Proteome Profiler™ Array

Human Protease Array Kit

Catalog Number ARY021

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.

TABLE OF CONTENTS

SECTION PAGE

MANUFACTURED AND DISTRIBUTED BY:

USA & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

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 Human Protease Array Kit is a rapid, sensitive, and economical tool to simultaneously detect protease differences between samples. The relative expression of 34 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, 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 Kit. 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 Human Protease 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 Human Protease 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 Protease Array	894547	4 nitrocellulose membranes each containing 34 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 before and during use.*	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.	
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	894548	1 vial of a 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 Rectangular Multi-dish	607544	Clear 4-well rectangular multi-dish.	Store at room temperature.
Transparency Overlay Template	607807	1 transparency overlay template for coordinate reference.	

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

OTHER SUPPLIES REQUIRED

- Aprotinin (Sigma, Catalog # A6279)
- Leupeptin (Tocris, Catalog # 1167)
- Pepstatin (Tocris, Catalog # 1190)
- Igepal® CA-630 (Sigma, Catalog # I3021)
- Pipettes and pipette tips
- Gloves
- Deionized or distilled water
- 1000 mL graduated cylinder
- 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 (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)

SUPPLIES REQUIRED FOR TISSUE LYSATE SAMPLES

- Protease Inhibitor Cocktail (Sigma, Catalog # P8340)
- Igepal® CA-630 (Sigma, Catalog # I3021)
- Sodium deoxycholate (Sigma, Catalog # D6750)
- Sodium dodecyl sulfate (Sigma, Catalog # L6026)

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 50-200 µL for serum, plasma, 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. 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 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.

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 place in Tissue Lysis buffer (0.5% Igepal, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 50 mM Tris-HCl (pH 7.5), and 150 mM NaCl) with Protease Inhibitor Cocktail. Homogenize tissue and centrifuge at 1000 x g for 10 minutes at 2-8 °C to remove cellular debris. Transfer supernate to a new tube. 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 proteins are found in saliva. It is recommended that a mask and gloves be used to protect kit reagents from contamination.*

Human Protease Array - Four nitrocellulose membranes each containing 34 different capture antibodies printed in duplicate. **Handle the membranes only with gloved hands and flat-tipped tweezers.**

Detection Antibody Cocktail - One vial of lyophilized biotinylated antibodies. Before use, 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 to prepare 1000 mL of 1X Wash Buffer.

Note: *Wash Buffer Concentrate may turn yellow over time.*

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.**

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 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 prtoeins 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. Using flat-tip tweezers, remove each membrane to be used from between the protective sheets and place in a well of the 4-Well Multi-dish. The number on the membrane should be facing upward.

   Note: *Upon contact with Array Buffer 6, the blue dye from the spots will disappear, but the capture antibodies are retained in their specific locations.*
4. Incubate for one hour on a rocking platform shaker. Orient the 4-Well Multi-dish so that each membrane rocks end to end in its well.
5. While the membranes are blocking, prepare samples by adding up to 1 mL of each sample to 0.5 mL of Array Buffer 4 in separate tubes. Adjust to a final volume of 1.5 mL with Array Buffer 6 as necessary.
6. Add 15 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. Dilute the Streptavidin-HRP in Array Buffer 6 using the dilution factor on the vial label. Pipette 2.0 mL of diluted Streptavidin-HRP into each well of the 4-Well Multi-dish.
12. Carefully remove each membrane from its wash container. Allow excess Wash Buffer to drain from the membrane. Return the membrane to the 4-Well Multi-dish containing the diluted Streptavidin-HRP. Cover the wells with the lid.
13. Incubate for 30 minutes at room temperature on a rocking platform shaker.

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 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

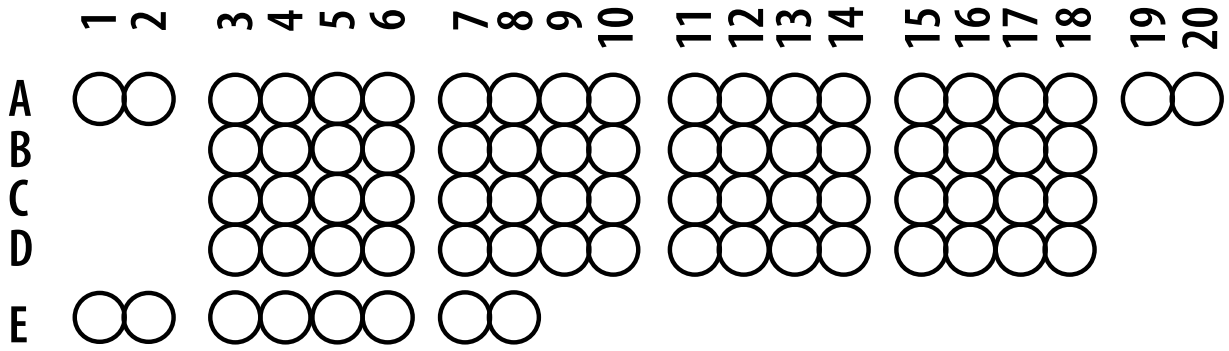
The positive signals seen on developed film can be quickly identified by placing the transparency overlay template on the array image and aligning it with the pairs of reference spots in three corners of each array. The stamped identification number on the array should be placed on the left hand side.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

