Proteome Profiler™ Array

Human Soluble Receptor Array Kit
Hematopoietic Panel

Catalog Number ARY011

For the parallel determination of the relative levels of soluble receptors and related proteins in hematopoietic cells.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Analyzing the expression profiles of soluble receptors expressed and released by hematopoietic cells is essential for understanding the roles these molecules play in cellular activation of migratory cells. The Human Soluble Receptor Array Kit is a rapid, sensitive, and economical tool to simultaneously detect changes in a multitude of proteins between samples. The relative expression levels of 105 soluble receptors and related proteins can be determined without performing numerous immunoprecipitations or Western blots. Each capture and detection antibody, directed to the extracellular domain, was carefully selected using both natural and recombinant proteins.

PRINCIPLE OF THE ASSAY

Capture and control antibodies have been spotted in duplicate on nitrocellulose membranes. Cell culture supernate, serum, or cell lysate samples are diluted and incubated overnight with each array part of the Hematopoietic Panel. This panel is comprised of the Hematopoietic Array and the Common Analytes Array, which is also used in the Non-Hematopoietic Array Panel (R&D Systems, Catalog # ARY012). The membranes are washed to remove unbound material followed by incubation with their specific cocktail of biotinylated detection antibodies. 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 the 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 Soluble Receptor 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 membranes before proceeding to the next step.
- Do not allow the membranes to dry out. This will cause high background.
- Avoid microbial contamination of reagents and buffers.
- For a procedure demonstration video, please visit: www.RnDSystems.com/ProteomeProfilerVideo.

PRECAUTIONS

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

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.

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 Soluble Receptor Array Hematopoietic Panel	893368	4 nitrocellulose membranes: 2 Part H each containing 48 different capture antibodies; and 2 Part C each containing 57 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.*
Lysis Buffer 17	895943	21 mL of a non-denaturing buffered solution with preservatives.	May be stored for up to 3 months at 2-8 °C.*
Array Buffer 1	895477	2 vials (21 mL/vial) of a buffered protein base with preservatives.	
Array Buffer 8	895050	21 mL of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	2 vials (21 mL/vial) of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Detection Antibody Cocktail H	893365	1 vial of a biotinylated antibody cocktail; lyophilized; red cap.	
Detection Antibody Cocktail C	893369	1 vial of a biotinylated antibody cocktail; lyophilized; blue cap.	
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	607676	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)
- Pipettes and pipette tips
- Gloves
- Deionized or distilled water
- Rocking platform shaker
- Microcentrifuge
- Plastic containers 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®

SAMPLE COLLECTION & STORAGE

Since the Human Soluble Receptor 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 to use for* ***each array part*** *are: 200-500 µL for cell culture supernates, 100-300 µg for cell lysates (250 µL maximum), and 10-50 µL for serum 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.

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 Soluble Receptor Array: Hematopoietic Panel - Four nitrocellulose membranes; the Hematopoietic Arrays (Part H; array numbers begin with H) contain 48 antibodies printed in duplicate. The Common Analyte Arrays (Part C; array numbers begin with C) contain 57 antibodies printed in duplicate. Both arrays contain four sample control antibodies. The two array parts should be used in parallel to generate a complete profile in one experiment. **Handle membranes only with gloved hands and flat-tipped tweezers.**

Detection Antibody Cocktail H (red cap) - One vial of lyophilized biotinylated antibodies for use on Part H arrays. Before use, reconstitute Detection Antibody Cocktail H in 100 µL of deionized or distilled water.

Detection Antibody Cocktail C (blue cap) - One vial of lyophilized biotinylated antibodies for use on Part C arrays. Before use, reconstitute Detection Antibody Cocktail C in 100 µL of deionized or distilled water.

1X Array Buffer 8/1 - Dilute 1 mL of Array Buffer 8 into 9 mL of Array Buffer 1. **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 for each membrane.**

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. The Human Soluble Receptor Array: Hematopoietic Panel is divided into two parts (H and C). For best results, incubate Parts H and C in aliquots of the same sample but in separate wells of the 4-Well Multi-dish.
3. Pipette 2.0 mL of 1X Array Buffer 8/1 into each well of the 4-Well Multi-dish to be used. 1X Array Buffer 8/1 serves as a block buffer.
4. 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 1X Array Buffer 8/1, the blue dye from the spots will disappear, but the capture antibodies are retained in their specific locations.*

