# Proteome Profiler™ Array

## Human XL Cytokine Array Kit

Catalog Number ARY022B

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

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

Cytokines, chemokines and growth factors are extracellular signaling molecules that mediate cell to cell communication. These molecules are released from cells and have critical roles in many biological processes such as cellular growth, differentiation, gene expression, migration, immunity and inflammation. In most biological processes, multiple cytokines operate in a large network, where the action of one cytokine is regulated by the presence or absence of other cytokines. The Human XL Cytokine Array Kit is a rapid, sensitive, and economic tool to simultaneously detect cytokine differences between samples. The relative expression levels of 105 soluble human proteins can be determined without performing numerous immunoassays.

## 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 Cytokine Array. 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.**
- If using a LI-COR, additional reagents and protocol modifications are required. Refer to https://www.rndsystems.com/resources/technical/use-proteome-profiler-arrays-li-cor-detection for more details.
- 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 Cytokine Array** | 898505 | 4 nitrocellulose membranes each containing 105 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 Cytokine Array** | 898506 | 1 vial of biotinylated antibody cocktail; lyophilized. | |
| **Streptavidin-HRP** | 893019 | 200 µL of streptavidin conjugated to horseradish-peroxidase. | |
| **Chemi Reagent 1** | 894287 | 1 vial (2.5 mL) | |
| **Chemi Reagent 2** | 894288 | 1 vial (2.5 mL) | |
| **4-Well Multi-dish** | 607544 | Clear 4-well rectangular multi-dish. | Store at room temperature. |
| **Transparency Overlay Template** | 607156 | 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 (1% Igepal CA-630, 20 mM Tris-HCl (pH 8.0), 137 mM NaCl, 2 mM EDTA, 200 mM Sodium orthovanadate, 5mM NaF)
- Aprotinin (Tocris™, Catalog # 4139)
- 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 Cytokine 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 17 supplemented with 10 µg/mL Aprotinin, 10 µg/mL Leupeptin, and 10 µg/mL Pepstatin. Solubilize cells at 1 x $10^7$ cells/mL in this buffer. Pipette up and down to resuspend and rock the lysates gently at 2-8 °C for 30 minutes. Microcentrifuge at 14,000 x g for 5 minutes, and transfer the supernate into a clean test tube. Quantitation of sample protein concentration using a total protein assay is recommended. Assay immediately or aliquot and store at ≤ -70 °C. Avoid repeated freeze-thaw cycles.

**Serum** - Allow blood samples to clot for 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 face mask and gloves be used to protect kit reagents from contamination.*

**Human XL Cytokine 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 Cytokine 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.

**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 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 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.

## 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 face 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. 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 1 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 arrays are blocking, prepare samples by diluting the desired quantity to a final volume of 1.5 mL with Array Buffer 6.
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.

14. Pipette 2 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 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.*

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.


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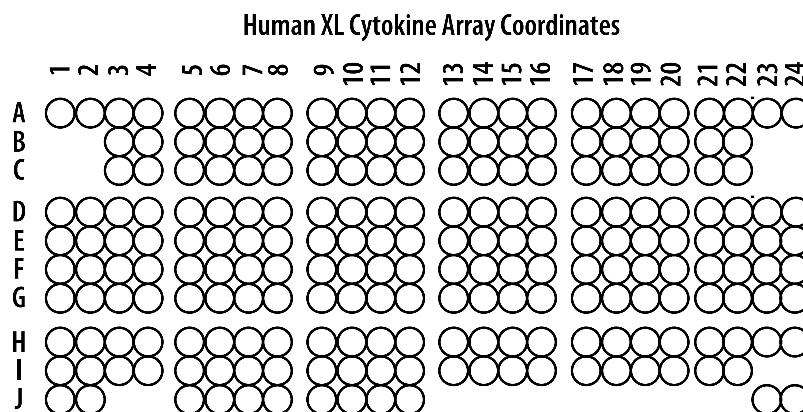
## DATA ANALYSIS

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.




