Proteome Profiler™ Array

Human XL Oncology Array Kit

Catalog Number ARY026

For the parallel determination of the relative levels of selected human cancer-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

Oncogenic transformation alters cellular protein expression and metabolism in ways that may be characteristic of the type or stage of cancer. The Proteome Profiler Human XL Oncology Array Kit is a rapid, sensitive, and economical tool to detect differences in cancer-related proteins between samples. The relative expression levels of 84 human cancer-related proteins can be determined simultaneously without performing numerous Western blots or ELISAs. Each capture and detection antibody was carefully selected using natural samples and recombinant proteins.

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 and incubated overnight with the Proteome Profiler Human XL Oncology Array Kit. The membrane is washed to remove unbound material followed by incubation with a cocktail of biotinylated detection antibodies. Streptavidin-HRP and chemiluminescent detection reagents are then 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 XL Oncology Array	894747	4 nitrocellulose membranes each containing 84 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 XL Oncology Array	894748	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	607897	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

- 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 XL Oncology 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, 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. Solubilize cells at 1 x 10^7 cells/mL in Lysis Buffer 17 (prepared as described in the Reagent Preparation section). 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 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 XL Oncology Array - Immediately before use, remove each membrane to be used from between the protective sheets with a flat-tipped tweezers. **Handle the membranes with gloved hands and flat-tipped tweezers only.**

Detection Antibody Cocktail - Before use, reconstitute the Human XL Oncology Detection Antibody Cocktail in 200 µL of deionized or distilled water.

1X Array Buffer 4/6 - *Array Buffer 4 may contain a precipitate. Mix well before and during use.* Add 4 mL of Array Buffer 4 to 8 mL of Array Buffer 6. Prepare fresh for each use.

Lysis Buffer 17 - Add 10 µg/mL Aprotinin, 10 µg/mL Leupeptin, and 10 µg/mL Pepstatin to the volume of lysis buffer required for cell lysate preparation. **Prepare fresh for each use.**

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.

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.

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 proteins 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. Pipet 2.0 mL of Array Buffer 6 into each well of the 4-Well Multi-dish. 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 by adding up to 0.5 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. Aspirate Array Buffer 6 from the wells of the 4-Well Multi-dish and add the prepared samples. Place the lid on the 4-Well Multi-dish.
7. 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.*
8. 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.
9. Wash each membrane with 1X Wash Buffer for 10 minutes on a rocking platform shaker. Repeat two times for a total of three washes.
10. For each array, add 30 µL of Detection Antibody Cocktail to 1.5 mL of 1X Array Buffer 4/6. Pipette 1.5 mL per well of diluted Detection Antibody Cocktail into the 4-Well Multi-dish.
11. Carefully remove each array from its wash container. Allow excess Wash Buffer to drain from the array. Return the array to the 4-Well Multi-dish containing the diluted Detection Antibody Cocktail, and cover with the lid.
12. Incubate for 1 hour on a rocking platform shaker.
13. Wash each array as described in steps 8 and 9.

ARRAY PROCEDURE *CONTINUED*

14. Pipette 2.0 mL of 1X Streptavidin-HRP into each well of the 4-Well Multi-dish.

15. 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.

16. Incubate for 30 minutes at room temperature on a rocking platform shaker.

17. Wash each array as described in steps 8 and 9.

    Note: *Complete the remaining steps without interruption.*

18. 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.

19. 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.*

20. 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.

21. Position paper towels on the top and sides of the plastic sheet protector containing the membranes and carefully squeeze out excess Chemi Reagent Mix.

22. 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.