5. Incubate for one hour on a rocking platform shaker. Orient the tray so that each membrane rocks end to end in its well.
6. While arrays are blocking, prepare samples for both parts of the array (H and C) by adding the desired quantity of sample (up to 500 µL for cell lysates, 100 µL for serum samples, or 1 mL for all other sample types) to 300 µL of Array Buffer 8. Adjust to a final volume of 3 mL with Array Buffer 1.
7. Aspirate 1X Array Buffer 8/1 from the wells of the 4-Well Multi-dish and add 1.5 mL of the prepared sample to the Part H array and 1.5 mL to the Part C array. 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 array and place into separate plastic containers with 20 mL of 1X Wash Buffer. The recommended container size for washing is approximately 11 x 8 x 2 cm (L x W x H). Rinse the 4-Well Multi-dish with deionized or distilled water and dry thoroughly.
10. Wash each array with 1X Wash Buffer for 10 minutes on a rocking platform shaker. Repeat two times for a total of three washes.
11. For each Part H array, dilute 30 µL of reconstituted Detection Antibody Cocktail H (red cap) to 1.5 mL with 1X Array Buffer 8/1. Pipette 1.5 mL per well of diluted Detection Antibody Cocktail H into the 4-Well Multi-dish.
12. Carefully remove each Part H 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 H.

13. For each Part C array, dilute 30 µL of reconstituted Detection Antibody Cocktail C (blue cap) to 1.5 mL with 1X Array Buffer 8/1. Pipette 1.5 mL per well of diluted Detection Antibody Cocktail C into the 4-Well Multi-dish.
14. Carefully remove each Part C 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 C, and cover with the lid.
15. Incubate for 2 hours at room temperature on a rocking platform shaker.
16. Wash the array as described in steps 9 and 10.
17. Dilute the Streptavidin-HRP in 1X Array Buffer 8/1 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.
18. 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.
19. Incubate for 30 minutes at room temperature on a rocking platform shaker.
20. Wash each array as described in steps 9 and 10.

    Note: *Complete the remaining steps without interruption.*
21. 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.
22. 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.*
23. 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.
24. Position paper towels on the top and sides of the plastic sheet protector containing the membranes and carefully squeeze out excess Chemi Reagent Mix.
25. 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.
26. 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.
27. 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.*
28. 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 the 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 protein.
4. Subtract an averaged background signal from each spot. Use a signal from a clear area of the array or negative control spots as a background value.
5. Compare corresponding signals on different arrays to determine the relative change in protein levels between samples.

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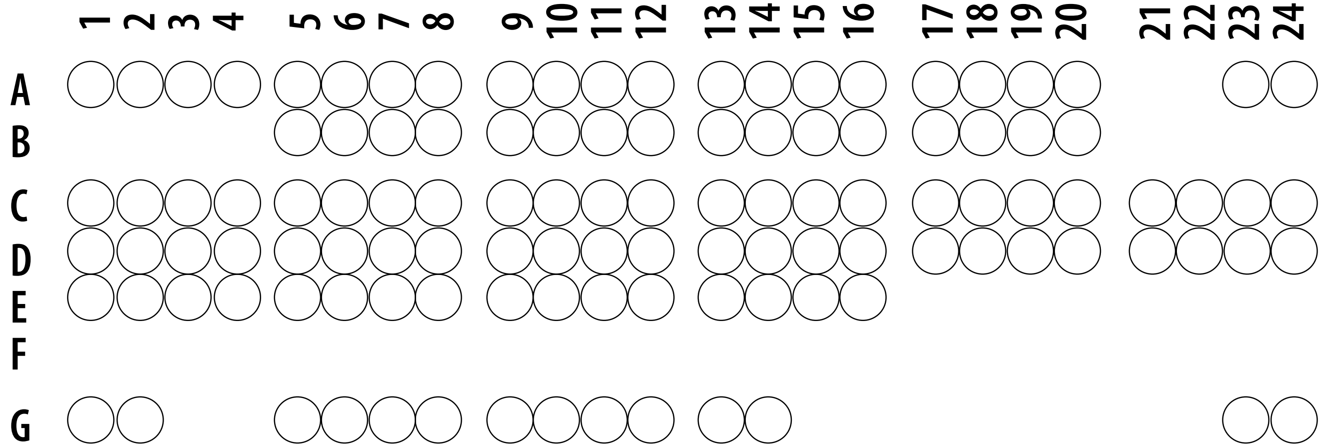
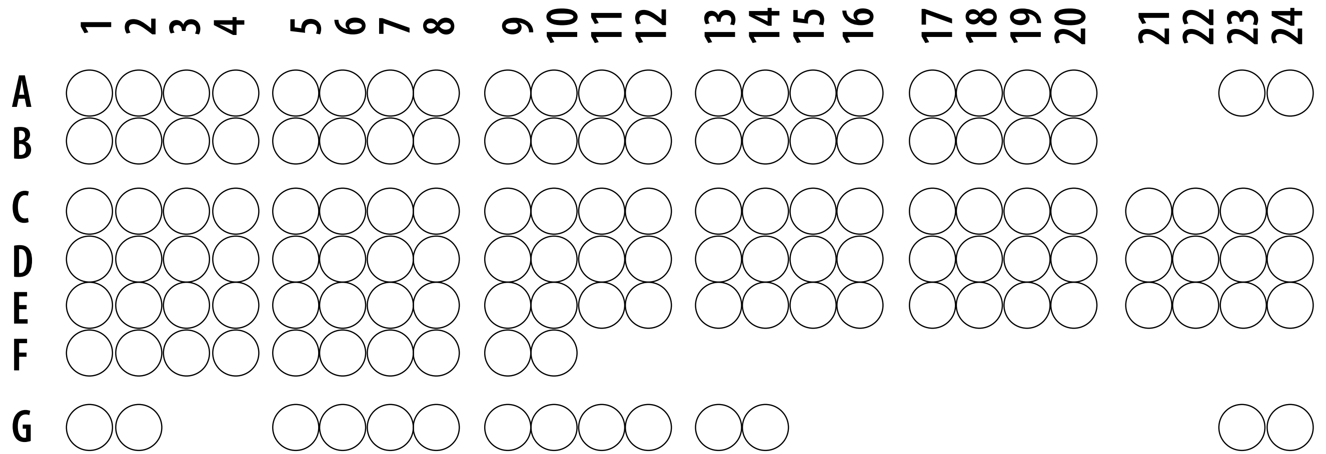
Human Hematopoietic Array Coordinates