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 Cytokine Array detects multiple cytokines, chemokines, growth factors and other soluble proteins in cell culture supernates.** Cells were untreated or treated as indicated below. 500 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.

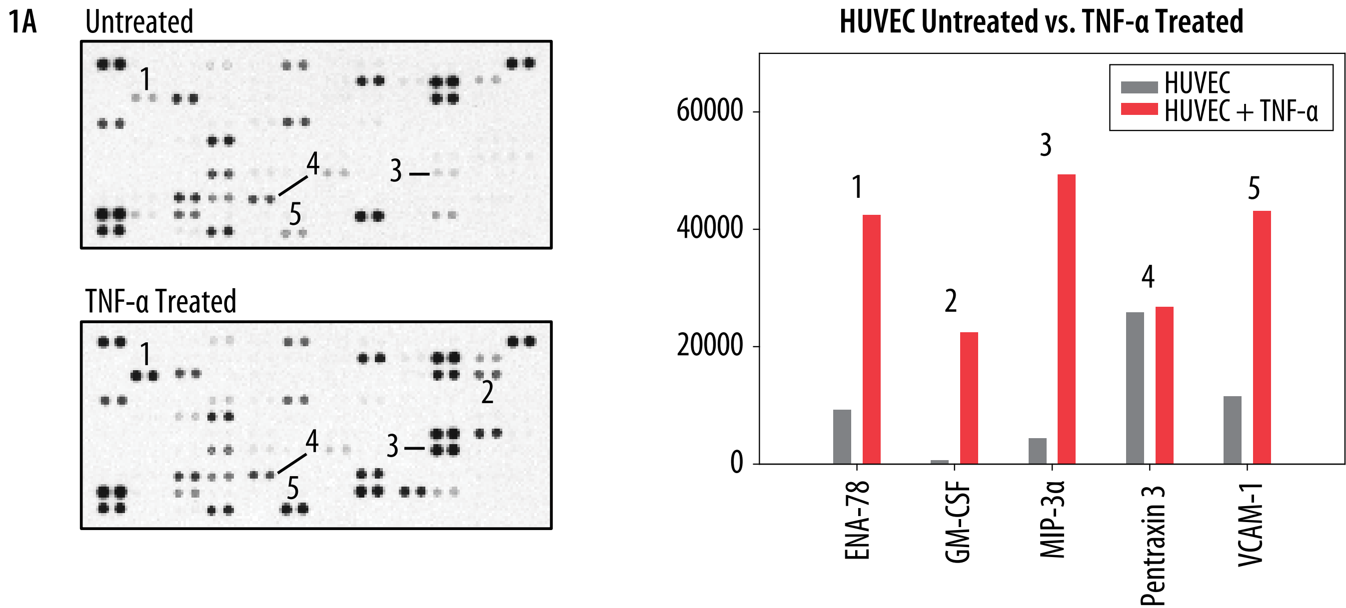



**Figure 1A:** HUVEC human umbilical vein endothelial cells were untreated or treated with 100 ng/mL recombinant human TNF-α (R&D Systems®, Catalog # 210-TA) for 24 hours.