23. 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.*

24. Expose membranes to X-ray film for 1-10 minutes. Multiple exposure times are recommended.

DATA ANALYSIS

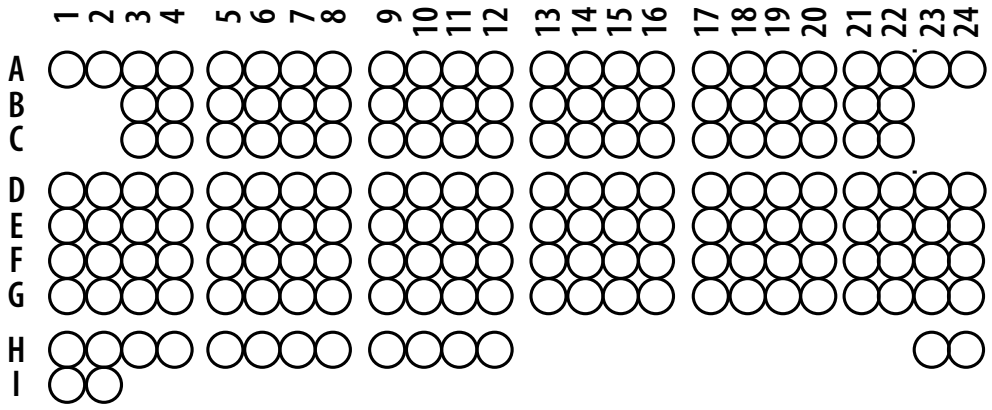
The positive signals seen on developed film can be quickly identified by placing the transparency overlay template on the array image and aligning it with the pairs of reference spots in three corners of each array. The stamped identification number on the array should be placed on the left hand side. 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 XL Oncology 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 XL Oncology Array detects multiple oncology-related proteins in cell culture supernates. 500 µL of cell culture supernate was run on each array. Data shown are from a five minute exposure to X-ray film. Profiles of mean spot pixel density were created using a transmission-mode scanner and image analysis software.

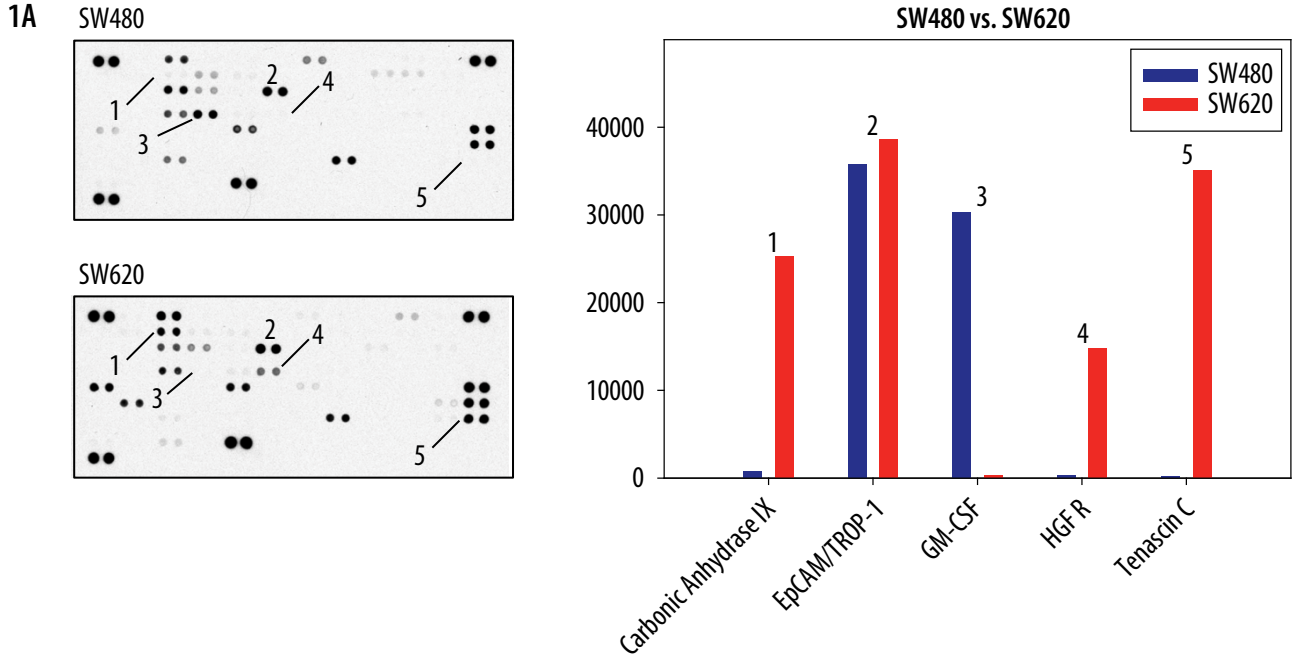


Figure 1A: Supernates collected from SW480 human colorectal adenocarcinoma and SW620 human colorectal adenocarcinoma metastatic site cells.

