# Proteome Profiler™ Array

## Human NFκB Pathway Array Kit

Catalog Number ARY029

For the parallel determination of the relative levels of selected NFκB Pathway proteins.

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

# TABLE OF CONTENTS




**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

The NFκB signaling pathway plays a central role in many physiological and pathological processes. Dysregulation of NFκB activity is associated with inflammatory disorders, autoimmune and metabolic diseases, as well as cancer. Profiling NFκB signaling pathway proteins may help to better understand their underlying mechanisms. The Human NFκB Pathway Array is a rapid, sensitive, and economical tool to simultaneously detect the relative levels of 41 proteins and 4 serine or tyrosine phosphorylation sites involved in NFκB signal transduction without performing numerous immunoprecipitations and Western blots. Each capture antibody was carefully selected using cell lysates prepared from cell lines known to express the target protein.

## 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 NFκB Pathway Array. The array is washed to remove unbound proteins, followed by incubation with a 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 a list and coordinates of analytes and controls.

## TECHNICAL HINTS

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- This kit should not be used beyond the expiration date on the kit label.
- **Do not mix or substitute reagents with those from other lots or sources. Substitution of some high intensity chemiluminescent reagents for Chemi Reagents 1 and 2 may cause either increased background or diminished signal, depending on the reagent.**
- Any variation in sample handling, buffers, operator, pipetting technique, washing technique, and incubation time or temperature can alter the performance of the kit.
- The Human NFκB Pathway 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 NFκB Pathway Array** | 894251 | 4 nitrocellulose membranes each containing 45 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 3** | 895008 | 21 mL of a buffered protein base with preservatives. | |
| **Array Buffer 6** | 893573 | 21 mL of a buffered protein base with preservatives. | |
| **Lysis Buffer 6** | 895561 | 21 mL of a denaturing buffered solution 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 NFκB Pathway Array** | 894252 | 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** | 607944 | 1 transparency overlay template for coordinate reference. | |

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

## PRECAUTIONS

Chemi Reagents 1 and 2 contain Boric Acid which is suspected of damaging fertility or the unborn child. Do not handle until all safety precautions in the MSDS have been read and understood.

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 MSDS on our website prior to use.

## OTHER SUPPLIES REQUIRED

- Aprotinin (Sigma, Catalog # A6279)
- Leupeptin (Tocris®, Catalog # 1167)
- Pepstatin (Tocris®, Catalog # 1190)
- Pipettes and pipette tips
- Gloves
- Phosphate-Buffered Saline (PBS)
- Deionized or distilled water
- Flat-tipped tweezers
- 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
- Absorbent lab wipes (KimWipes® or equivalent)
- Paper towels
- Autoradiography cassette
- Film developer
- X-ray film (Kodak® BioMax™ Light-1, Catalog # 1788207) or equivalent
- Flatbed scanner with transparency adapter capable of transmission mode
- Computer capable of running image analysis software and Microsoft® Excel

## 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 NFκB Pathway Array Kit 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. The suggested starting range for cell lysates is 200-500 µg.*

**Cell Lysates -** Rinse cells with PBS and remove any remaining PBS before adding lysis buffer. Solubilize the cells at 1 x $10^7$ cells/mL in Lysis Buffer 6. 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 concentrations using a total protein assay is recommended. The maximum allowable lysate volume is 250 µL/array. Cell lysates should be used immediately or aliquoted and stored at ≤ -70 °C. Avoid repeated freeze-thaw cycles. Thawed lysates should be kept on ice prior to use.

## REAGENT PREPARATION

**Bring all reagents to room temperature before use.**

**Human NFκB Pathway Array** - Four nitrocellulose membranes each containing 45 different capture antibodies printed in duplicate. **Handle arrays only with gloved hands and flat-tipped tweezers.**

**Detection Antibody Cocktail** - One vial of lyophilized biotinylated antibodies. Before use, reconstitute the Human NFκB Pathway Detection Antibody Cocktail in 100 µL of deionized or distilled water.

**1X Wash Buffer** - If crystals have formed in the concentrate, warm the bottles to room temperature and mix gently until the crystals have completely dissolved. Add 40 mL of 25X Wash Buffer Concentrate to 960 mL of deionized or distilled water to prepare 1000 mL of 1X Wash Buffer.

**Chemi Reagent Mix** - Chemi Reagent 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.** Discard any remaining after use.