Human Protease Array Coordinates



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

PROFILING PROTEINS IN CELL CULTURE SUPERNATES

The Human Protease Array detects multiple proteases in cell culture supernates. Cells were 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.

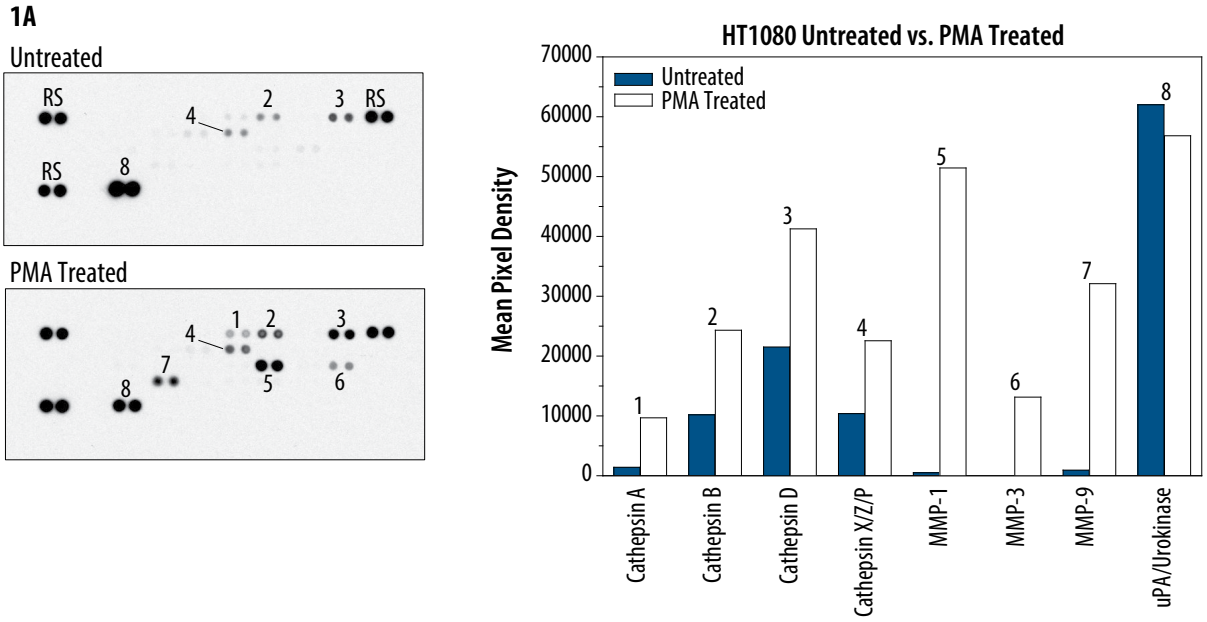


Figure 1A: HT1080 human fibrosarcoma cells were untreated or treated with 100 nM PMA for 24 hours. RS=Reference Spot.