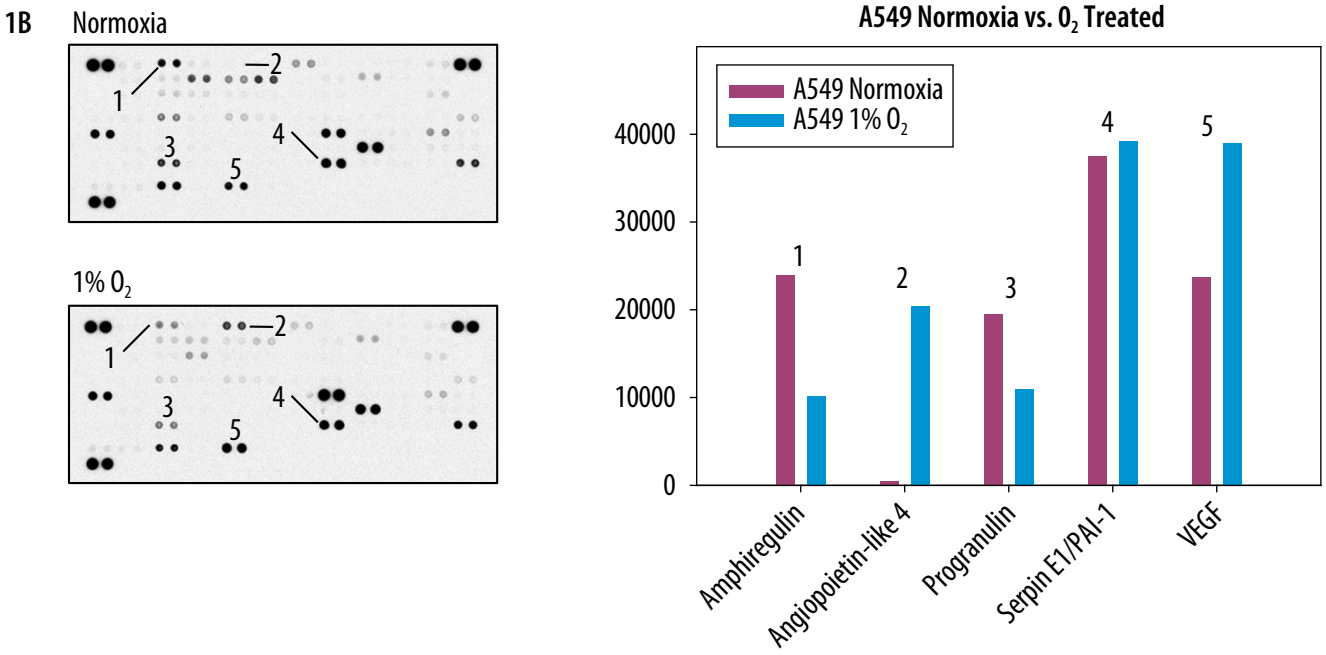


Figure 1B: Supernates collected from A549 human lung carcinoma cells were either cultured in normoxia or 1% O_2 for 3 days.

PROFILING PROTEINS IN CELL LYSATES

The Human XL Oncology Array detects multiple oncology-related proteins in cell lysates. 200 µg of cell lysate was run on each array. Data shown are from a five minute exposure to X-ray film. Profiles of mean spot pixel density were created using a transmission-mode scanner and image analysis software.