**Array Buffer 3/6** – Mix equal volumes of Array Buffer 3 and Array Buffer 6 to make Array Buffer 3/6. **Make fresh for each day of the assay procedure.**

## 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 3/6 into each well of the 4-Well Multi-dish to be used. Array Buffer 3/6 serves as a block buffer.
3. Using flat-tip tweezers, remove each membrane to be used from between the protective sheets and place in a well of the 4-Well Multi-dish. The array number should be facing upward.

   **Note:** *Upon contact with Array Buffer 3/6, 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 tray so that each array rocks end to end in its well.
5. While the membranes are blocking, prepare samples by adding the desired quantity of lysate to Array Buffer 1 for a total volume of 1.5 mL. Maximum lysate volume is 250 µL.
6. Aspirate Array Buffer 3/6 from the wells of the 4-Well Multi-dish. Add prepared samples and place the lid on the 4-Well Multi-dish.
7. Incubate overnight at 2-8 °C on a rocking platform.

   **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, dilute 15 µL of reconstituted Detection Antibody Cocktail to 1.5 mL with Array Buffer 3/6. Pipette 1.5 mL per well of diluted Detection Antibody Cocktail into the 4-Well Multi-dish.
11. Carefully remove each membrane from its wash container. Allow excess buffer to drain from the membrane. Return the membrane to the 4-Well Multi-dish containing the diluted Detection Antibody Cocktail. Cover the wells with the lid.
12. Incubate for 1 hour at room temperature on a rocking platform shaker.
13. Wash each array as described in steps 8 and 9.
14. Dilute the Streptavidin-HRP in Array Buffer 3/6 using the dilution factor on the vial label. Pipette 2.0 mL into each well of the 4-Well Multi-dish.

## ARRAY PROCEDURE *CONTINUED*

15. Carefully remove each membrane from the wash container. Allow excess Wash Buffer to drain from the membrane. Return the array to the 4-Well Multi-dish containing the diluted Streptavidin-HRP, and cover with the lid. Incubate for 30 minutes 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

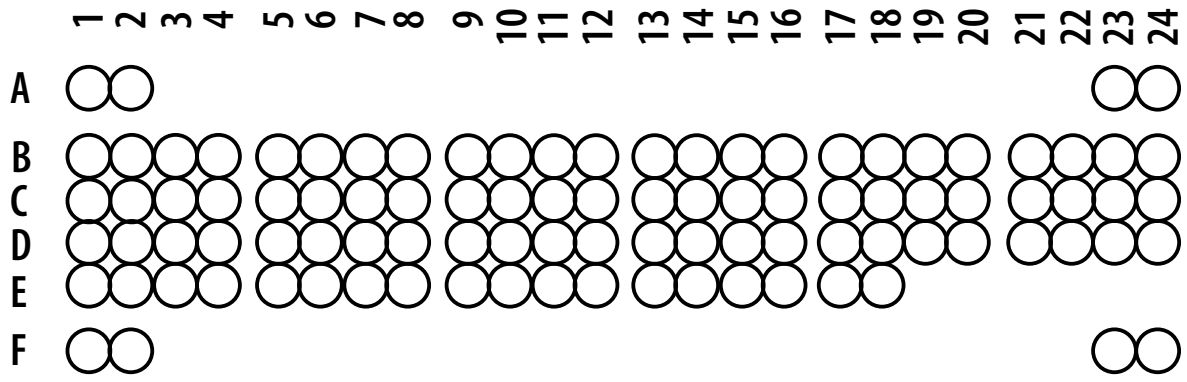
The positive signals seen on developed film can be quickly identified by placing the transparency overlay template on the array image and aligning it with the pairs of reference spots in three corners of each array. The stamped identification number on the array should be placed on the left hand side.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

### Human NFκB Pathway 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 NFkB Pathway Array detects multiple proteins 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.