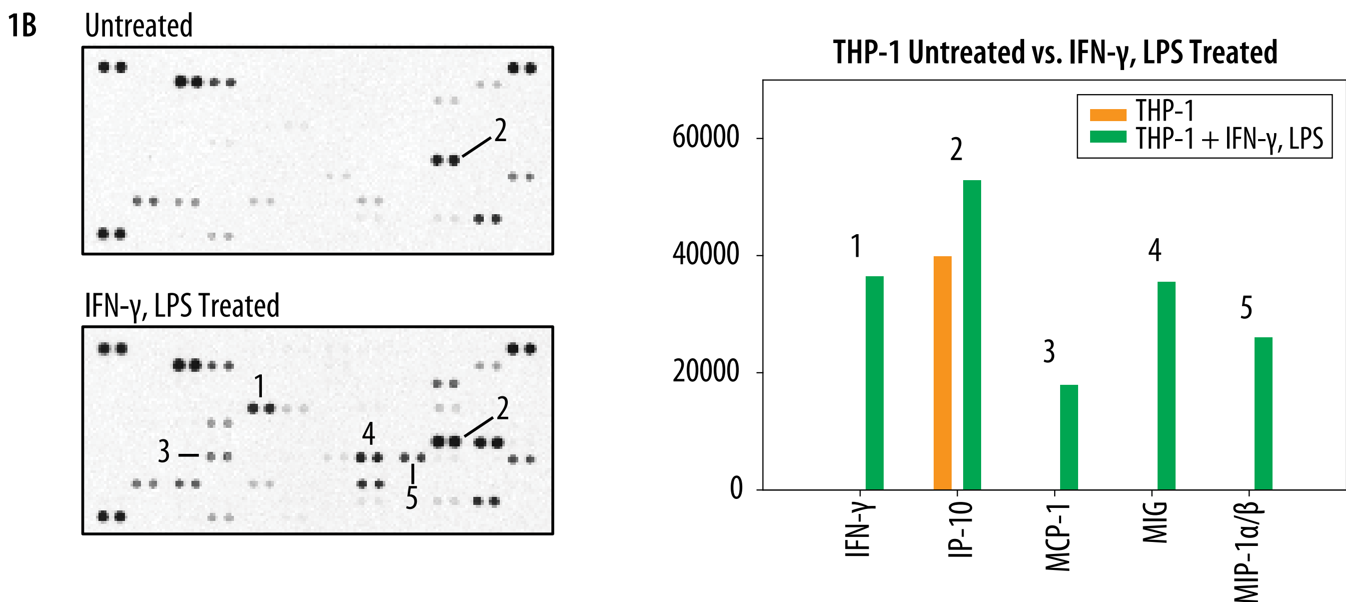



**Figure 1B:** THP-1 human acute monocytic leukemia cells were untreated or treated with 1.0 µg/mL of recombinant human IFN-γ (R&D Systems, Catalog # 285-IF) for 16 hours then 1.0 µg/mL of LPS for 8 hours.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## PROFILING PROTEINS IN CELL LYSATES

**The Human XL Cytokine Array detects multiple cytokines, chemokines, growth factors and other soluble 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.