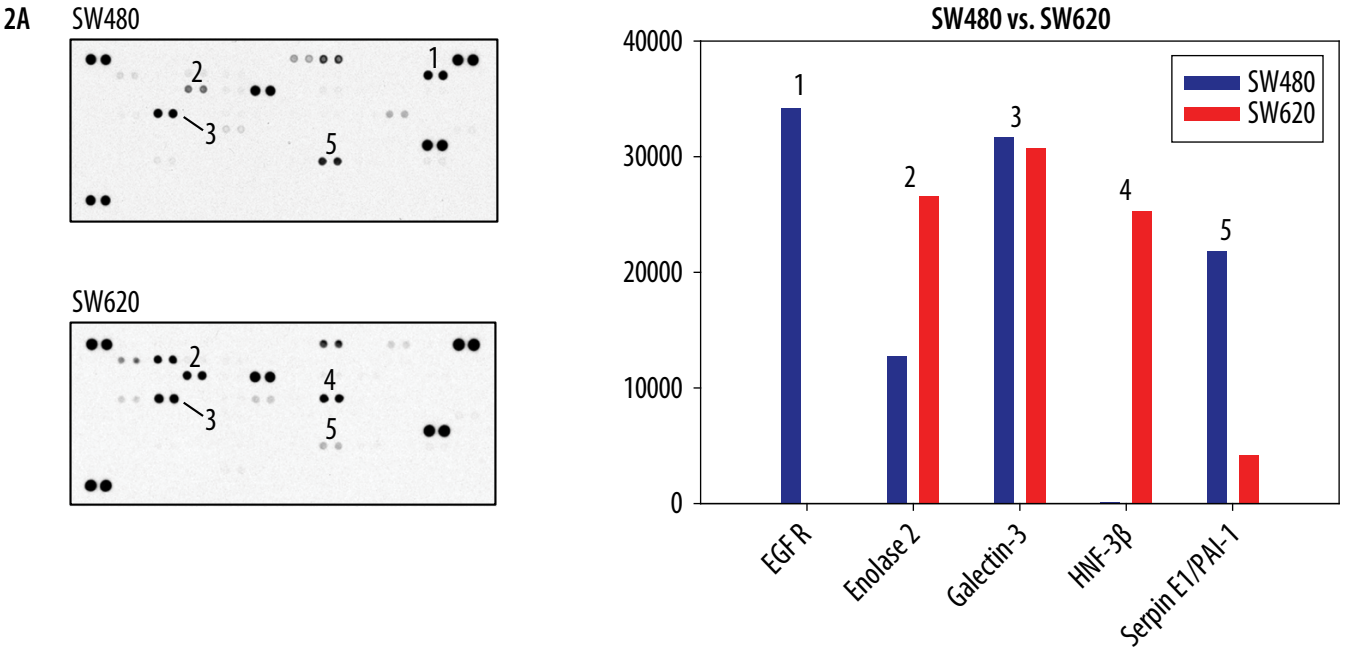


Figure 2A: Lysates from SW480 human colorectal adenocarcinoma and SW620 human colorectal adenocarcinoma metastatic site cells.