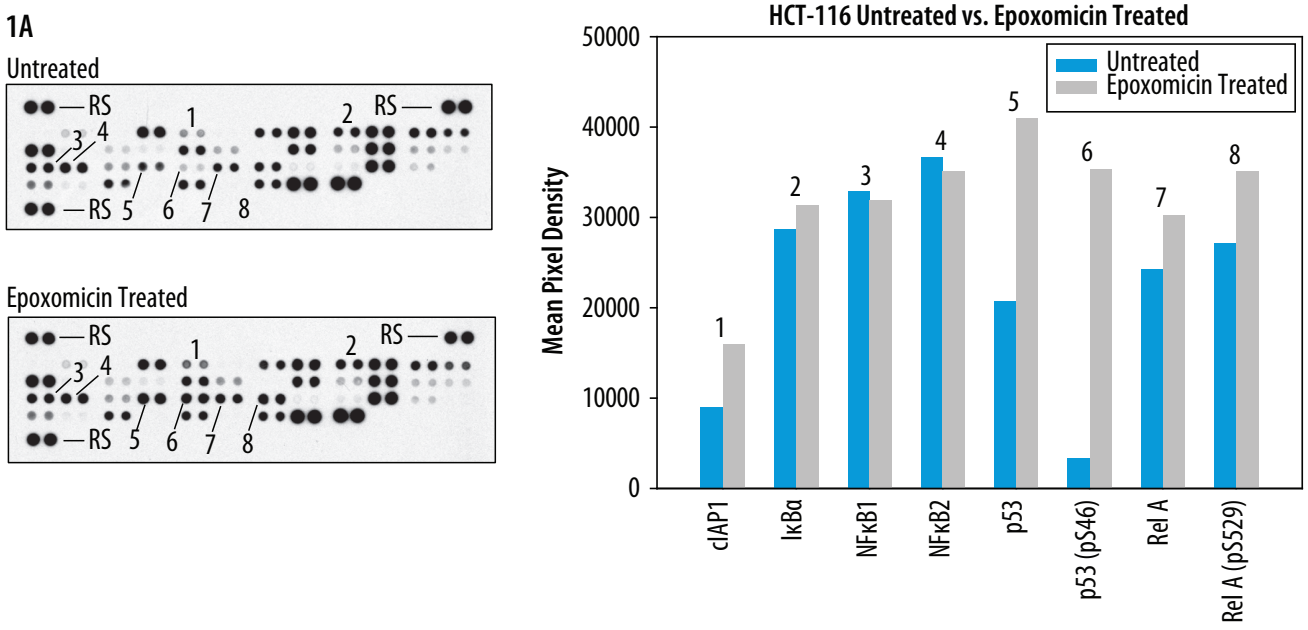



**Figure 1A:** HCT-116 human colorectal carcinoma cells were untreated or treated with 1 µM Epoxomicin (Boston Biochem, Catalog # I-110) for 6 hours (500 µg lysate, 5 minute exposure). RS = Reference Spots.

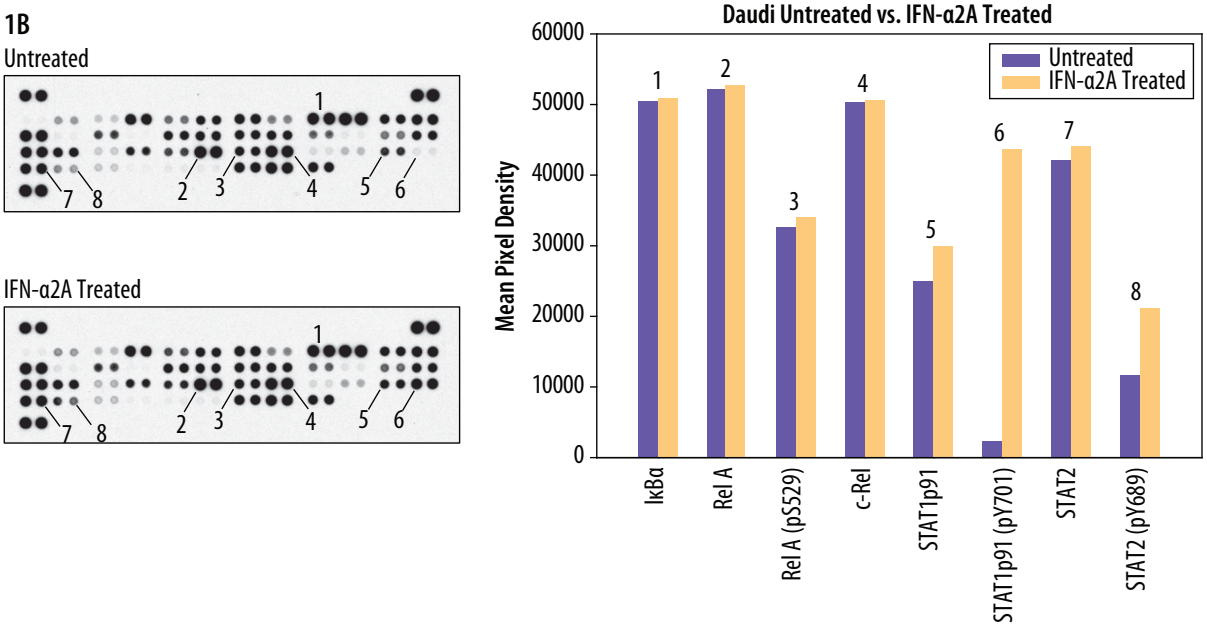
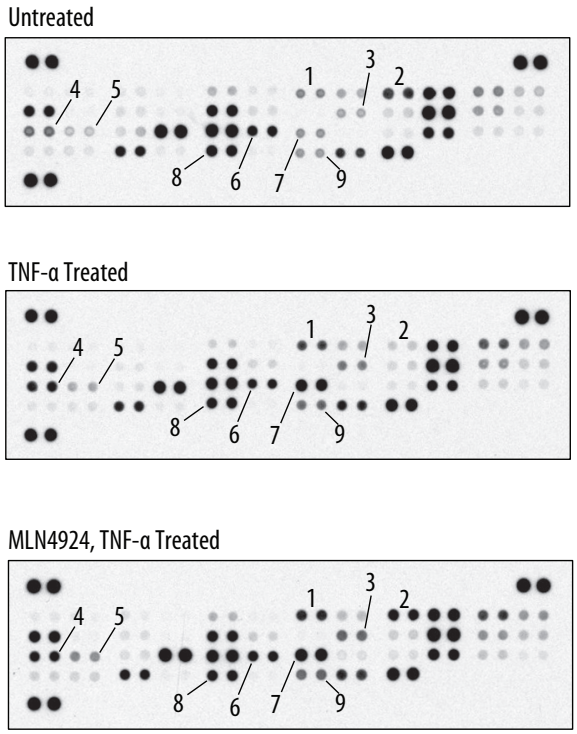



