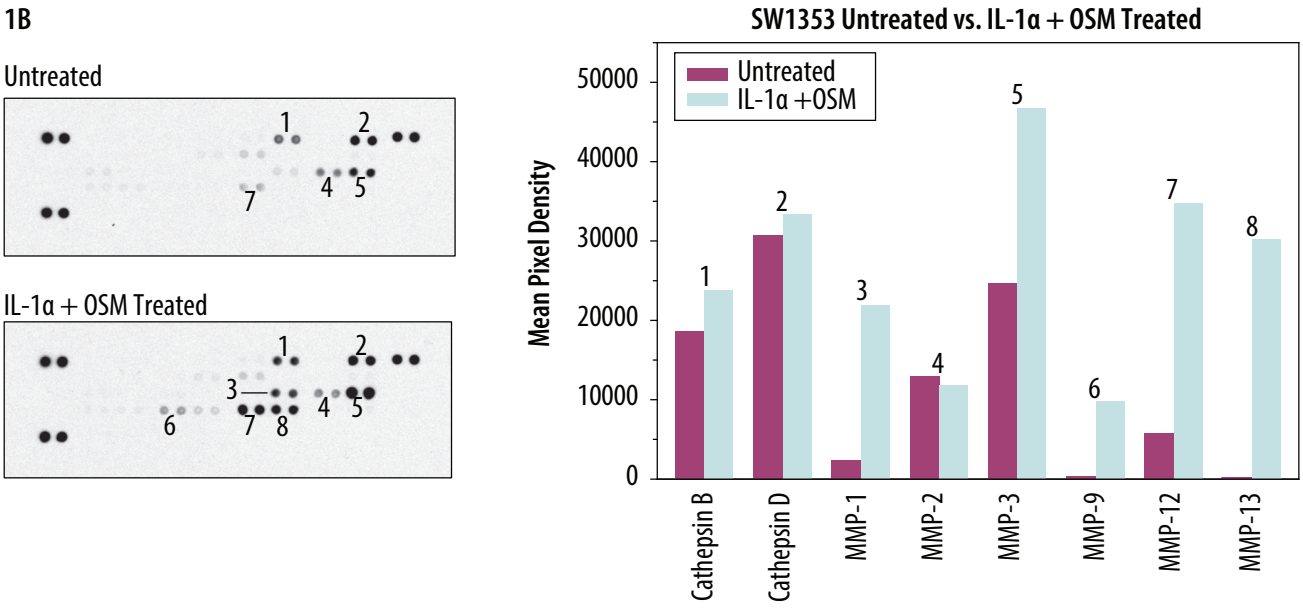


Figure 1B: SW1353 human chondrosarcoma cells were untreated or treated with 5 ng/mL of Recombinant Human IL-1α (R&D Systems, # 200-LA) and 10 ng/mL of Recombinant Human Oncostatin M (OSM) (R&D Systems, # 295-OM) for 24 hours.

PROFILING PROTEINS IN CELL LYSATES

The Human Protease Array detects multiple proteins in cell lysates. Cells were either untreated or treated as indicated below. 200 µg of cell lysate 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.