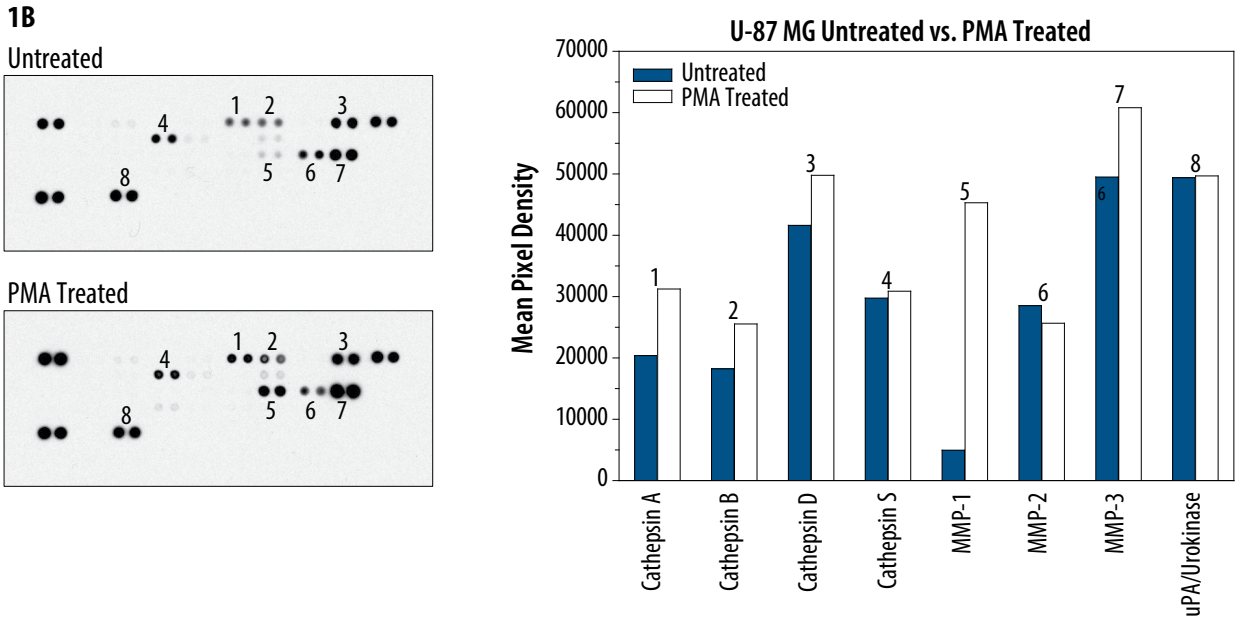


Figure 1B: U-87 MG human glioblastoma/astrocytoma cells were untreated or treated with 100 nM PMA for 24 hours.

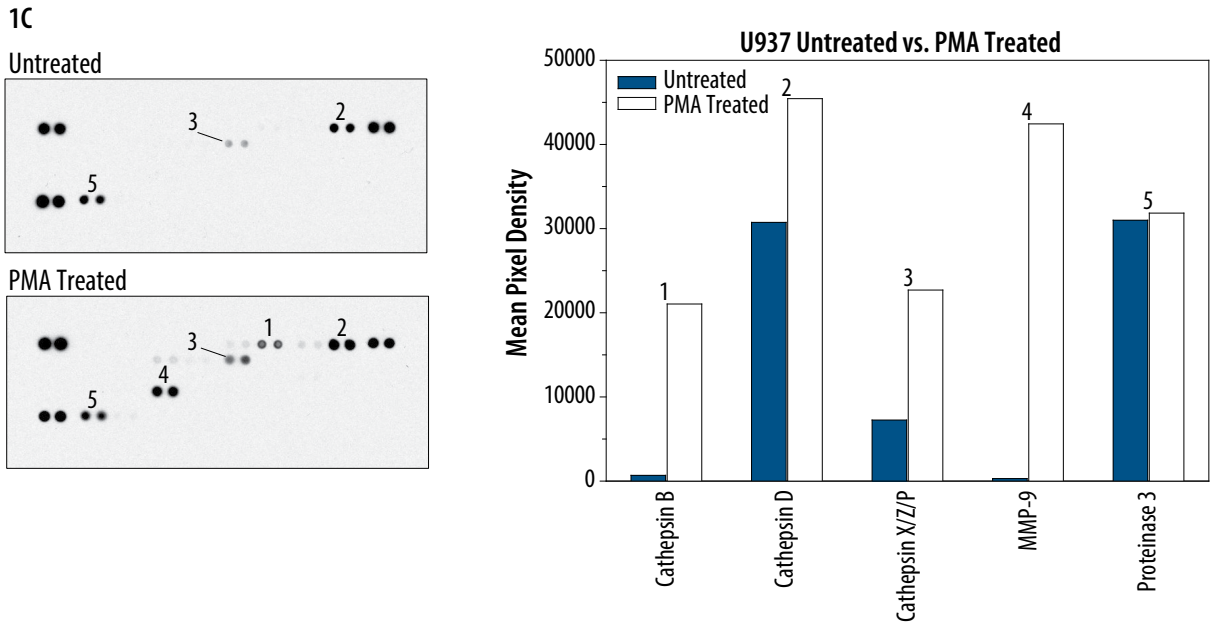


Figure 1C: U937 human histiocytic lymphoma cells were untreated or treated with 200 nM PMA for 16 hours.