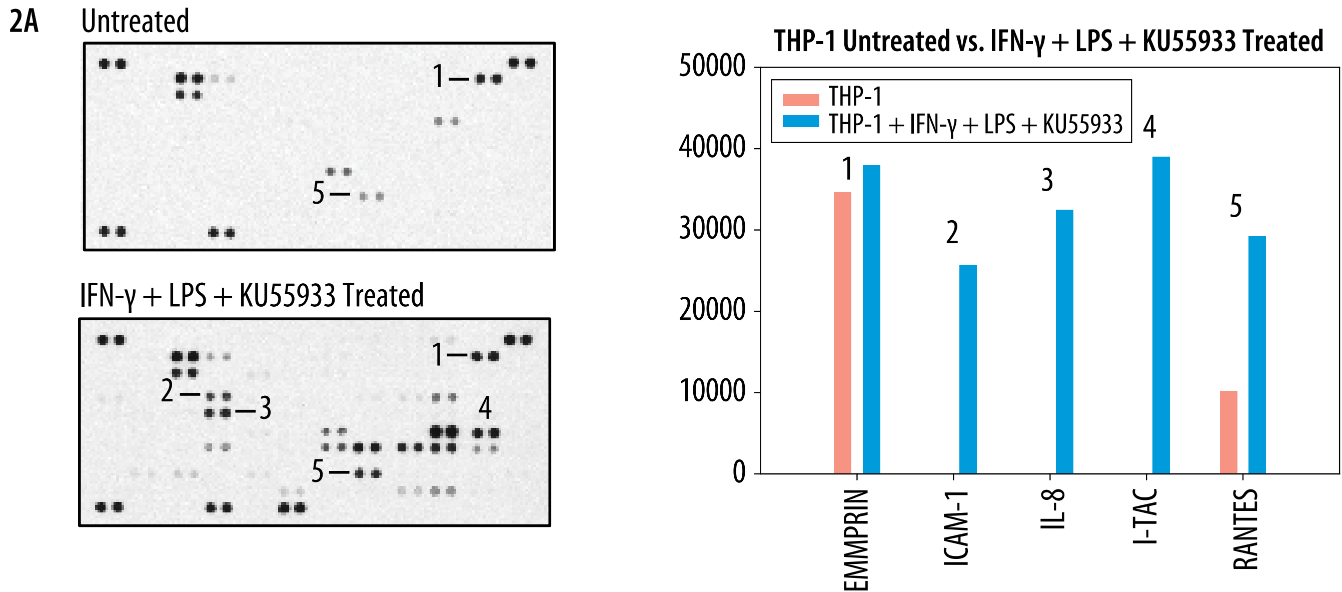



**Figure 2A:** THP-1 human acute monocytic leukemia cells were untreated or treated with 40 ng/mL of recombinant human IFN-γ (R&D Systems®, Catalog # 285-IF), 500 ng/mL of LPS, and 10 µM KU 55933 (Tocris™, Catalog #3544) for 48 hours.

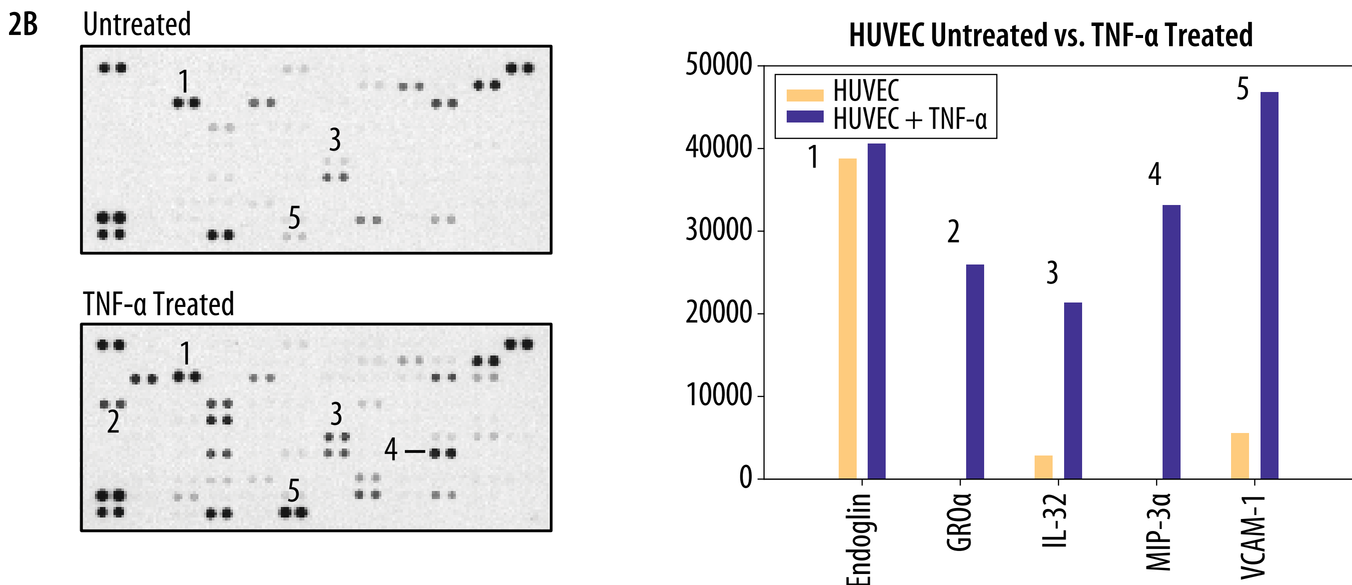
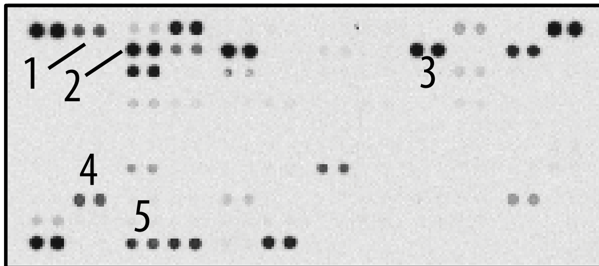