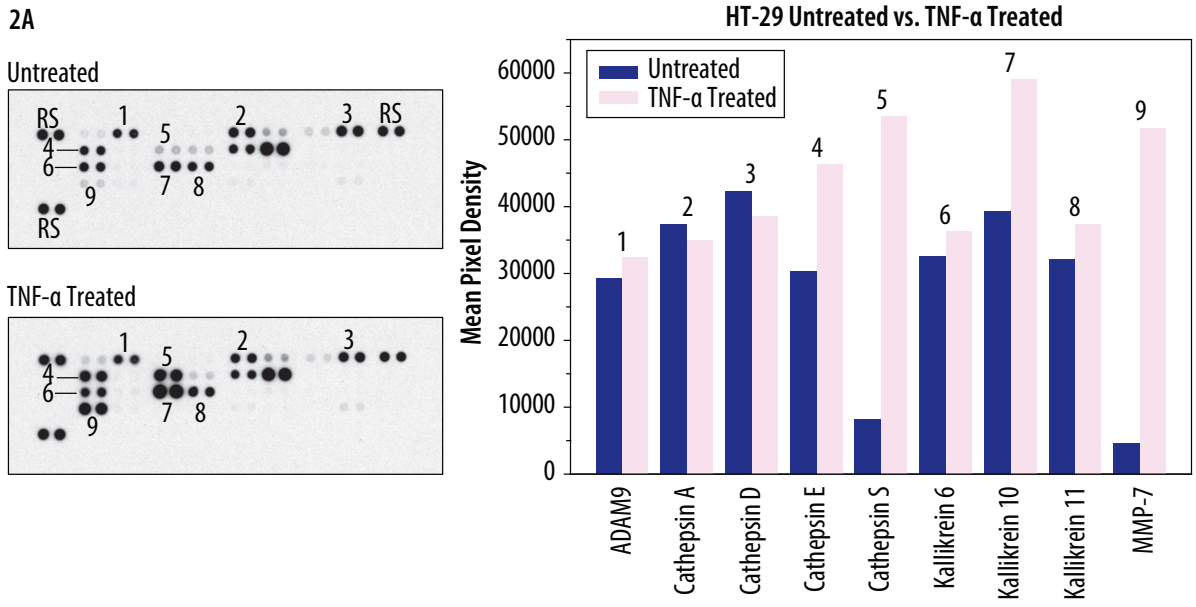


Figure 2A: HT-29 human colon adenocarcinoma cells were untreated or treated with 10 ng/mL of Recombinant Human TNF-α (R&D Systems®, # 210-TA) for 24 hours.

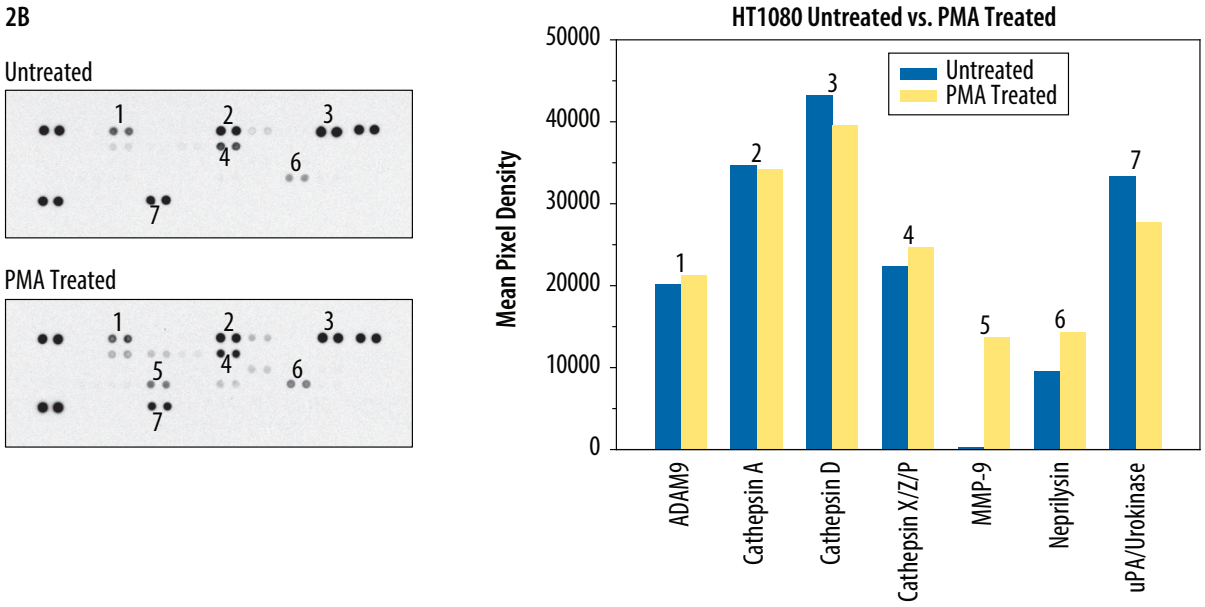
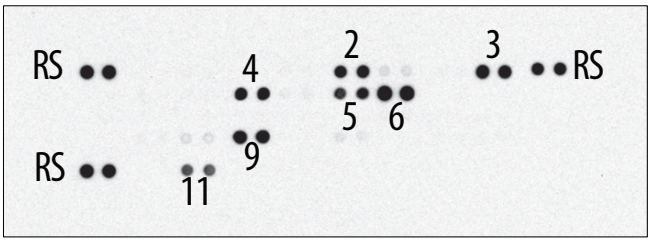