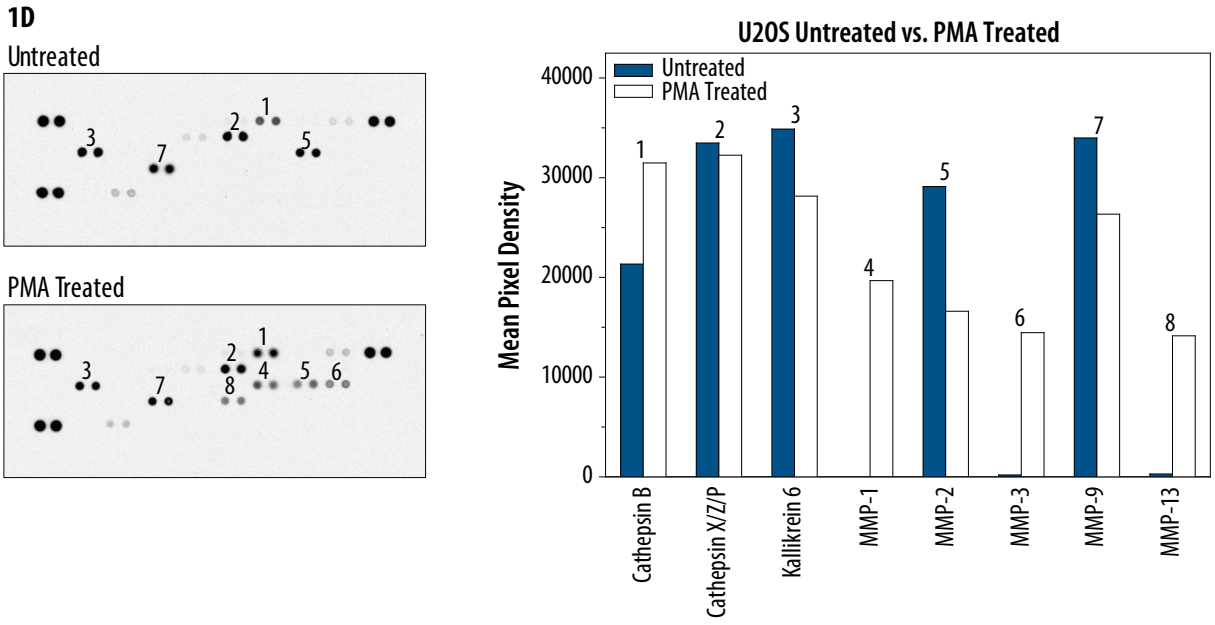


Figure 1D: U2OS human osteosarcoma cells were untreated or treated with 50 nM PMA for 24 hours.

PROFILING PROTEINS IN CELL LYSATES

The Human Protease 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. 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.

