Proteome Profiler™ Array

Human Ubiquitin Array Kit

Catalog Number ARY027

For the parallel determination of the relative level of ubiquitination of selected human 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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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 ubiquitinated proteins is essential for understanding their roles in normal cellular responses and the development of disease states. The Human Ubiquitin Array Kit is a rapid, sensitive, and economical tool to simultaneously detect changes in ubiquitinated proteins between samples. The relative ubiquitination levels of 49 different proteins can be determined without performing numerous immunoprecipitations and Western blots. Each capture antibody was carefully selected using lysate samples prepared from cell lines known to express the target proteins.

PRINCIPLE OF THE ASSAY

Capture and control antibodies have been spotted in duplicate on nitrocellulose membranes. Cell lysates are diluted and incubated overnight with the Human Cell Ubiquitin Array. The membrane is washed to remove unbound proteins, followed by incubation with a biotinylated pan anti-ubiquitin detection antibody. Streptavidin-HRP and chemiluminescent detection reagents are applied, and a signal is produced at each capture spot corresponding to the relative amount of ubiquitinated 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 Ubiquitin Array	894867	4 nitrocellulose membranes each containing 49 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 1	895477	21 mL of a buffered protein base with preservatives.	May be stored for up to 3 months at 2-8 °C.*
Array Buffer 2 5X Concentrate	895478	21 mL of a concentrated buffered protein base with preservatives.	
Lysis Buffer 6	895561	21 mL of a denaturing buffered solution.	
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, Human Ubiquitin Array	894868	1 vial of biotinylated detection antibody; 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	607908	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
- 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

SUPPLIES REQUIRED FOR CELL LYSATE SAMPLES

- Phosphate-Buffered Saline (PBS)
- Aprotinin (Sigma, Catalog # A6279)
- Leupeptin (Tocris, Catalog # 1167)
- Pepstatin (Tocris, Catalog # 1190)

PRECAUTIONS

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

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.

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 Ubiquitin 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: 200-300 µg for samples obtained from ligand treated cells and 25-100 µg for samples from cells treated with proteasome inhibitors.*

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 6 (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.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Human Ubiquitin 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 - Before use, reconstitute the Human Ubiquitin Detection Antibody in 100 µL of deionized or distilled water.

1X Array Buffer 2 - Add 2 mL of Array Buffer 2 to 8 mL of deionized or distilled water. Prepare fresh for each use.

Lysis Buffer 6 - 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 1X Array Buffer 2. See vial label for dilution factor.

ARRAY PROCEDURE

Bring all reagents to room temperature before use. Keep samples on ice. To avoid contamination, wear gloves while performing the procedures.

1. Prepare all reagents and samples as directed in the previous sections.
2. Pipette 2.0 mL of Array Buffer 1 into each well of the 4-Well Multi-dish to be used. Array Buffer 1 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 1, 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 tray 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. Adjust to a final volume of 1.5 mL with Array Buffer 1 as necessary.
6. Aspirate Array Buffer 1 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 15 µL of Detection Antibody to 1.5 mL of 1X Array Buffer 2. Pipette 1.5 mL per well of diluted Detection Antibody 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, 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. Dilute the Streptavidin-HRP in 1X Array Buffer 2 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.

ARRAY PROCEDURE *CONTINUED*

14. 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. Place the lid on the 4-Well Multi-dish.

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

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

    Note: *Complete the remaining steps without interruption.*

17. 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.

18. 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.*

19. 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.

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

21. 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.