**Figure 2B:** HUVEC human umbilical vein endothelial cells were untreated or treated with 100 ng/mL of recombinant human TNF-α (R&D Systems, Catalog # 210-TA) for 24 hours.

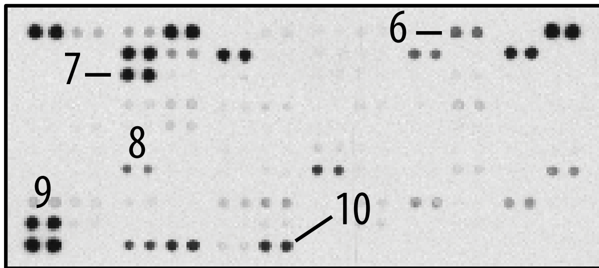
## PROFILING PROTEINS IN TISSUE LYSATES

**The Human XL Cytokine Array detects multiple cytokines, chemokines, growth factors and other soluble proteins in tissue lysates.** 200 µg of tissue lysate was run on each array, Data shown are from a 10 minute exposure to X-ray film.

Kidney

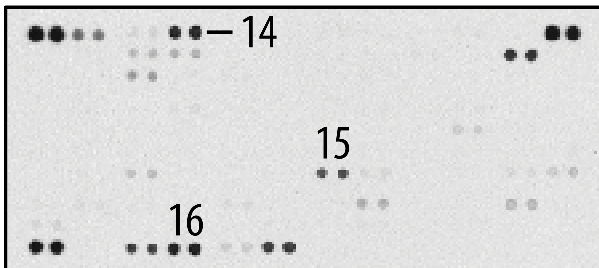



Liver




Pancreas

Thymus




| | |
|---|---|
| 1 | Adiponectin/Acrp30 |
| 2 | Chitinase 3-like 1 |
| 3 | DPPIV/CD26 |
| 4 | Osteopontin |
| 5 | Vitamin D BP |
| 6 | CD14 |
| 7 | Endoglin |
| 8 | Lipocalin-2/NGAL |
| 9 | Serpin E1/PAI-1 |
| 10 | VCAM-1 |
| 11 | EMMPRIN |
| 12 | GDF-15 |
| 13 | IGFBP-2 |
| 14 | Angiogenin |
| 15 | MIF |
| 16 | CD31/PECAM-1 |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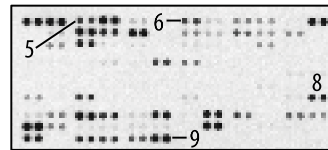
## PROFILING PROTEINS IN PBMC SUPERNATES & BODY FLUIDS

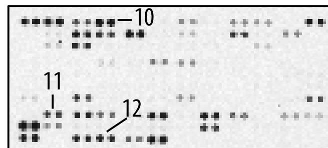
**The Human XL Cytokine Array detects multiple cytokines, chemokines, growth factors and other soluble proteins in PBMC supernates, serum, plasma, milk, urine, and saliva samples.** The sample type, quantity used per array, and exposure duration to X-ray film are listed below. Data shown are from a 5 minute exposure to X-ray film.

