#13788 Store at 4°C

PathScan®Immune Cell Signaling Antibody Array Kit (Fluorescent Readout)

1 Kit (32 multiplexed assays)

www.cellsignal.com

Support: 877-678-TECH (8324)
www.cellsignal.com/support

Orders: 877-616-CELL (2355)
orders@cellsignal.com

New 07/14

For Research Use Only. Not For Use In Diagnostic Procedures.

Species Cross-Reactivity: H

Description: The PathScan® Immune Cell Signaling Antibody Array Kit (Fluorescent Readout) uses glass slides as the planar surface and is based upon the sandwich immunoassay principle. The array kit allows for the simultaneous detection of 19 signaling molecules that are involved in the regulation of the immune and the inflammatory responses. Target-specific capture antibodies have been spotted in duplicate onto nitrocellulose-coated glass slides. Each kit contains two slides allowing for the interrogation of 32 different samples and the generation of 608 data points in a single experiment. Cell lysates are incubated on the slide followed by a biotinylated detection antibody cocktail. DyLight™ 680-linked Streptavidin is then used to visualize the bound detection antibody. A fluorescent image of the slide can then be captured with a digital imaging system and spot intensities quantified using array analysis software.

Specificity/Sensitivity: PathScan® Immune Cell Signaling Antibody Array Kit (Fluorescent Readout) detects the target proteins as specified on the Array Target Map. No substantial cross-reactivity has been observed between targets. This kit is optimized for cell lysates diluted to a total protein concentration between 0.2 and 1 mg/ml (see kit protocol). All sandwich assays have been validated for human derived samples. This kit detects proteins from the indicated species as determined through in-house testing, but may also detect homologous proteins from other species.

Products Included	Quantity	Cap Color
Array Slides	2 slides	
Multi-Well Gasket	2 gaskets	
Sealing Tape	2 sheets	
20X Array Wash Buffer	15 ml	White
Array Blocking Buffer	5 ml	Red
Array Diluent Buffer	15 ml	Blue
10X Detection Antibody Cocktail	300 µl	White
10X DyLight™ 680-linked Streptavidin	300 µl	Brown
*Cell Lysis Buffer #7018	30 ml	Clear

*Kit should be stored at 4°C with the exception of 1X Cell Lysis Buffer, which is stored at –20°C (packaged separately).

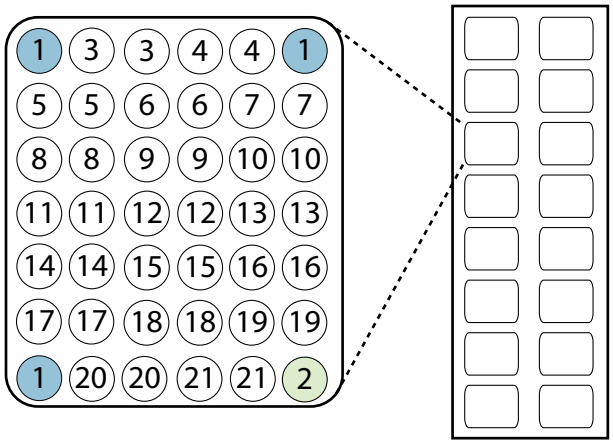


Figure 1. Target map of the PathScan® Immune Cell Signaling Antibody Array Kit (Fluorescent Readout) #13788.

Immune Cell Signaling

	Target	Site	Modification
1	Positive Control	N/A	N/A
2	Negative Control	N/A	N/A
3	p44/42 MAPK (Erk1/2)	Thr202/Tyr204	Phosphorylation
4	Akt	Ser473	Phosphorylation
5	p38 MAPK	Thr180/Tyr182	Phosphorylation
6	SAPK/JNK	Thr183/Tyr185	Phosphorylation
7	Caspase-7	Asp198	Cleavage
8	Iκbα	Total	N/A
9	Iκbα	Ser32/36	Phosphorylation
10	TAK1	Ser412	Phosphorylation
11	Stat1	Tyr701	Phosphorylation
12	Stat1	Ser727	Phosphorylation
13	Stat3	Tyr705	Phosphorylation
14	Stat3	Ser727	Phosphorylation
15	Stat5a	Tyr694	Phosphorylation
16	Stat6	Tyr641	Phosphorylation
17	Lck	Tyr416	Phosphorylation
18	Syk	Tyr352	Phosphorylation
19	Zap-70	Tyr319	Phosphorylation
20	Rig-I	Total	N/A
21	IRF-3	Total	N/A

Thank you for your recent purchase. If you would like to provide a review visit www.cellsignal.com/comments.