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

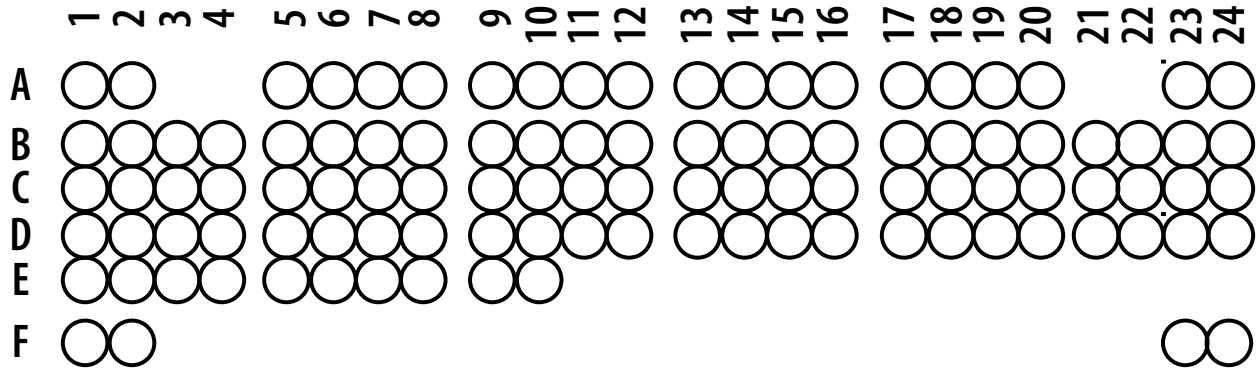
The positive signals seen on developed film can be quickly identified by placing the transparency overlay template on the array image and aligning it with the pairs of reference spots in three corners of each array. The stamped identification number on the array should be placed on the left hand side.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

The Human Ubiquitin Array Kit detects multiple analytes in cell lysates. Cells were either untreated or treated as indicated below. The amount of cell lysate used on each array and the duration of exposure to X-ray film is indicated below. Profiles of mean spot pixel density were created using a transmission-mode scanner and image analysis software.

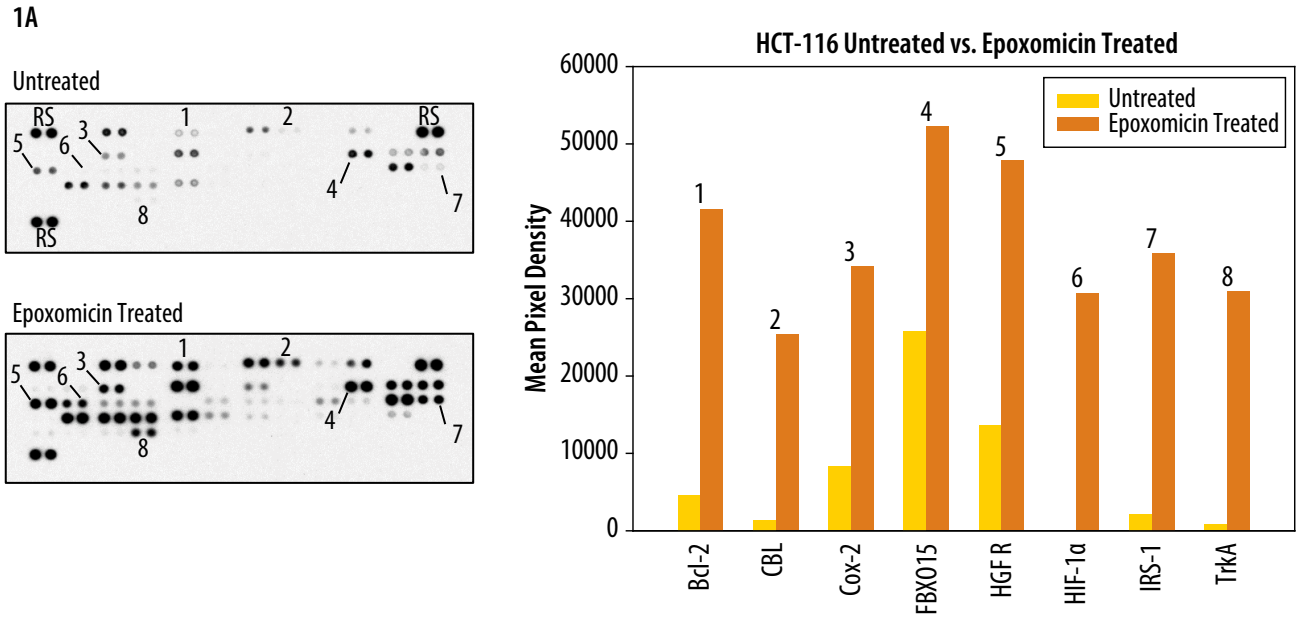


Figure 1A: HCT-116 human colorectal carcinoma cells were untreated or treated with 1 mM Epoxomicin (Boston Biochem, Catalog # I-110) for 6 hours (50 µg lysate, 2 minute exposure). RS =Reference Spots.