**A.** PBMC supernates, 500 µL per array

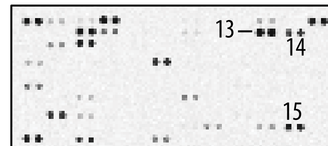
**B.** Serum, 100 µL per array



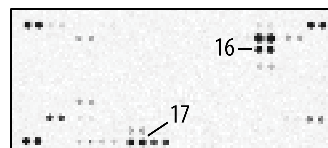

**C.** EDTA plasma, 100 µL per array



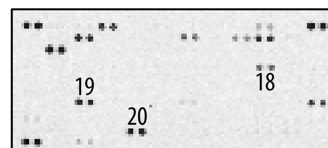

**D.** Human milk, 100 µL per array




**E.** Urine, 200 µL per array




**F.** Saliva, 50 µL per array



# PROFILING PROTEINS IN PBMC SUPERNATES & BODY FLUIDS *CONTINUED*

| | | MEAN PIXEL DENSITY | | | | | |
|---|---|---|---|---|---|---|---|
| | | PBMCs | Serum | Plasma | Human Milk | Urine | Saliva |
| 1 | Chitinase 3-like 1 | 38,785 | 44,540 | 34,879 | 40,570 | 8130 | 28,686 |
| 2 | ENA-78 | 39,794 | 12,682 | 4565 | 12,975 | 0 | 40,515 |
| 3 | IL-8 | 27,783 | 0 | 0 | 237 | 0 | 62 |
| 4 | IP-10 | 45,734 | 1783 | 232 | 62 | 0 | 0 |
| 5 | Apolipoprotein A-1/ApoA1 | 0 | 28,028 | 23,106 | 2654 | 11 | 854 |
| 6 | BAFF | 0 | 30,150 | 12,879 | 1078 | 0 | 0 |
| 7 | IGFBP-2 | 0 | 27,167 | 14,158 | 32,686 | 84 | 29 |
| 8 | MMP-9 | 27,738 | 31,977 | 22,428 | 136 | 468 | 23,223 |
| 9 | VCAM-1 | 0 | 47,719 | 45,110 | 16,320 | 28,393 | 833 |
| 10 | Angiogenin | 0 | 47,722 | 40,289 | 42,595 | 932 | 27,746 |
| 11 | OPN | 815 | 15,637 | 27,966 | 30,585 | 24,666 | 225 |
| 12 | CD31/PECAM-1 | 566 | 27,375 | 31,242 | 69 | 3996 | 383 |
| 13 | EGF | 0 | 9516 | 557 | 48,334 | 44,864 | 29,225 |
| 14 | EMMPRIN | 160 | 12,716 | 14,311 | 29,598 | 8647 | 783 |
| 15 | VEGF | 0 | 1326 | 171 | 34,747 | 0 | 456 |
| 16 | GDF-15 | 0 | 9637 | 4674 | 476 | 37,249 | 0 |
| 17 | TIM-3 | 196 | 24,522 | 18,702 | 0 | 38,175 | 387 |
| 18 | IL-1ra | 5362 | 0 | 0 | 59 | 6287 | 17,005 |
| 19 | Lipocalin-2/NGAL | 14,785 | 27,299 | 26,634 | 5042 | 8225 | 30,562 |
| 20 | TFF3 | 0 | 2585 | 667 | 277 | 9505 | 33,795 |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# APPENDIX