**Figure 1B:** Daudi human Burkitt's lymphoma cells were untreated or treated with 500 U/mL IFN-α2A (R&D Systems®, Catalog # 11100-1) for five minutes (1 mg lysate, 10 minute exposure).



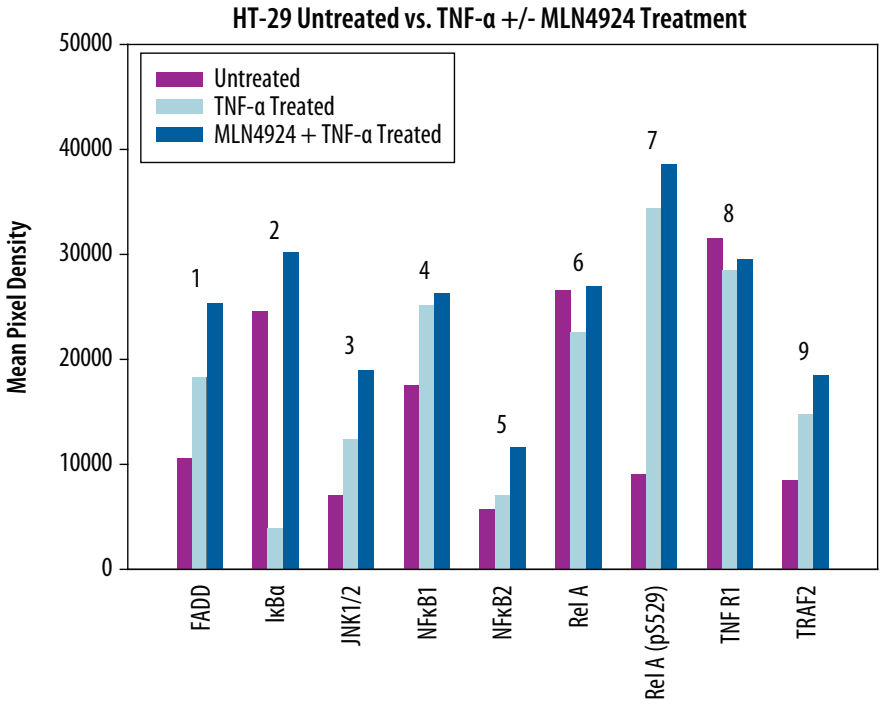




**Figure 1C:** HT-29 human colorectal carcinoma cells were untreated or treated with 50 ng/mL recombinant human TNF-α (R&D Systems®, Catalog # 210-TA) for ten minutes with or without pretreatment with 5 µM MLN4924 (Boston Biochem, Catalog # I-502) for 1 hour (400 µg lysate, 5 minute exposure).