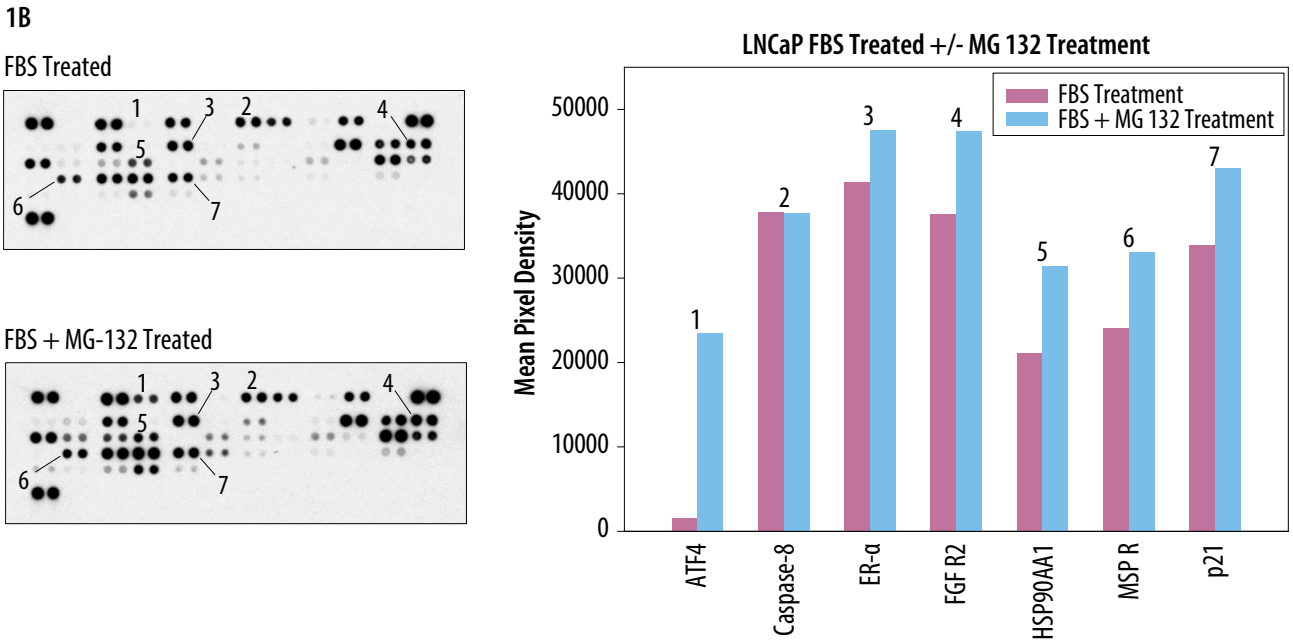


Figure 1B: LNCaP human prostate cancer cells were serum-starved for 18 hours followed by addition of 10% fetal bovine serum with or without 25 µM MG 132 (Tocris, Catalog # 1748) for 5 hours (50 µg lysate, 5 minute exposure).