Figure 2B: HT1080 human fibrosarcoma cells were untreated or treated with 100 nM PMA (Tocris™, # 1201) for 24 hours.

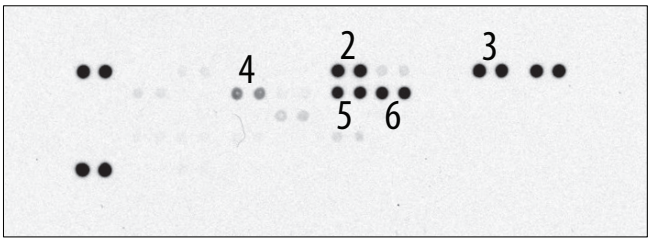
PROFILING PROTEASES IN TISSUE LYSATES

The Human Protease 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.

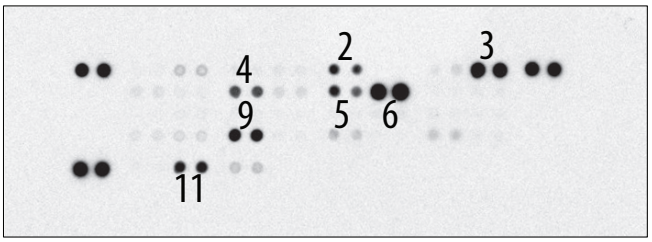
Liver



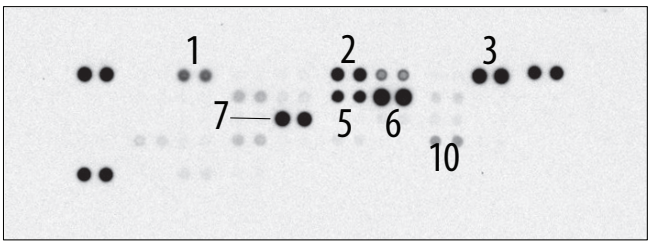
Pancreas



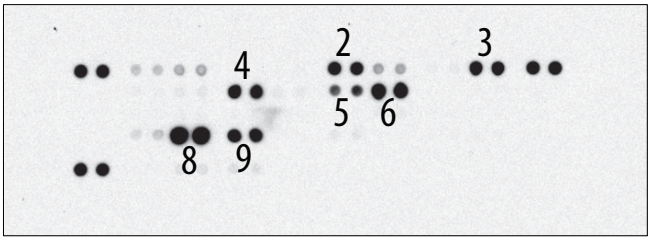
Placenta



Prostate

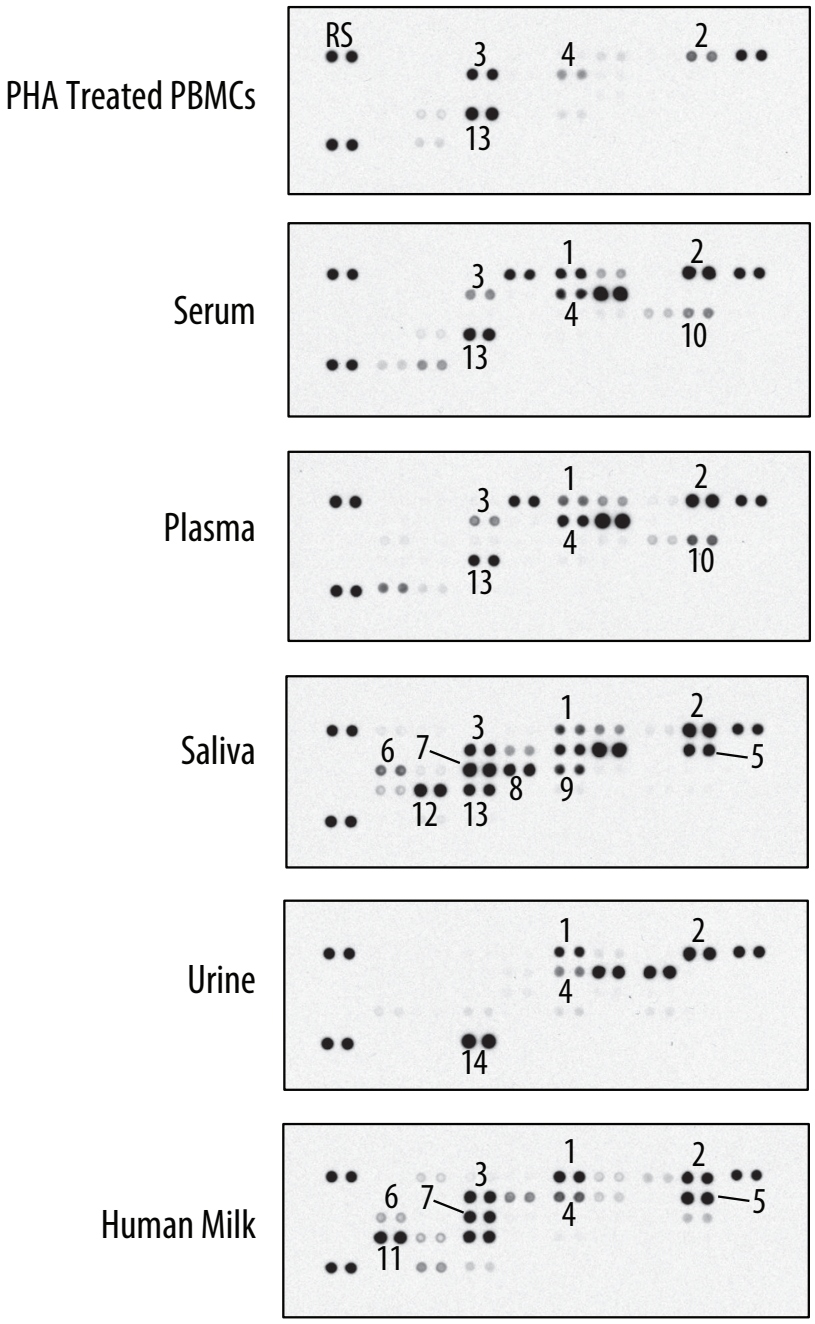


Spleen



1	ADAM9
2	Cathepsin A
3	Cathepsin D
4	Cathepsin S
5	Cathepsin X/Z/P
6	DPPIV/CD26
7	Kallikrein 11
8	MMP-8
9	MMP-9
10	Neprilysin/CD10
11	Proteinase 3

PROFILING PROTEINS IN PBMC SUPERNATES AND BODY FLUIDS



The Human Protease Array detects multiple proteins in PBMCs, 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.

PBMCs - Treated with 10 µg/mL PHA for five days; 200 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	Cathepsin A	1311	35,253	26,120	27,437	29,770	38,704
2	Cathepsin D	26,172	50,471	49,871	52,218	47,379	39,790
3	Cathepsin S	35,673	19,293	21,694	40,294	345	42,170
4	Cathepsin X/Z/P	17,913	31,524	38,044	35,689	20,950	29,257
5	Kallikrein 5	0	107	444	44,617	0	45,808
6	Kallikrein 6	0	278	2607	28,337	0	14,081
7	Kallikrein 10	360	624	1305	51,394	127	40,877
8	Kallikrein 11	164	0	135	42,870	1211	994
9	Kallikrein 13	28	184	890	29,595	27	247
10	MMP-3	0	21,761	28,381	714	0	10,455
11	MMP-7	352	431	606	10,433	3134	45,203
12	MMP-8	4609	3716	653	47,921	799	11,698
13	MMP-9	47,024	43,824	37,358	40,806	3320	42,512
14	uPA/Urokinase	527	213	380	909	54,874	6964