Human Common Analytes Array Coordinates



These images are not to scale. They are for coordinate reference only.
Please use the transparency overlay for analyte identification.

PROFILING SOLUBLE RECEPTORS IN CELL CULTURE SUPERNATES

The Human Soluble Receptor Array detects multiple analytes in cell culture supernates.

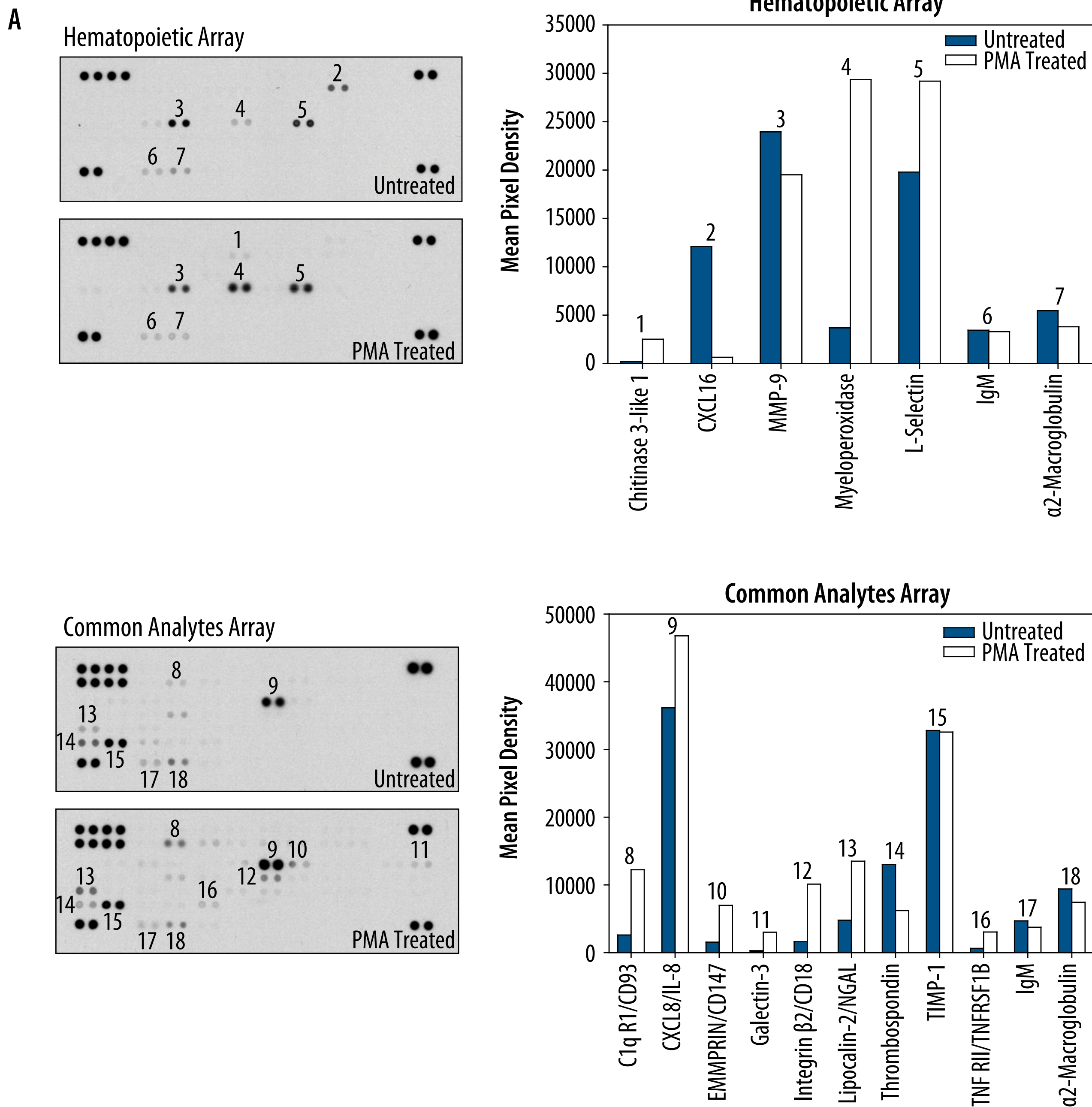


Figure 1A: Peripheral blood mononuclear cells (PBMCs) were untreated or treated with 50 ng/mL of PMA for 24 hours. 