Cell Signaling TECHNOLOGY®

Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

Background: The vertebrate immune response relies on both a non-specific, innate immune system and an antigen-specific, adaptive immune response. Organisms rely on cell-mediated and antibody-mediated responses to detect pathogens and protect against infection and disease. Inflammation is an immune response in which circulating immune system cells localize to sites of injury or infection. Regulation of inflammation is essential, as unchecked inflammation can result in permanent damage to tissues and organs. A variety of signaling proteins are known to regulate the immune and inflammatory response.

Akt and p44/42 MAPK (Erk1/2) kinases are key regulators of cell fate that generally transmit growth promoting and survival signals. These two kinases serve as major signal integration hubs and very few cellular processes occur without their involvement. Both p38 MAPK and SAPK/JNK kinases are activated through a dual phosphorylation mechanism in response to pro-inflammatory cytokines, stressful conditions, or genotoxic stress. Caspase-7 is an intracellular protease involved in apoptosis and is part of a cellular sub-organelle called the inflammasome. Caspase-7 is activated by cleavage at Asp198. Activation of the essential immune system regulator NF-κB is triggered by a diverse group of extracellular signals promoted by inflammatory cytokines, growth factors, and chemokines. The proteasome-mediated degradation of the NF-κB/Rel inhibitor IκBα results from phosphorylation of IκBα at Ser32 and Ser36 and targeting of IκBα to the proteasome. The TAK1 kinase responds to a variety of cytokines to regulate cellular kinases and activate the NF-κB pathway; phosphorylation of TAK1 at Ser412 by PKA regulates kinase activity.

Stat family transcription factors are activated by a variety of cytokines, chemokines, or growth factors to regulate the immune response. Phosphorylation of Stat proteins at specific tyrosine or serine residues leads to Stat protein dimerization, nuclear translocation, and binding of the transcription factor to genes that regulate the immune response. The range and specificity of responses regulated by Stat proteins is determined in part by the tissue-specific expression of different cytokine receptors and by the combinatorial coupling of various Stat members to different receptors. Lck, Syk, and Zap-70 are Src family tyrosine kinases that couple the activated B cells and T cell immunoreceptors to downstream signaling events that mediate the immune response. Rig-1 is a Toll-receptor signaling complex component that plays a role in the antiviral innate immune response. The transcription factor IRF-3 plays a role in regulating interferon (IFN) and IFN-inducible gene expression in response to viral infection.

Selected References:

(1) Sojka, D.K. et al. (2009) *Immunol Res* 45, 239-50.

(2) Si-Tahar, M. et al. (2009) *Clin Exp Immunol* 156, 194-8.

(3) Girardin, S.E. and Philpott, D.J. (2009) *Semin Immunol* 21, 173-4.

(4) Lukic, M.L. et al. (2009) *Mol Immunol* 47, 1-2.

(5) Gaestel, M. et al. (2009) *Nat Rev Drug Discov* 8, 480-99.

(6) Nag, K. and Chaudhary, A. (2009) *Curr Signal Transduct Ther* 4, 76-81.

(7) Müller-Hübenthal, B. et al. (2009) *Anticancer Res* 29, 4795-805.

(8) Yu, H. et al. (2009) *Nat Rev Cancer* 9, 798-809.

(9) Grivennikov, S.I. et al. (2010) *Cell* 140, 883-99.

(10) Maletzki, C. and Emmrich, J. (2010) *Dig Dis* 28, 574-8.

(11) Davis, B.K. et al. (2011) *Annu Rev Immunol* 29, 707-35.

(12) Newton, K. and Dixit, V.M. (2012) *Cold Spring Harb Perspect Biol* 4.

(13) Mantovani, A. and Garlanda, C. (2013) *Nat Immunol* 14, 768-70.