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

| Coordinate | Analyte/Control | Entrez Gene ID | Alternate Nomenclature |
|---|---|---|---|
| A1, A2 | Reference Spots | N/A | RS |
| A3, A4 | Adiponectin | 9370 | Acrp30 |
| A5, A6 | Apolipoprotein A-I | 335 | ApoA1 |
| A7, A8 | Angiogenin | 283 | ——— |
| A9, A10 | Angiopoietin-1 | 284 | Ang-1, ANGPT1 |
| A11, A12 | Angiopoietin-2 | 285 | Ang-2, ANGPT2 |
| A13, A14 | BAFF | 10673 | BLyS, TNFSF13B |
| A15, A16 | BDNF | 627 | Brain-derived Neurotrophic Factor |
| A17, A18 | Complement Component C5/C5a | 727 | C5/C5a |
| A19, A20 | CD14 | 929 | ——— |
| A21, A22 | CD30 | 943 | TNFRSF8 |
| A23, A24 | Reference Spots | N/A | RS |
| B3, B4 | CD40 ligand | 959 | CD40L, TNFSF5, CD154, TRAP |
| B5, B6 | Chitinase 3-like 1 | 1116 | CHI3L1, YKL-40 |
| B7, B8 | Complement Factor D | 1675 | Adipsin, CFD |
| B9, B10 | C-Reactive Protein | 1401 | CRP |
| B11, B12 | Cripto-1 | 6997 | Teratocarcinoma-derived Growth Factor |
| B13, B14 | Cystatin C | 1471 | CST3, ARMD11 |
| B15, B16 | Dkk-1 | 22943 | Dickkopf-1 |
| B17, B18 | DPPIV | 1803 | CD26, DPP4, Dipeptidyl-peptidase IV |
| B19, B20 | EGF | 1950 | Epidermal Growth Factor |
| B21, B22 | EMMPRIN | 682 | CD147, Basigin |
| C3, C4 | ENA-78 | 6374 | CXCL5 |
| C5, C6 | Endoglin | 2022 | CD105, ENG |
| C7, C8 | Fas Ligand | 356 | TNFSF6, CD178, CD95L |
| C9, C10 | FGF basic | 2247 | FGF-2 |
| C11, C12 | FGF-7 | 2252 | KGF |
| C13, C14 | FGF-19 | 9965 | ——— |
| C15, C16 | Flt-3 Ligand | 2323 | FLT3LG |
| C17, C18 | G-CSF | 1440 | CSF3 |
| C19, C20 | GDF-15 | 9518 | MIC-1 |
| C21, C22 | GM-CSF | 1437 | CSF2 |
| D1, D2 | GROα | 2919 | CXCL1, MSGA-α |
| D3, D4 | Growth Hormone | 2688 | GH, Somatotropin |
| D5, D6 | HGF | 3082 | Scatter Factor, SF |
| D7, D8 | ICAM-1 | 3383 | CD54 |
| D9, D10 | IFN-γ | 3458 | IFNG |
| D11, D12 | IGFBP-2 | 3485 | ——— |

# APPENDIX *CONTINUED*

| Coordinate | Analyte/Control | Entrez Gene ID | Alternate Nomenclature |
|---|---|---|---|
| D13, D14 | IGFBP-3 | 3486 | ——— |
| D15, D16 | IL-1α | 3552 | IL-1F1 |
| D17, D18 | IL-1β | 3553 | IL-1F2 |
| D19, D20 | IL-1ra | 3557 | IL-1F3 |
| D21, D22 | IL-2 | 3558 | ——— |
| D23, D24 | IL-3 | 3562 | ——— |
| E1, E2 | IL-4 | 3565 | ——— |
| E3, E4 | IL-5 | 3567 | ——— |
| E5, E6 | IL-6 | 3569 | ——— |
| E7, E8 | IL-8 | 3576 | CXCL8 |
| E9, E10 | IL-10 | 3586 | ——— |
| E11, E12 | IL-11 | 3589 | ——— |
| E13, E14 | IL-12 p70 | 3593 | ——— |
| E15, E16 | IL-13 | 3596 | ——— |
| E17, E18 | IL-15 | 3600 | ——— |
| E19, E20 | IL-16 | 3603 | ——— |
| E21, E22 | IL-17A | 3605 | IL-17, CTLA8 |
| E23, E24 | IL-18 Bpa | 10068 | ——— |
| F1, F2 | IL-19 | 29949 | ——— |
| F3, F4 | IL-22 | 50616 | IL-TIF |
| F5, F6 | IL-23 | 51561 | IL-23A, SGRF |
| F7, F8 | IL-24 | 11009 | C49A, FISP, MDA-7, MOB-5, ST16 |
| F9, F10 | IL-27 | 246778 | ——— |
| F11, F12 | IL-31 | 386653 | ——— |
| F13, F14 | IL-32 | 9235 | ——— |
| F15, F16 | IL-33 | 90865 | C9orf26, DVS27, NF-HEV |
| F17, F18 | IL-34 | 146433 | C16orf77 |
| F19, F20 | IP-10 | 3627 | CXCL10 |
| F21, F22 | I-TAC | 6373 | CXCL11, SCYB9B |
| F23, F24 | Kallikrein 3 | 354 | PSA, KLK3 |
| G1, G2 | Leptin | 3952 | OB |
| G3, G4 | LIF | 3976 | ——— |
| G5, G6 | Lipocalin-2 | 3934 | NGAL, LCN2, Siderocalin |
| G7, G8 | MCP-1 | 6347 | CCL2, MCAF |
| G9, G10 | MCP-3 | 6354 | CCL7, MARC |
| G11, G12 | M-CSF | 1435 | CSF1 |
| G13, G14 | MIF | 4282 | ——— |
| G15, G16 | MIG | 4283 | CXCL9 |