500 µL of cell culture supernate was run on each array. Data shown are from a 5 minute exposure to X-ray film.

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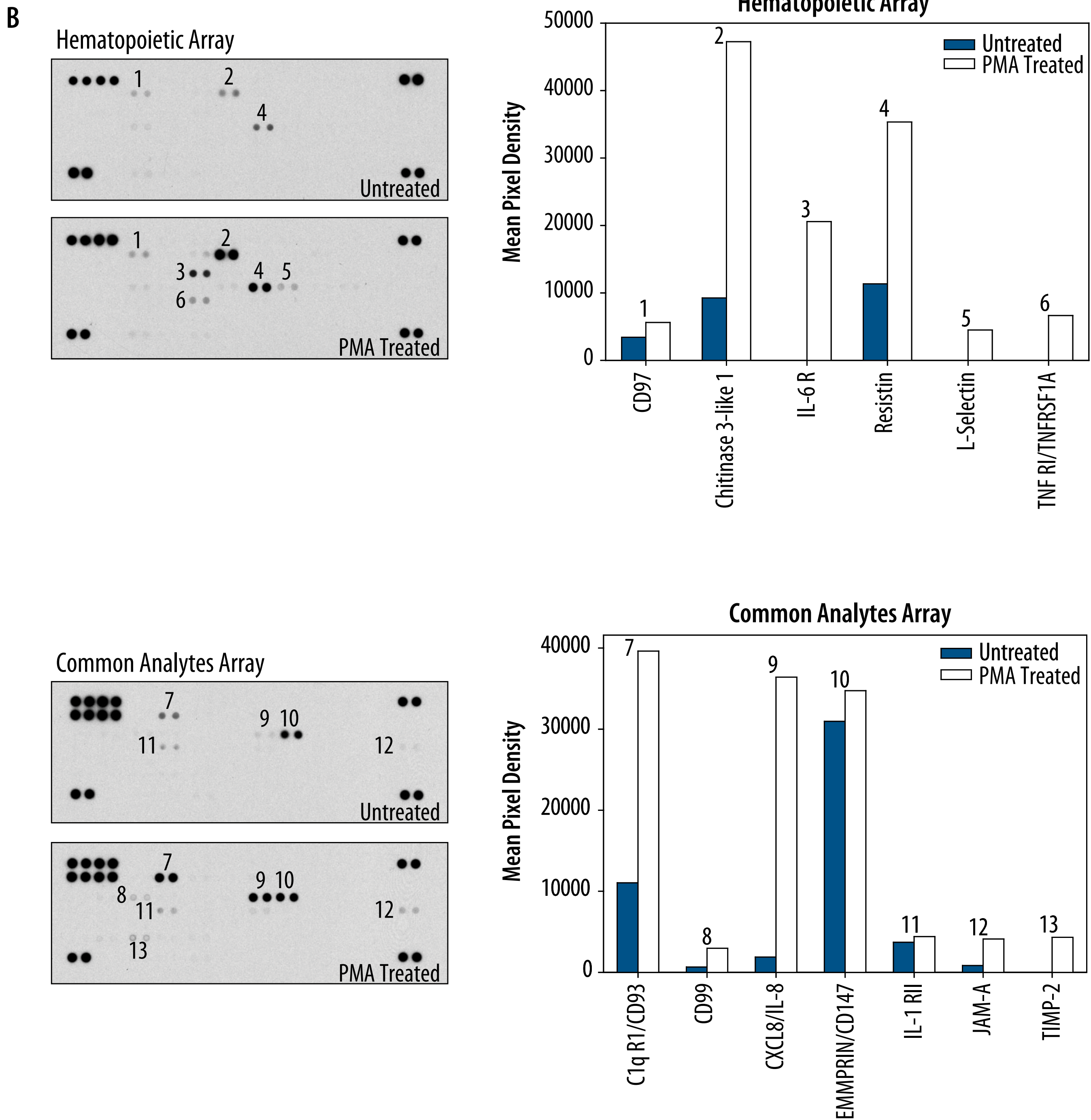

Figure 1B: U937 human histiocytic lymphoma cells were untreated or treated with 100 ng/mL of PMA for 1 hour. 500 µL of cell culture supernate was run on each array. Data shown are from a 10 minute exposure to X-ray film.