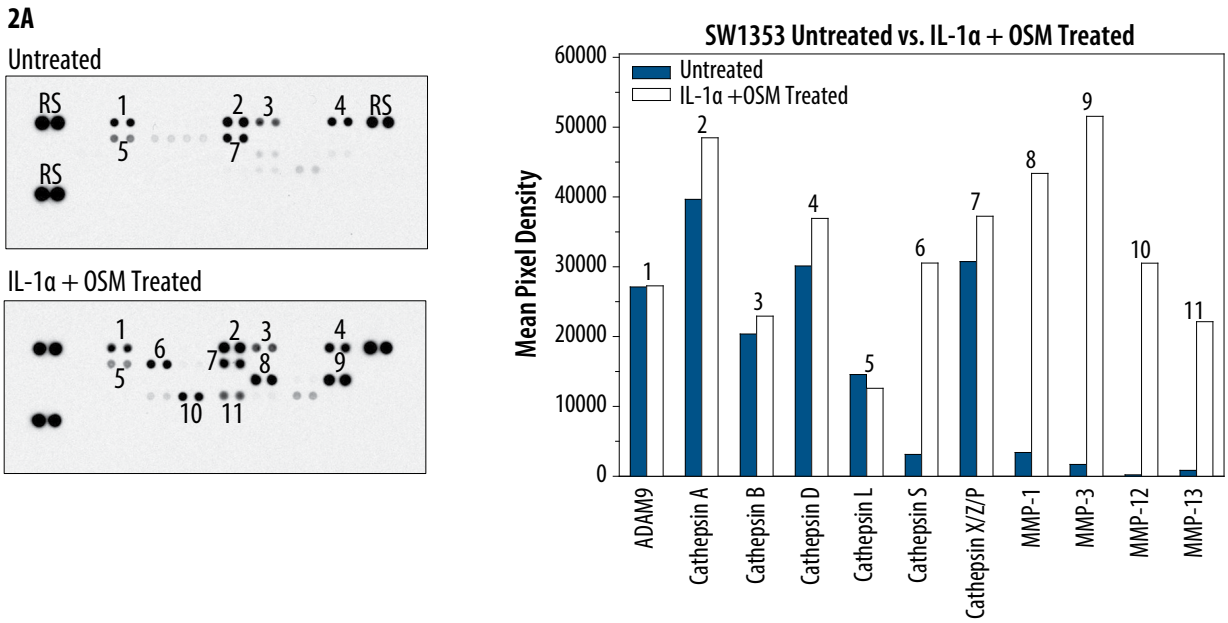


Figure 2A: SW1353 human chondrosarcoma cells were untreated or treated with 5 ng/mL recombinant human IL-1α (R&D Systems, Catalog # 200-LA) and 10 ng/mL recombinant human Oncostatin M (OSM) (R&D Systems, Catalog # 295-OM) for 24 hours. RS=Reference Spot

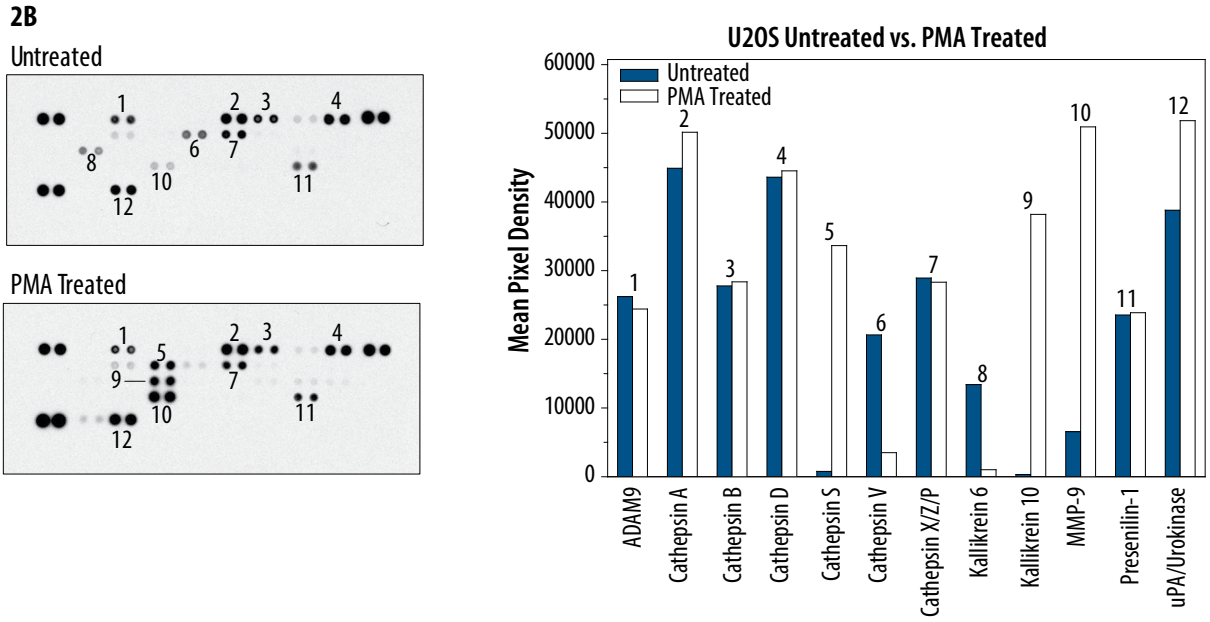


Figure 2B: U2OS human osteosarcoma cells were untreated or treated with 50 nM PMA for 24 hours.