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

| Coordinate | Analyte/Control | Entrez Gene ID | Alternate Nomenclature |
|---|---|---|---|
| G17, G18 | MIP-1α/MIP-1β | 6348/6351 | CCL3/CCL4 |
| G19, G20 | MIP-3α | 6364 | CCL20, Exodus-1, LARC |
| G21, G22 | MIP-3β | 6363 | CCL19, ELC |
| G23, G24 | MMP-9 | 4318 | CLG4B, Gelatinase B |
| H1, H2 | Myeloperoxidase | 4353 | MPO, Lactoperoxidase |
| H3, H4 | Osteopontin | 6696 | OPN |
| H5, H6 | PDGF-AA | 5154 | ——— |
| H7, H8 | PDGF-AB/BB | 5154/5155 | ——— |
| H9, H10 | Pentraxin 3 | 5806 | PTX3, TSG-14 |
| H11, H12 | PF4 | 5196 | CXCL4 |
| H13, H14 | RAGE | 177 | ——— |
| H15, H16 | RANTES | 6352 | CCL5 |
| H17, H18 | RBP-4 | 5950 | ——— |
| H19, H20 | Relaxin-2 | 6019 | RLN2, RLXH2 |
| H21, H22 | Resistin | 56729 | ADSF, FIZZ3, RETN |
| H23, H24 | SDF-1α | 6387 | CXCL12, PBSF |
| I1, I2 | Serpin E1 | 5054 | PAI-I, PAI-1, Nexin |
| I3, I4 | SHBG | 6462 | ABP |
| I5, I6 | ST2 | 9173 | IL-1 R4, IL1RL1, ST2L |
| I7, I8 | TARC | 6361 | CCL17 |
| I9, I10 | TFF3 | 7033 | ITF, TFI |
| I11, I12 | TfR | 7037 | CD71, TFR1, TFRC, TRFR |
| I13, I14 | TGF-α | 7039 | TGFA |
| I15, I16 | Thrombospondin-1 | 7057 | THBS1, TSP-1 |
| I17, I18 | TNF-α | 7124 | TNFSF1A |
| I19, I20 | uPAR | 5329 | PLAUR |
| I21, I22 | VEGF | 7422 | BEGFA |
| J1, J2 | Reference Spots | N/A | RS |
| J5, J6 | Vitamin D BP | 2638 | VDB, DBP, VDBP |
| J7, J8 | CD31 | 5175 | PECAM-1 |
| J9, J10 | TIM-3 | 84868 | HAVCR2 |
| J11, J12 | VCAM-1 | 7412 | CD106 |
| J23, J24 | Negative Controls | N/A | Control (-) |

# NOTES

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