PROFILING SOLUBLE RECEPTORS IN CELL LYSATES

The Human Soluble Receptor Array detects multiple analytes in cell lysates.

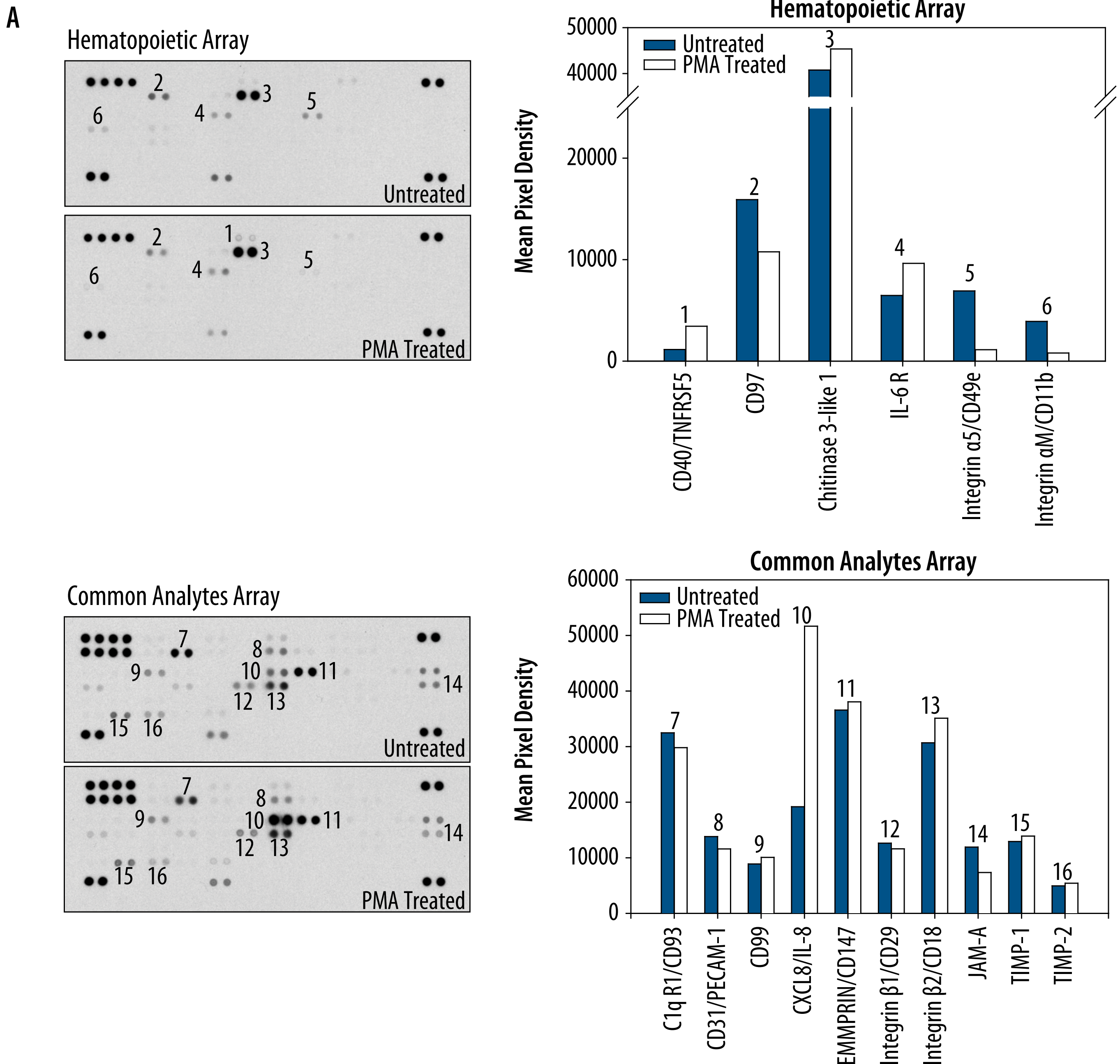

Figure 2A: THP-1 human acute monocytic leukemia cells were either untreated or treated with 100 ng/mL of PMA for 6 hours. 200 µg of cell lysate was run on each array. Data shown are from a 5 minute exposure to X-ray film.