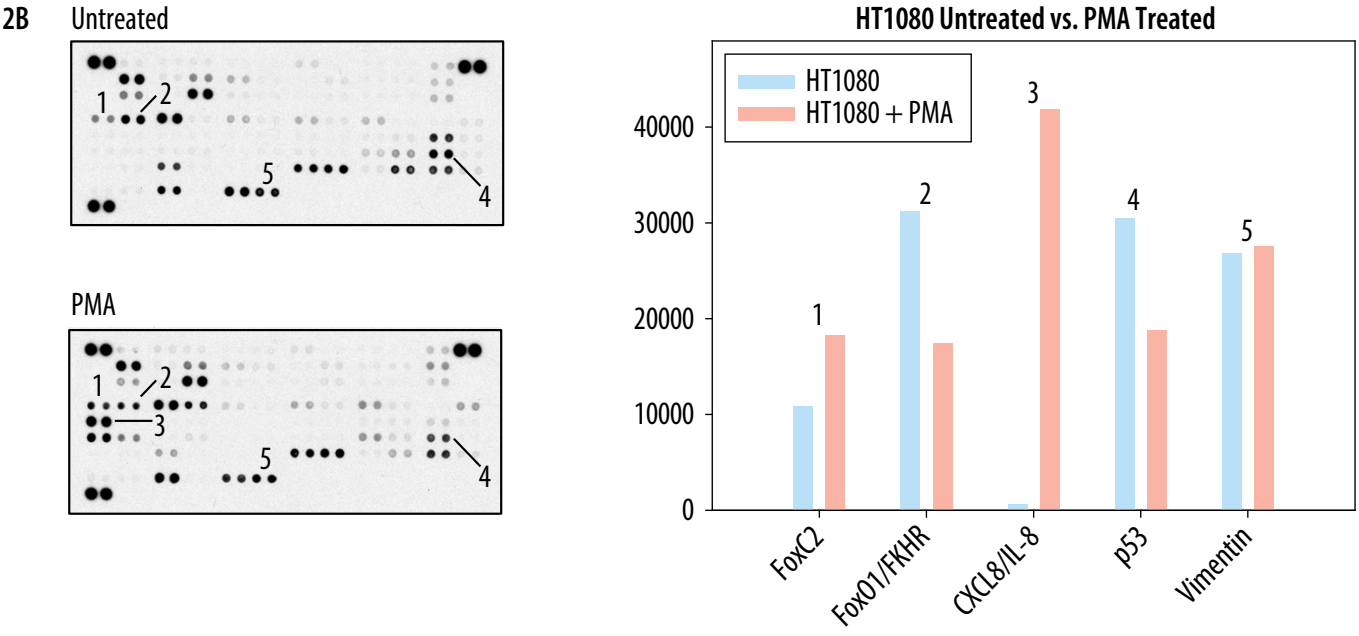
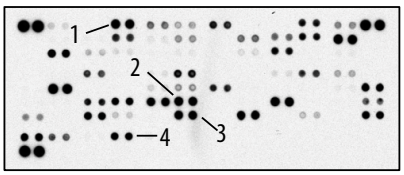


Figure 2B: Lysates from HT1080 human fibrosarcoma cells were untreated or treated with 100 nM PMA (Tocris, Catalog # 1201) for 48 hours.

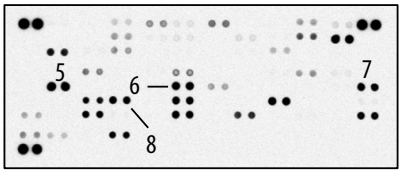
PROFILING PROTEINS IN BODY FLUIDS & TISSUE LYSATES

The Human XL Oncology Array detects multiple oncology-related proteins in serum, plasma, human milk, placenta tissue lysate, and lung tissue lysate samples. The sample type and quantity used are listed below. Data shown are from a ten minute exposure to X-ray film.