1C

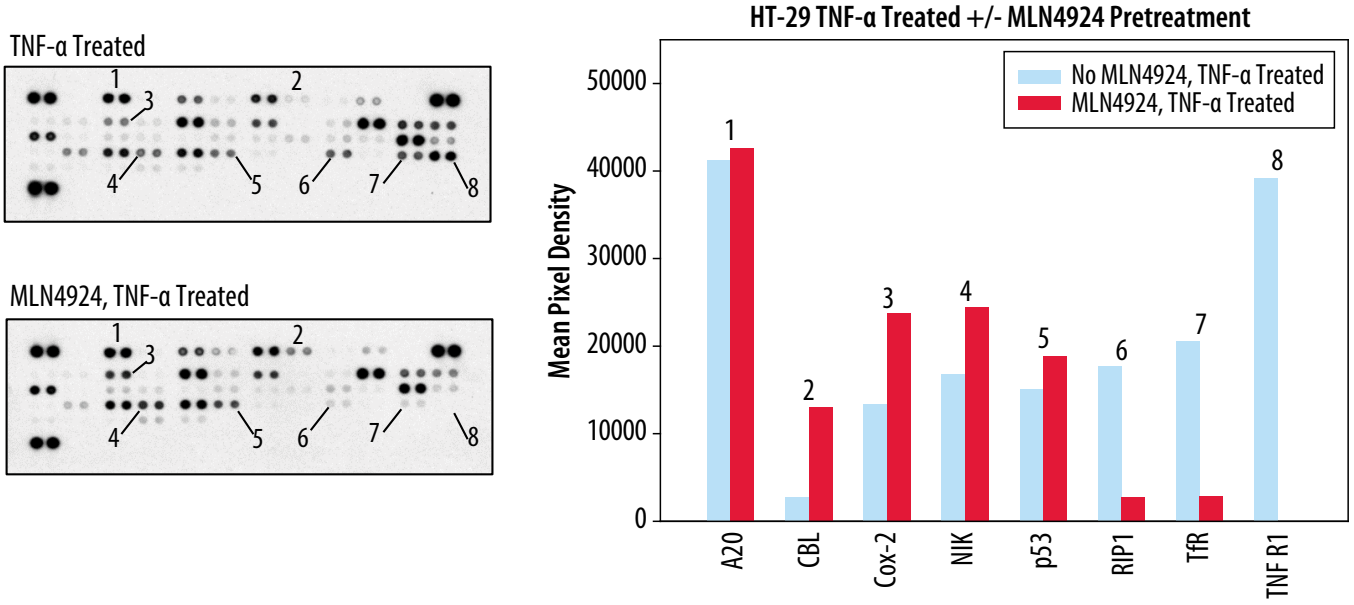


Figure 1C: HT-29 human colorectal carcinoma cells were treated with 50 ng/mL of recombinant human TNF-α (R&D Systems, Catalog # 210-TA) for ten minutes with or without pretreatment with 5 µM MLN4924 for 1 hour (300 µg lysate, 10 minute exposure).

1D

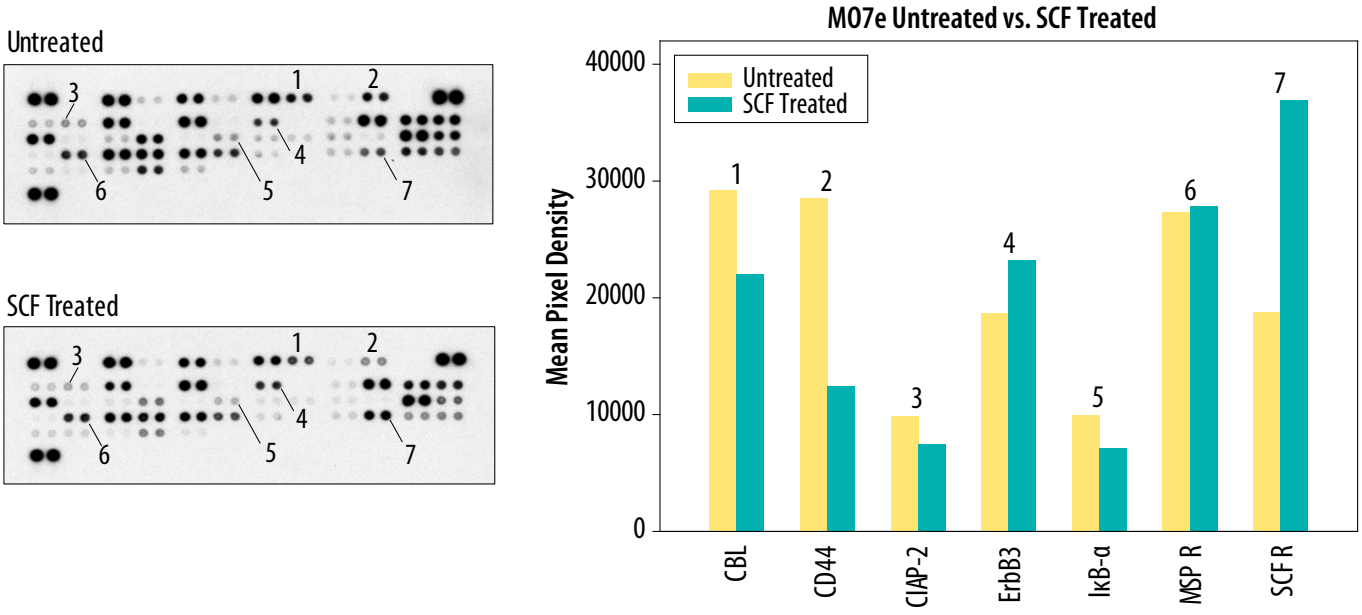


Figure 1D: MO7e human acute megakaryocytic leukemia cells were untreated or treated with 250 ng/mL of recombinant human SCF (R&D Systems, Catalog # 255-SC) for 5 minutes (300 µg lysate, 10 minute exposure).