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B

Hematopoietic Array

1 2 3 4 Untreated

1 2 3 4 PMA Treated

Hematopoietic Array

Untreated
PMA Treated

Mean Pixel Density

0 10000 20000 30000 40000 50000 60000

CD97 | Integrin α5/CD49e | Resistin | Siglec-6

Common Analytes Array

5 6 7 8 9 10 11 12 13 Untreated

5 6 7 8 9 10 11 12 13 PMA Treated

Common Analytes Array

Untreated
PMA Treated

Mean Pixel Density

0 10000 20000 30000 40000

ADAM9 | C1q R1/CD93 | CD31/PECAM-1 | CD99 | CXCL8/IL-8 | EMMPRIN/CD147 | IL-1 RII | Integrin β1/CD29 | Integrin β2/CD18

Figure 2B: U937 human histiocytic lymphoma cells were either untreated or treated with 100 ng/mL of PMA for 1 hour. 200 µg of cell lysate was run on each array. Data shown are from a 5 minute exposure to X-ray film.

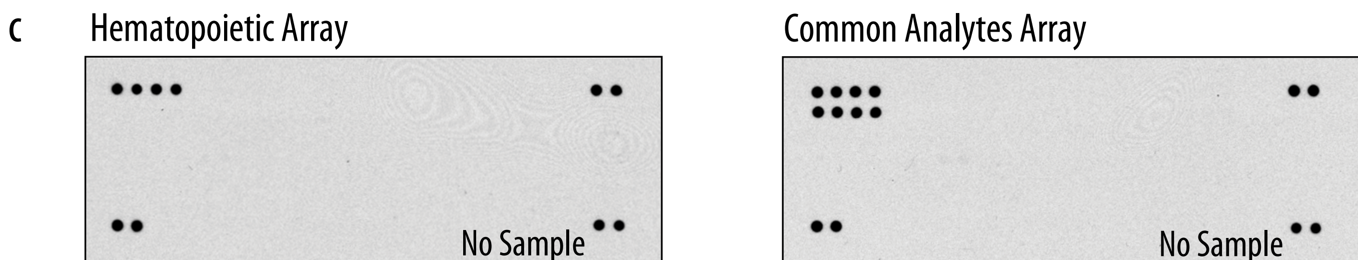

Figure 2C: Arrays incubated with buffer only (no sample) are shown. Images are from a 5 minute exposure to X-ray film.