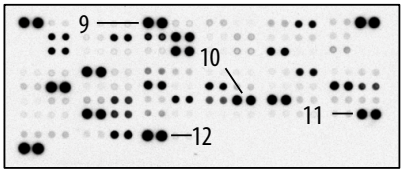
A. Serum, 200 µL per array



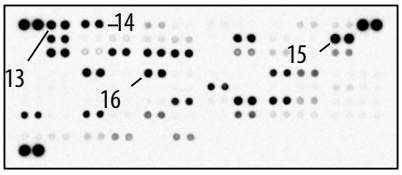
B. Plasma, 200 µL per array



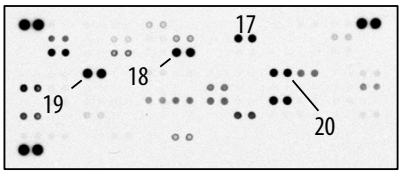
C. Human Milk, 200 µL per array



D. Placenta Tissue Lysate, 200 µg per array



E. Lung Tissue Lysate, 200 µg per array



		MEAN PIXEL DENSITY				
		Serum	Plasma	Human Milk	Placenta	Lung
1	Angiopoietin-1	42,353	3412	2394	275	0
2	MSP/MST1	40,981	28,925	27,902	20,297	12,332
3	E-Selectin	39,092	28,565	1114	332	0
4	VCAM-1	28,995	21,985	35,543	7953	596
5	IL-18 BPa	48,767	41,246	57,786	415	419
6	Leptin	9830	33,320	1159	335	0
7	Mesothelin	31,179	29,580	20,599	587	8566
8	MMP-3	27,935	27,981	24,747	408	100
9	Angiopoietin-like 4	15,157	9867	55,396	5431	4309
10	Nectin-4	102	4	47,890	35,111	162
11	Tenascin C	30,382	26,323	50,029	722	1243
12	VEGF	794	242	58,394	44	0
13	α-Fetoprotein	3889	843	2905	38,183	1072
14	Amphiregulin	0	9	1567	31,186	47
15	EGF R	44,675	37,844	3013	45,782	3515
16	HCG	1303	286	11,516	31,142	1090
17	Decorin	15,456	2207	6432	32,469	30,961
18	EpCAM/TROP-1	984	249	48,069	30,163	38,866
19	Galectin-3	18,281	10,080	53,719	34,431	44,849
20	HO-1/HMOX1/HSP32	1715	430	1821	19615	32,524

APPENDIX

Refer to the table below for the Human XL Oncology Array coordinates.

Coordinate	Analyte/Control	Entrez Gene ID	Alternate Nomenclature
A1, A2	Reference Spots	N/A	———
A3, A4	α-Fetoprotein	174	AFP, DSCAM2
A5, A6	Amphiregulin	374	AREG
A7, A8	Angiopoietin-1	284	ANGPT1
A9, A10	Angiopoietin-like 4	51129	ANGPTL4
A11, A12	ENPP-2/Autotaxin	5168	ATX, Lysophosphatidic Acid, NPP2, PDNP2
A13, A14	Axl	558	Ark, Ufo
A15, A16	BCL-x	598	BCL2L1
A17, A18	CA125/MUC16	94025	MUC16
A19, A20	E-Cadherin	999	Arc-1, CAD1, Cadherin-1, CD324, CDH1, Cell-CAM120/80, ECAD, L-CAM
A21, A22	VE-Cadherin	1003	Cadherin-5, CD144, CDH5
A23, A24	Reference Spots	N/A	———
B3, B4	CapG	822	AFCP
B5, B6	Carbonic Anhydrase IX	768	CA9, G250, MN, RCC
B7, B8	Cathepsin B	1508	CTSB
B9, B10	Cathepsin D	1509	CTSD
B11, B12	Cathepsin S	1520	CTSS
B13, B14	CEACAM-5	1048	CD66e, CEA
B15, B16	Decorin	1634	DCN, DSPG2, PG-II, PSG2, SLRR1B
B17, B18	Dkk-1	22943	Dickkopf-1
B19, B20	DLL1	28514	Delta 1
B21, B22	EGF R/ErbB1	1956	ErbB, ErbB1, HER-1
C3, C4	Endoglin/CD105	2022	CD105, ENG
C5, C6	Endostatin	80781	COL18A1
C7, C8	Enolase 2	2026	ENO2; γ-Enolase; NSE
C9, C10	eNOS	4846	NOS3
C11, C12	EpCAM/TROP1	4072	17-1A, CD326, GA733-2, gp40, KS1/4, M4S1, TACSTD1
C13, C14	ERα/NR3A1	2099	ESR1, NR3A1
C15, C16	ErbB2	2064	CD340, HER2, Neu Oncogene, NGL, TKR1
C17, C18	ErbB3/Her3	2065	HER3
C19, C20	ErbB4	2066	HER4
C21, C22	FGF basic	2247	FGF2, FGF-2, FGF2AS, GFG1, HBGH-2, NUDT6, Prostatropin
D1, D2	FoxC2	2303	Fkh14, LD, MFH1
D3, D4	FoxO1/FKHR	2308	FKH1, FKHR
D5, D6	Galectin-3	3958	AGE-R3, CBP35, GAL3, L29, LGALS3, Mac-2
D7, D8	GM-CSF	1437	CSF2
D9, D10	CG α/β (HCG)	1081 (α)/1082 (β)	CGB, CGB3, Choriogonadotropin
D11, D12	HGF R/c-Met	4233	MET