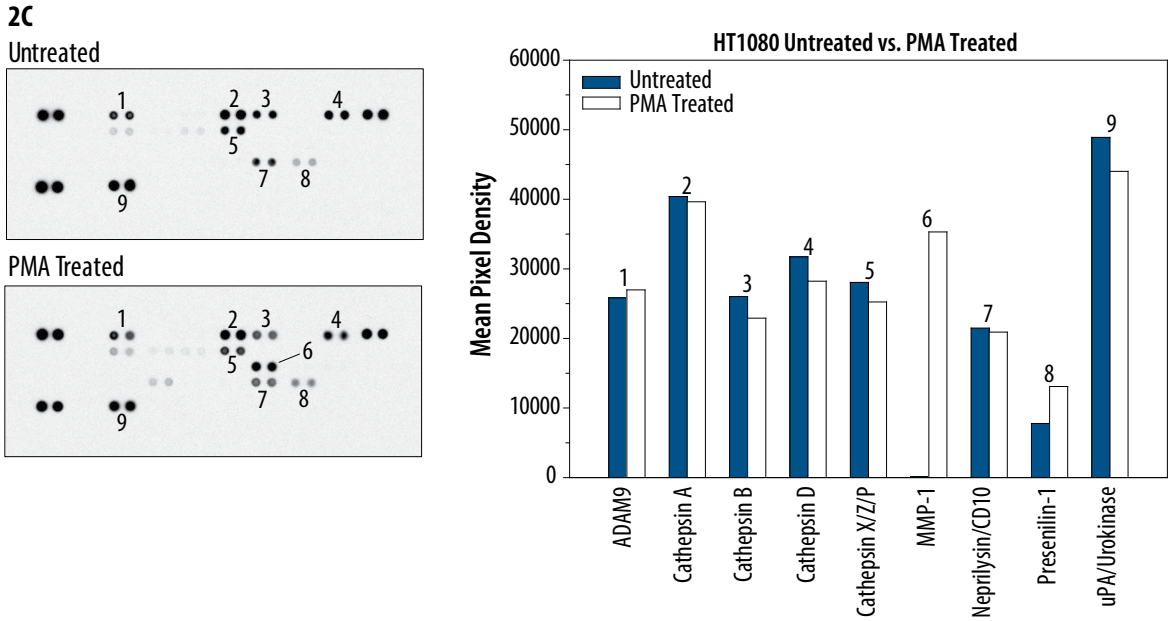


Figure 2C: HT1080 human fibrosarcoma cells were untreated or treated with 100 nM PMA for 24 hours.

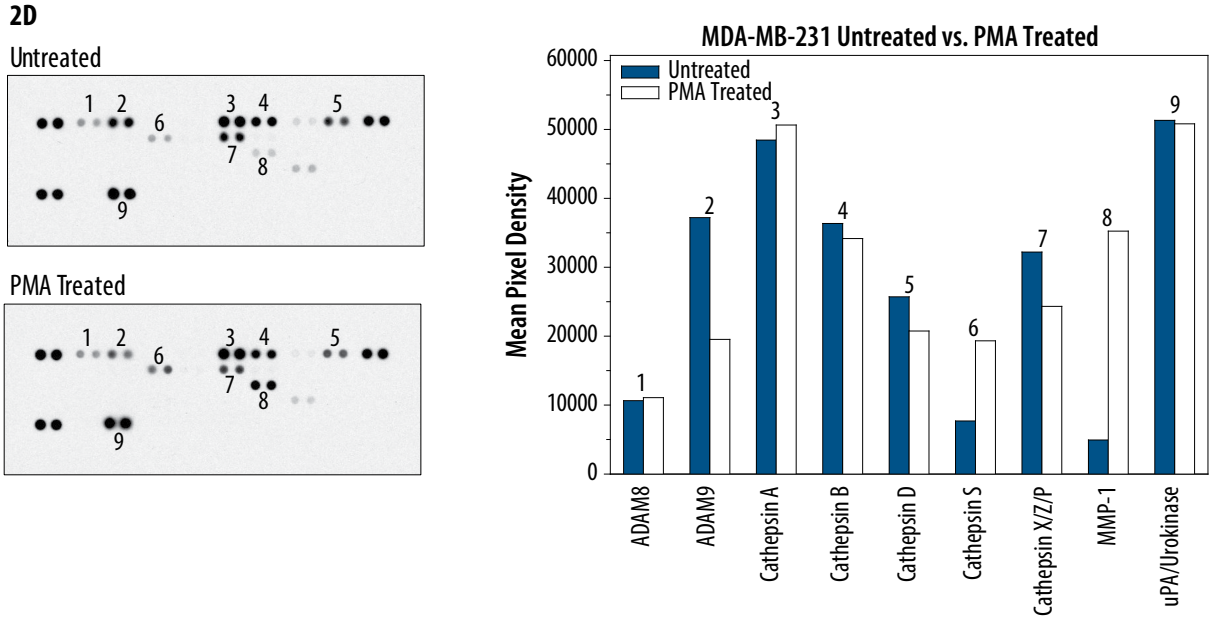


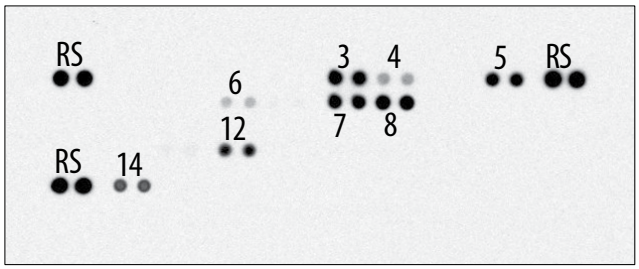
Figure 2D: MDA-MB-231 human breast cancer cells were untreated or treated with 50 nM PMA for 24 hours.