PROFILING SOLUBLE RECEPTORS IN SERUM

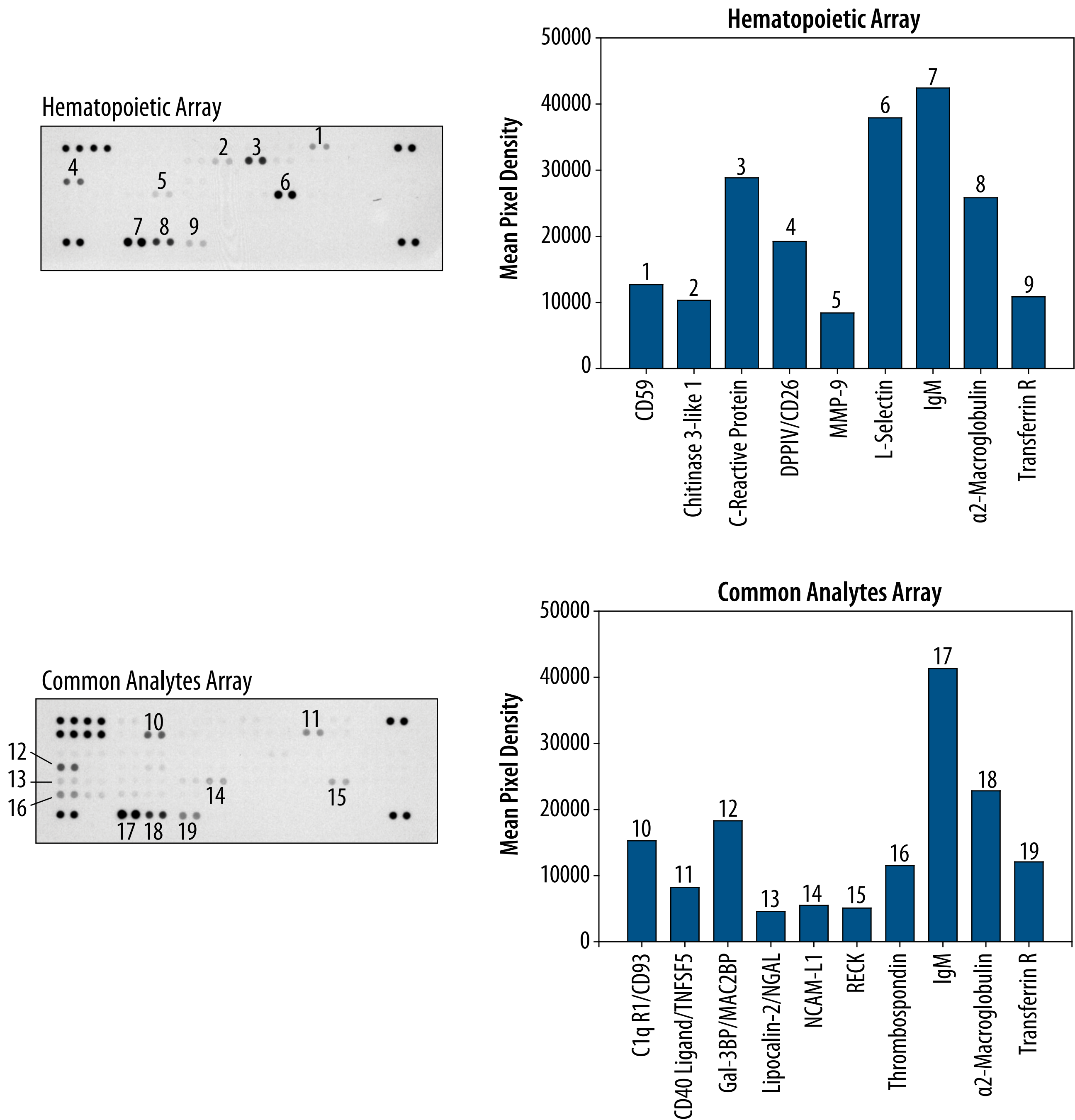

Figure 3: The Human Soluble Receptor Array detects multiple analytes in serum. 25 µL of serum from a normal donor was run on each array. Data shown are from a 1 minute exposure to X-ray film.

APPENDIX

Refer to the table below for the Human Hematopoietic Array (Part H) coordinates.

Coordinate	Analyte/Control	Alternate Nomenclature	Entrez Gene ID
A1, A2, A3, A4	Reference Spots	——	——
A5, A6	CD5	——	921
A7, A8	CD6	——	923
A9, A10	CD30/TNFRSF8	——	943
A11, A12	CD40/TNFRSF5	——	958
A13, A14	CD43	Sialophorin	6693
A15, A16	CD48/SLAMF2	——	962
A17, A18	CD59	——	966
A19, A20	CD84/SLAMF5	——	8832
A23, A24	Reference Spots	——	——
B5, B6	CD97	——	976
B7, B8	CD163	——	9332
B9, B10	CD229/SLAMF3	hly9	4063
B11, B12	Chitinase 3-like 1	YKL40	1116
B13, B14	C-Reactive Protein	——	1401
B15, B16	CRTAM	——	56253
B17, B18	CXCL16	CXCLG16	58191
B19, B20	DNAM-1	——	10666
C1, C2	DPPIV/CD26	——	1803
C3, C4	IFN-γ R2	——	3460
C5, C6	IL-2 Rα	——	3559
C7, C8	IL-2 Rβ	——	3560
C9, C10	IL-6 R	——	3570
C11, C12	Integrin α3/CD49c	ITGA3	3675
C13, C14	Integrin α4/CD49d	ITGA4	3676
C15, C16	Integrin α5/CD49e	ITGA5	3678
C17, C18	Integrin α6/CD49f	ITGA6	3655
C19, C20	Integrin α9	ITGA9	3680
C21, C22	Integrin αE/CD103	ITGAE	3682
C23, C24	Integrin αL/CD11a	ITGAL	3683
D1, D2	Integrin αM/CD11b	ITGAM	3684
D3, D4	Integrin αX/CD11c	ITGAX	3687
D5, D6	LAG-3	——	3902
D7, D8	MMP-9 (total)	——	4318
D9, D10	MMR	——	4360
D11, D12	Myeloperoxidase	MPO	4353
D13, D14	Resistin	——	56729

continued on next page....

Coordinate	Analyte/Control	Alternate Nomenclature	Entrez Gene ID
D15, D16	L-Selectin	CD62L	6402
D17, D18	Siglec-5	CD170	8778
D19, D20	Siglec-6	CD327, CD33L1	946
D21, D22	Siglec-7	CD328	27036
D23, D24	Siglec-9	CD329	27180
E1, E2	Siglec-10	——	89790
E3, E4	TIM-3	HAVCR2, KIM-3	84868
E5, E6	TLR2	CD282	7097
E7, E8	TLR4	CD284	7099
E9, E10	TNF RI/TNFRSF1A	CD120a	7132
E11, E12	TRACP/PAP/ACP5	TRAP	54
E13, E14	TRANCE/TNFSF11	CD254, RANKL	8600
E15, E16	TREM-1	——	54210
G1, G2	Reference Spots	——	——
G5, G6	IgM (Sample Control)*	——	3507
G7, G8	α2-Macroglobulin (Sample Control)*	A2M	2
G9, G10	Transferrin R (Sample Control)*	CD71	7037
G11, G12	Vimentin (Sample Control)*	VIM	7431
G13, G14	PBS (Negative Control)	——	——
G23, G24	Reference Spots	——	——

*Sample controls are included to allow for the detection of proteins commonly present in cell culture supernates, cell lysates, and serum. If these endogenous proteins are present in a particular sample, positive signals indicate that the sample has been incubated with the array and the assay procedure has been performed correctly.