APPENDIX *CONTINUED*

Coordinate	Analyte/Control	Entrez Gene ID	Alternate Nomenclature
D13, D14	HIF-1α	3091	HIF1A
D15, D16	HNF-3β	3170	FoxA2
D17, D18	HO-1/HMOX1	3162	HSP32
D19, D20	ICAM-1/CD54	3383	———
D21, D22	IL-2 Rα	3559	CD25, IL2RA
D23, D24	IL-6	3569	BSF-2, IFN-β2, MGI-2A
E1, E2	CXCL8/IL-8	3576	GCP1, IL8, LAI, MDNCF, NAP1, NCF, TCF, TSG1
E3, E4	IL-18 BPa	10068	IL18BP
E5, E6	Kallikrein 3/PSA	354	KLK3
E7, E8	Kallikrein 5	25818	KLK5, KLK-L2, SCTE
E9, E10	Kallikrien 6	5653	KLK6, Neurosin, Protease M, PRSS18, PRSS9, SP59, Zyme
E11, E12	Leptin	3952	LEP, OB
E13, E14	Lumican	4060	LDC, LUM, SLRR2D
E15, E16	CCL2/MCP-1	6347	MCAF
E17, E18	CCL8/MCP-2	6355	———
E19, E20	CCL7/MCP-3	6354	MARC
E21, E22	M-CSF	1435	CSF1, CSF-1
E23, E24	Mesothelin	10232	CAK1, MPF, MSLN, SMR
F1, F2	CCL3/MIP-1α	6348/6351	LD78a; MIP-1 alpha
F3, F4	CCL20/MIP-3α	6364	exodus-1; LARC; MIP-3 alpha
F5, F6	MMP-2	4313	Gelatinase A
F7, F8	MMP-3	4314	Stromelysin-1
F9, F10	MMP-9	4318	CLG4B, Gelatinase B, GELB
F11, F12	MSP/MST1	4485	HGFL, MST1, SF2
F13, F14	MUC-1	4582	CD227, Episialin, H23AG, KL-6, Mucin-1, PEM, PEMT
F15, F16	Nectin-4	81607	LNIR, PRR4, PVRL4
F17, F18	Osteopontin (OPN)	6696	Eta-1, Spp1
F19, F20	p27/Kip1	1027	CDKN1B
F21, F22	p53	7157	BCC7, LFS1, TP53, TRP53
F23, F24	PDGF-AA	5154	———
G1, G2	CD31/PECAM-1	5175	PECAM1
G3, G4	Progesterone R/NR3C3	5241	———
G5, G6	Progranulin	2896	Acrogranin, GEP, GP88, GRN, PCDGF, PEPI, PGRN, Proepithelin
G7, G8	Prolactin	5617	PRL
G9, G10	Prostasin/Prss8	5652	———
G11, G12	E-Selectin/CD62E	6401	ELAM1, LECAM2, SELE
G13, G14	Serpin B5/Maspin	5268	PI5
G15, G16	Serpin E1/PAI-1	5054	Nexin, PLANH1

APPENDIX *CONTINUED*

Coordinate	Analyte/Control	Entrez Gene ID	Alternate Nomenclature
G17, G18	Snail	6615	SLUGH2, SNAH, SNAI1
G19, G20	SPARC	6678	BM-40, Osteonectin
G21, G22	Survivin	332	API4, BIRC5
G23, G24	Tenascin C	3371	Cytotactin, HXB, Tenascin J1, TNC
H1, H2	Thrombospondin-1	7057	THBS1, TSP-1
H3, H4	Tie-2	7010	———
H5, H6	u-Plasminogen Activator/Urokinase	5328	PLAU, uPA
H7, H8	VCAM-1/CD106	7412	———
H9, H10	VEGF	7422	VAS, Vasculotropin, VEGFA, VPF
H11, H12	Vimentin	7431	VIM
I1, I2	Negative Control	N/A	———
I23, I24	Reference Spots	N/A	———