APPENDIX

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

Coordinate	Analyte/Control	Entrez Gene ID	Alternate Nomenclature
A1, A2	Reference Spots	NA	RS
A5, A6	A20	7128	OTUD7C, TNFA1P2, TNFAIP3
A7, A8	ATF4	468	TAXREB67, TXREB
A9, A10	Bcl-2	596	———
A11, A12	β-TrCP1	8945	BTRC, BTRCP, E3RSIkappaB, FBW1A, FBXW1A, FWD1
A13, A14	Caspase-8	841	CASP8, MCH5, FLICA, MACHα1
A15, A16	CBL	867	CBL2, RNF55
A17, A18	Cyclin D1	NA	RS
A19, A20	CD44	595	Bcl-1, CCND1, PRAD1
A23, A24	Reference Spots	960	ECMR-III, HCELL, LHR, MDU2, MDU3, MIC4, HUTCH-I, Pgp1
B1, B2	cIAP-1	329	BIRC2, HIAP-2, MIHB
B3, B4	cIAP-2	330	BIRC3, HIAP-1, MIHC
B5, B6	COX-2	5743	PGHS-2, PHS-II, PTGS2
B7, B8	EGF R	1956	ErbB, ErbB1, HER1
B9, B10	ER-α	2099	ESR1, NR3A1
B11, B12	ErbB2	2064	CD340, HER2, Neu Oncogene, NGL, TKR1
B13, B14	ErbB3	2065	HER3
B15, B16	ErbB4	2066	HER4
B17, B18	Fatty Acid Synthase	2194	FAS, FASN
B19, B20	F-box protein 15	201456	FBXO15, FBX15
B21, B22	FBXW7	55294	AGO, Cdc4, FBW6, Fbw7, FBX30, FBXO30, FBXW6, SCF(Fbw7); SEL10
B23, B24	FGF R2α/FGF R2β	2263	α and β isoforms
C1, C2	HGF R	4233	c-MET, MET
C3, C4	HIF-1α	3091	HIF1A
C5, C6	HSP70	3303	HSP70-1A, HSP72, HSPA1A
C7, C8	HSP90	3320	HSP90A, HSP90AA1, HSP90N, HSPC1, HSPCA, HSPCAL1, HSPCAL4, HSPN
C9, C10	IGF-I R	3480	CD221, IGF1R
C11, C12	IκB-α	4792	MAD-3, NFKBIA
C13, C14	IκB-ε	4794	NFKBIE, IKBE
C15, C16	IKKγ	8517	FIP3P, IKBKG, IKKAP1, NEMO
C17, C18	Insulin R	3643	CD220, INSR
C19, C20	IRAK1	3654	Pelle
C21, C22	IRF3	3661	———
C23, C24	IRS1	3667	———
D1, D2	M-CSF R	1436	CD115, c-fms, CSF1R
D3, D4	MSP R	4486	CD136, MST1R, Ron
D5, D6	Nrf2	4780	NFE2L2
D7, D8	NIK	9020	FTDCR1B, HSNIK, MAP3K14

APPENDIX *CONTINUED*

Coordinate	Analyte/Control	Entrez Gene ID	Alternate Nomenclature
D9, D10	p21	1026	CDKN1A, CIP1
D11, D12	p53	7157	BCC7, LFS1, TP53, TRP53
D13, D14	PDGF Rα	5156	CD140a, PDGFRA
D15, D16	PDGF Rβ	5159	CD140b, PDGFRB
D17, D18	RIP1	8737	RIPK1
D19, D20	SCF R	3815	CD117, c-Kit
D21, D22	TfR	7037	CD71, TFR1, TFRC, TRFR
D23, D24	TNF RI	7132	CD120a, TNFRSF1A
E1, E2	TRAF-2	7186	TRAP3
E3, E4	TRAF-3	7187	CAP1, CD40bp, CRAF1, LAP1
E5, E6	TRAF-6	7189	RNF85
E7, E8	TrkA	4914	NTRK1, NTRK-1
E9, E10	VEGF R3	2324	FLT4, Flt-4
F1, F2	Reference Spots	NA	RS
F23, F24	Negative Control	NA	Controls (-)

NOTES

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