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APPENDIX *CONTINUED*

Refer to the table below for the Human Common Analytes Array (Part C) coordinates.

Coordinate	Analyte/Control	Alternate Nomenclature	Entrez Gene ID
A1, A2, A3, A4	Reference Spots	——	——
A5, A6	ACE	CD143	1636
A7, A8	ADAM8	CD156	101
A9, A10	ADAM9	——	8754
A11, A12	ADAM10	CD156c	102
A13, A14	ALCAM/CD166	——	214
A15, A16	Amphiregulin	AR	374
A17, A18	APP (pan)	——	351
A19, A20	BACE-1	——	23621
A23, A24	Reference Spots	——	——
B1, B2, B3, B4	Reference Spots	——	——
B5, B6	BCAM	CD239	4059
B7, B8	C1q R1/CD93	——	22918
B9, B10	CD9	——	928
B11, B12	CD23/Fc ε RII	——	2208
B13, B14	CD31/PECAM-1	——	5175
B15, B16	CD36/SR-B3	FAT	948
B17, B18	CD40 Ligand/TNFSF5	CD154	959
B19, B20	CD44H	——	960
C1, C2	CD58/LFA-3	——	965
C3, C4	CD90/Thy1	——	7070
C5, C6	CD99	——	4267
C7, C8	CD155/PVR	——	5817
C9, C10	CEACAM-1/CD66a	——	634
C11, C12	CX3CL1/Fractalkine	Neurotactin	6376
C13, C14	CXCL8/IL-8	NAP-1	3576
C15, C16	EMMPRIN/CD147	BSG	682
C17, C18	Endoglin/CD105	——	2022
C19, C20	Epiregulin	——	2069
C21, C22	Galectin-1	GAL1	3956
C23, C24	Galectin-3	GAL3	3958
D1, D2	Galectin-3BP/MAC-2BP	——	3959
D3, D4	HB-EGF	——	1839
D5, D6	ICAM-2/CD102	——	3384
D7, D8	IL-1 RII	CD121b	7850
D9, D10	IL-15 Rα	——	3601
D11, D12	Integrin β1/CD29	ITGB1	3688

continued on next page....

Coordinate	Analyte/Control	Alternate Nomenclature	Entrez Gene ID
D13, D14	Integrin β2/CD18	ITGB2	3689
D15, D16	Integrin β3/CD61	ITGB3	3690
D17, D18	Integrin β4/CD104	ITGB4	3691
D19, D20	Integrin β5	ITGB5	3693
D21, D22	Integrin β6	ITGB6	3694
D23, D24	JAM-A	CD321	50848
E1, E2	Lipocalin-2/NGAL	——	3934
E3, E4	LOX-1/SR-E1	CLEC8A	4973
E5, E6	MD-1	LY86	9450
E7, E8	MMP-2 (total)	——	4313
E9, E10	NCAM-1/CD56	——	4684
E11, E12	NCAM-L1	L1CAM, CD171	3897
E13, E14	Osteopontin	OPN	6696
E15, E16	PAR1	——	2149
E17, E18	Pref-1/DLK-1/FA1	——	8878
E19, E20	RECK	——	8434
E21, E22	Stabilin-1	CLEVER-1, FEEL-1	23166
E23, E24	TACE/ADAM17	CD156b	6868
F1, F2	Thrombospondin	THBS, TSP	7057
F3, F4	TIMP-1	——	7076
F5, F6	TIMP-2	——	7077
F7, F8	TIMP-3	——	7078
F9, F10	TNF RII/TNFRSF1B	CD120b	7133
G1, G2	Reference Spots	——	——
G5, G6	IgM (Sample Control)*	——	3507
G7, G8	α2-Macroglobulin (Sample Control)*	A2M	2
G9, G10	Transferrin R (Sample Control)*	CD71	7037
G11, G12	Vimentin (Sample Control)*	VIM	7431
G13, G14	PBS (Negative Control)	——	——
G23, G24	Reference Spots	——	——

*Sample controls are included to allow for the detection of proteins commonly present in cell culture supernates, cell lysates, and serum. If these endogenous proteins are present in a particular sample, positive signals indicate that the sample has been incubated with the array and the assay procedure has been performed correctly.

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