PROFILING PROTEINS IN TISSUE LYSATES

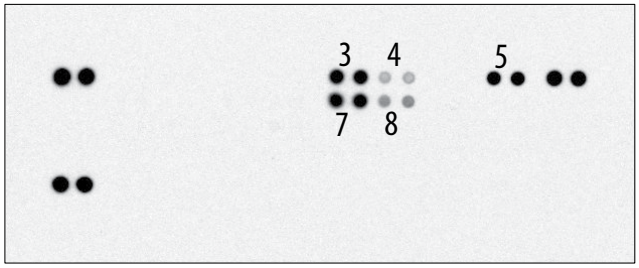
The Human Protease Array detects multiple analytes in tissue lysates.

200 µg of tissue lysate was run on each array. Data shown are from a five minute exposure to X-ray film. RS=Reference Spot.

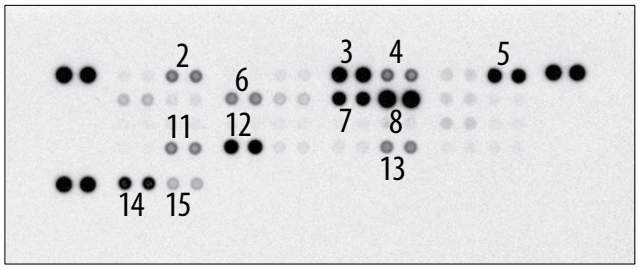
Liver



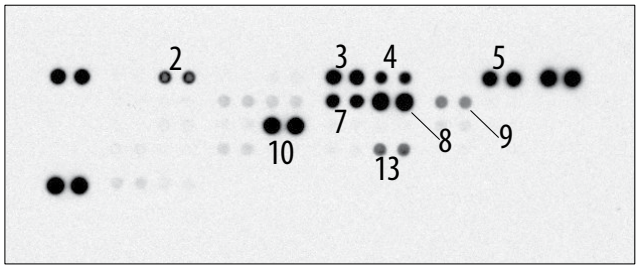
Pancreas



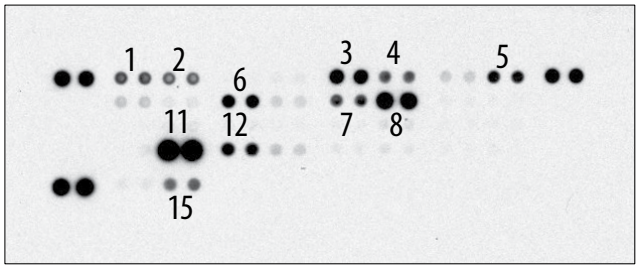
Placenta



Prostate



Spleen



1	ADAM8
2	ADAM9
3	Cathepsin A
4	Cathepsin B
5	Cathepsin D
6	Cathepsin S
7	Cathepsin X/Z/P
8	DPPIV/CD26
9	Kallikrein 3/PSA
10	Kallikrein 11
11	MMP-8
12	MMP-9
13	Neprilysin/CD10
14	Proteinase 3
15	uPA/Urokinase
RS	Reference Spots