PROTEASE ARRAY APPENDIX

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

Coordinate	Analyte/Control	Entrez Gene ID	Isoform Specificity	Alternate Nomenclature
A1, A2	Reference Spots	N/A	N/A	RS
A3, A4	ADAM8	101	Ectodomain	MS2, CD156a
A5, A6	ADAM9	8754	Ectodomain	MDC9, meltrin g
A7, A8	ADAMTS1	9510	Proform	METH1
A9, A10	ADAMTS13	11093	Active	von Willebrand factor-cleaving protease
A11, A12	Cathepsin A	5476	Proform & Active	CTSA, Lysosomal Carboxypeptidase A
A13, A14	Cathepsin B	1508	Proform	CTSB, APPS, CPSB
A15, A16	Cathepsin C	1075	Proform & Active	CTSC
A17, A18	Cathepsin D	1509	Proform & Active	CTSD, CPSD
A19, A20	Reference Spots	N/A	N/A	RS
B3, B4	Cathepsin E	1510	Proform & Active	CTSE, CATE
B5, B6	Cathepsin L	1514	Proform & Active	CTSL, CATL, MEP, Cathepsin L1
B7, B8	Cathepsin S	1520	Proform & Active	CTSS
B9, B10	Cathepsin V	1515	Proform & Active	CTSV, CTSL2, CTSU, Cathepsin L2
B11, B12	Cathepsin X/Z/P	1522	Proform & Active	CTSX, CTSZ
B13, B14	DPPIV/CD26	1803	Ectodomain	ADABP, ADCP2
B15, B16	Kallikrein 3/PSA	354	Proform & Active	KLK3, hK3, KLK2A1
B17, B18	Kallikrein 5	25818	Proform & Active	KLK5, SCTE, KLKL2
C3, C4	Kallikrein 6	5653	Proform & Active	KLK6
C5, C6	Kallikrein 7	5650	Proform & Active	KLK7, SCCE, PRSS6, hK7
C7, C8	Kallikrein 10	5655	Proform & Active	KLK10, NES1, PRSSL1
C9, C10	Kallikrein 11	11012	Proform & Active	KLK11, TLSP, PRSS20
C11, C12	Kallikrein 13	26085	Proform & Active	KLK13, KLKL4
C13, C14	MMP-1	4312	Proform & Active	Collagenase 1, Interstitial Collagenase
C15, C16	MMP-2	4313	Proform & Active	Gelatinase A
C17, C18	MMP-3	4314	Proform & Active	Stromelysin-1
D3, D4	MMP-7	4316	Proform & Active	Matrilysin, PUMP 1
D5, D6	MMP-8	4317	Proform & Active	Collagenase 2, Neutrophil Collagenase
D7, D8	MMP-9	4318	Proform & Active	Gelatinase B, CLG4B, GELB
D9, D10	MMP-10	4319	Proform & Active	Stromelysin-2
D11, D12	MMP-12	4321	Proform & Active	Macrophage Elastase
D13, D14	MMP-13	4322	Proform	Collagenase 3
D15, D16	Neprilysin/CD10	4311	Ectodomain	MME, NEP, CALLA
D17, D18	Presenilin	5663	N-Terminal Fragment	PSEN1, AD3, PS-1
E1, E2	Reference Spots	N/A	N/A	RS
E3, E4	Proprotein Convertase 9	255738	Active	PCSK9, PC9, NARC1
E5, E6	Proteinase 3	5657	Active	PRTN3, Myeloblastin
E7, E8	uPA/Urokinase	5328	Proform & Active	Urokinase-type Plasminogen Activator, PLAU
E9, E10	Negative Control	N/A	N/A	Control (-)

03.15 753177.2 04/24

For research use only. Not for use in diagnostic procedures.