# APPENDIX

Refer to the table below for the Human NFκB Pathway Array coordinates.

| Coordinate | Target/Control | Entrez Gene ID | Alternate Nomenclature |
|---|---|---|---|
| A1, A2, A23, A24 | Reference Spots | NA | RS |
| B1, B2 | ASC | 29108 | PYCARD/CARD5 |
| B3, B4 | BCL10 | 8915 | CLAP/CIPER/CARMEN/c-E10 |
| B5, B6 | CARD6 | 84674 | CINCIN1 |
| B7, B8 | CD40/TNFRSF5 | 958 | CDW40 |
| B9, B10 | cIAP1/BIRC2 | 329 | ——— |
| B11, B12 | cIAP2/BIRC3 | 330 | ——— |
| B13, B14 | FADD/MORT1 | 8772 | GIG3 |
| B15, B16 | Fas/TNFRSF6/CD95 | 355 | ——— |
| B17, B18 | IκBα | 4792 | NFKBIA/MAD-3/NFKBI/IKBA |
| B19, B20 | IκBε | 4794 | NFKBIE/IKBE |
| B21, B22 | IKK1/IKKα/CHUK | 1147 | IKKA/IKBKA/TCF16 |
| B23, B24 | IKK2/IKKβ | 3551 | IKKB/NFKBIKB/IMD15 |
| C1, C2 | IKKγ/NEMO | 8517 | IKBKG/FIP3/IMD33 |
| C3, C4 | IL-1 RI | 3554 | CD121A/IL-1R-alpha |
| C5, C6 | IL-17 RA | 23765 | CD217/CANDF5/CDw217 |
| C7, C8 | IL-18 Rα | 8809 | CD218a/IL18RA/IL1 RRP |
| C9, C10 | IRAK1 | 3654 | IRAK/pelle |
| C11, C12 | IRF5 | 3663 | SLEB10 |
| C13, C14 | IRF8 | 3394 | ICSBP/IMD32A |
| C15, C16 | JNK1/2 | 5599/5601 | SAPK1/MAPK8; SAPK1a/MAPK9 |
| C17, C18 | JNK2 | 5601 | SAPK1a/MAPK9 |
| C19, C20 | LTBR/TNFRSF3 | 4055 | CD18 |
| C21, C22 | Metadherin/AEG-1 | 92140 | LYRIC/MTDH |
| C23, C24 | MYD88 | 4615 | ——— |
| D1, D2 | NFκB1 | 4790 | p50/p105 |
| D3, D4 | NFκB2 | 4791 | p52/p100 |
| D5, D6 | NGF R/TNFRSF16 | 4804 | CD271/p75NTR |
| D7, D8 | p53 | 7157 | ——— |
| D9, D10 | p53 (pS46) | 7157 | ——— |
| D11, D12 | RelA/p65 | 5970 | NFKB3 |
| D13, D14 | RelA/p65 (pS529) | 5970 | NFKB4 |
| D15, D16 | c-Rel | 5966 | ——— |
| D17, D18 | SHARPIN | 81858 | SIPL1 |
| D19, D20 | SOCS6 | 9306 | CIS4/SSI4/SOCS4/STATI4 |
| D21, D22 | STAT1p91 | 6772 | ISGF-3/STAT91 |
| D23, D24 | STAT1 (pY701) | 6772 | ISGF-3/STAT91 |

## APPENDIX *CONTINUED*

| Coordinate | Target/Control | Entrez Gene ID | Alternate Nomenclature |
|---|---|---|---|
| E1, E2 | STAT2 | 6773 | P113/STAT113 |
| E3, E4 | STAT2 (pY689) | 6773 | P113/STAT113 |
| E5, E6 | STING/TMEM173 | 340061 | ——— |
| E7, E8 | TLR2 | 7097 | TIL4/CD282 |
| E9, E10 | TNF RI/TNFRSF1A | 7132 | CD120a/TNFAR |
| E11, E12 | TNF RII/TNFRSF1B | 7133 | CD120b/p75 |
| E13, E14 | TRAF2 | 7186 | TRAP/TRAP3 |
| E15, E16 | TRAIL R1/DR4 | 8797 | TNFRSF10A/CD261 |
| E17, E18 | TRAIL R2/DR5 | 8795 | TNFRSF10B/CD262/KILLER/TRICK2 |
| F1, F2 | Reference Spots | NA | RS |
| F23, F24 | Negative Control | NA | Controls (-) |

## NOTES

# NOTES

## NOTES