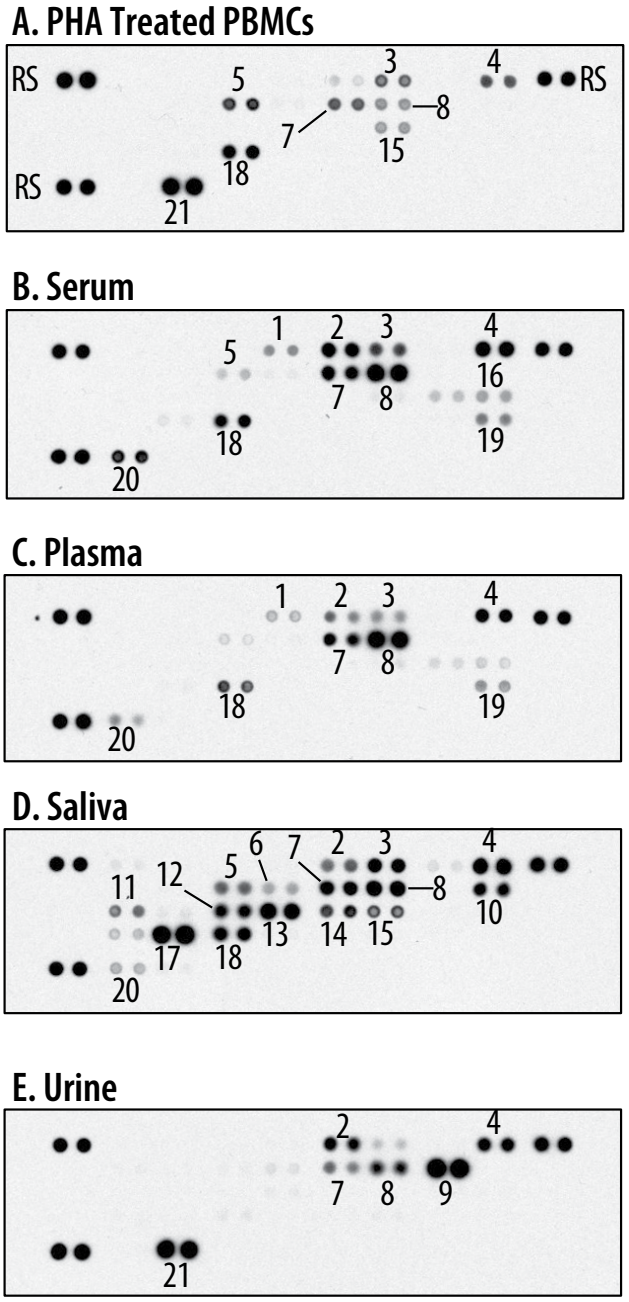


Figure 3: The Human Protease Array detects multiple proteases in PBMCs, serum, plasma, saliva, and urine samples. The sample type, quantity used per array, and exposure duration to X-ray film are listed below. RS=Reference Spot.

A. PBMCs were treated with 10 µg/mL PHA for 5 days; 200 µL of cell culture supernate per array (5 minute exposure).

B. Serum; 100 µL per array (2 minute exposure).

C. Heparin plasma; 100 µL per array (2 minute exposure).

D. Saliva; 100 µL per array (2 minute exposure).

E. Urine; 200 µL per array (2 minute exposure).

PROFILING PROTEINS IN PBMCs & BODY FLUIDS *CONTINUED*

		MEAN PIXEL DENSITY				
		PBMCs	Serum	Plasma	Saliva	Urine
1	ADAMTS13	574	10,804	6846	915	292
2	Cathepsin A	3649	40,371	14,288	21,571	33,542
3	Cathepsin B	19,117	26,718	13,738	38,496	5770
4	Cathepsin D	21,227	44,657	33,252	47,746	36,077
5	Cathepsin S	25,847	5444	2648	23,899	667
6	Cathepsin V	1009	1028	280	10,959	2101
7	Cathepsin X/Z/P	20,988	38,121	31,635	39,357	16,354
8	DPPIV/CD26	12,169	54,213	55,445	45,424	32,087
9	Kallikrein 3/PSA	410	107	0	115	59,258
10	Kallikrein 5	662	100	0	34,173	256
11	Kallikrein 6	486	0	0	16,503	0
12	Kallikrein 10	348	258	130	37,412	93
13	Kallikrein 11	197	38	0	50,763	804
14	Kallikrein 13	284	243	0	27,602	165
15	MMP-1	11,085	2177	1815	22,005	8
16	MMP-3	422	10,509	3409	327	120
17	MMP-8	726	1188	520	58,675	283
18	MMP-9	35,550	32,093	20,479	39,917	1279
19	Proprotein Convertase 9/PCSK9	372	14,556	10,373	0	0
20	Proteinase 3	503	28,094	9645	7896	241
21	uPA/Urokinase	55,091	175	116	1083	57,107

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 γ
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-12	4321	Proform & Active	Macrophage Elastase
D11, D12	MMP-13	4322	Proform	Collagenase 3
D13, D14	Neprilysin/CD10	4311	Ectodomain	MME, NEP, CALLA
D15, D16	Presenilin-1	5663	N-Terminal Fragment	PSEN1, AD3, PS-1
D17, D18	Proprotein Convertase 9	255738	Active	PC9, NARC1
E1, E2	Reference Spots	N/A	N/A	RS
E3, E4	Proteinase 3	5657	Active	PRTN3, Myeloblastin
E5, E6	uPA/Urokinase	5328	Proform & Active	Urokinase-type Plasminogen Activator, PLAU
E7, E8	Negative Control	N/A	N/A	Control (-)

NOTES

NOTES