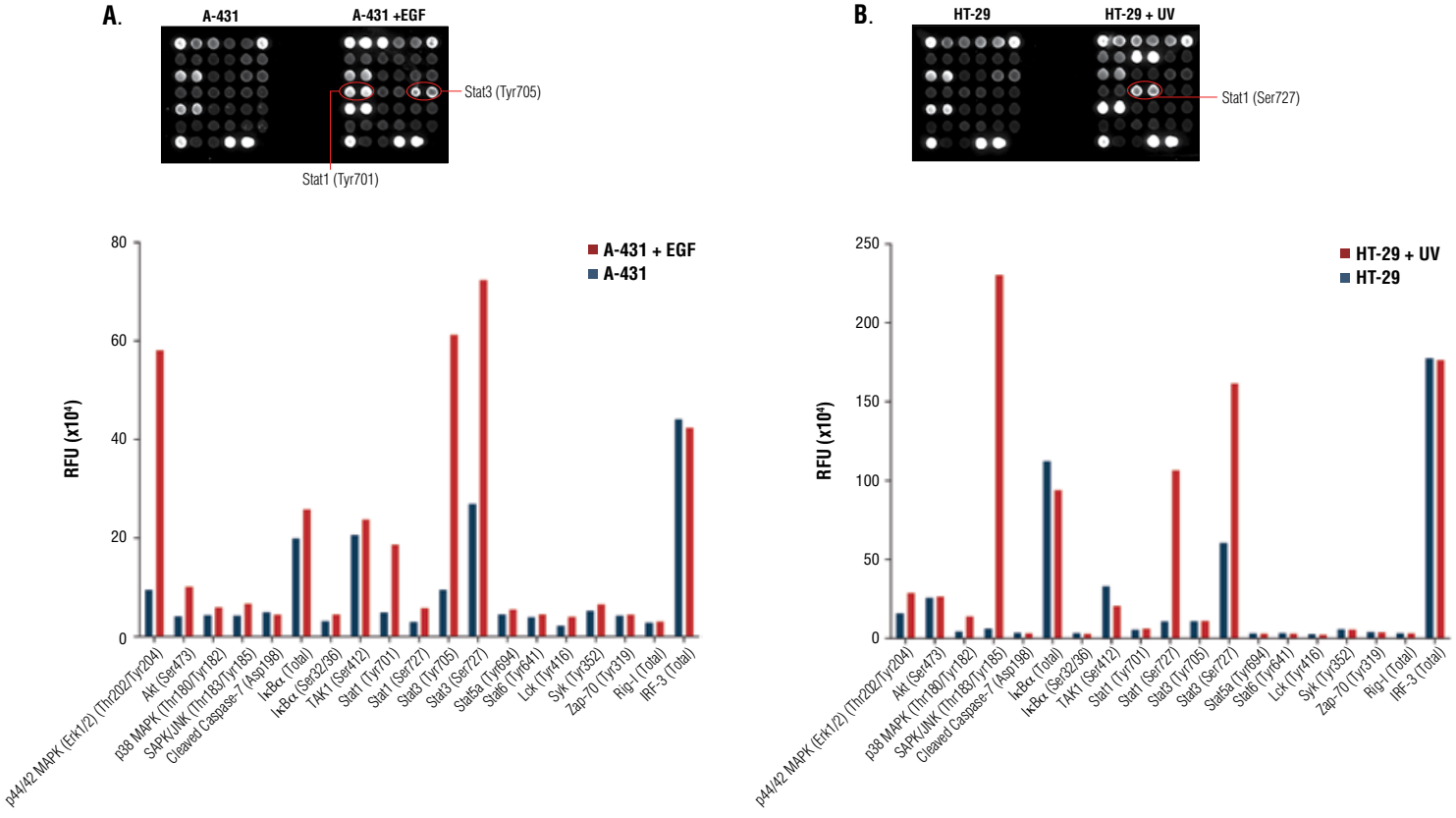


Figure 2. A-431 cells were grown to 90% confluency, serum starved overnight, and either untreated or treated with Human Epidermal Growth Factor (hEGF) #8916 (100 ng/ml, 5 min; Panel A). HT-29 cells were grown to 80% confluency and either untreated or UV-irradiated and allowed to recover for 60 min (Panel B). Cell lysates were analyzed using the PathScan® Immune Cell Signaling Antibody Array Kit (Fluorescent Readout) #13788. Array fluorescent images were acquired using the LI-COR® Biosciences Odyssey® imaging system (upper) and fluorescence intensities for each spot were quantified using the LI-COR® Image Studio v2.0 array analysis software and depicted as bar graphs (lower).

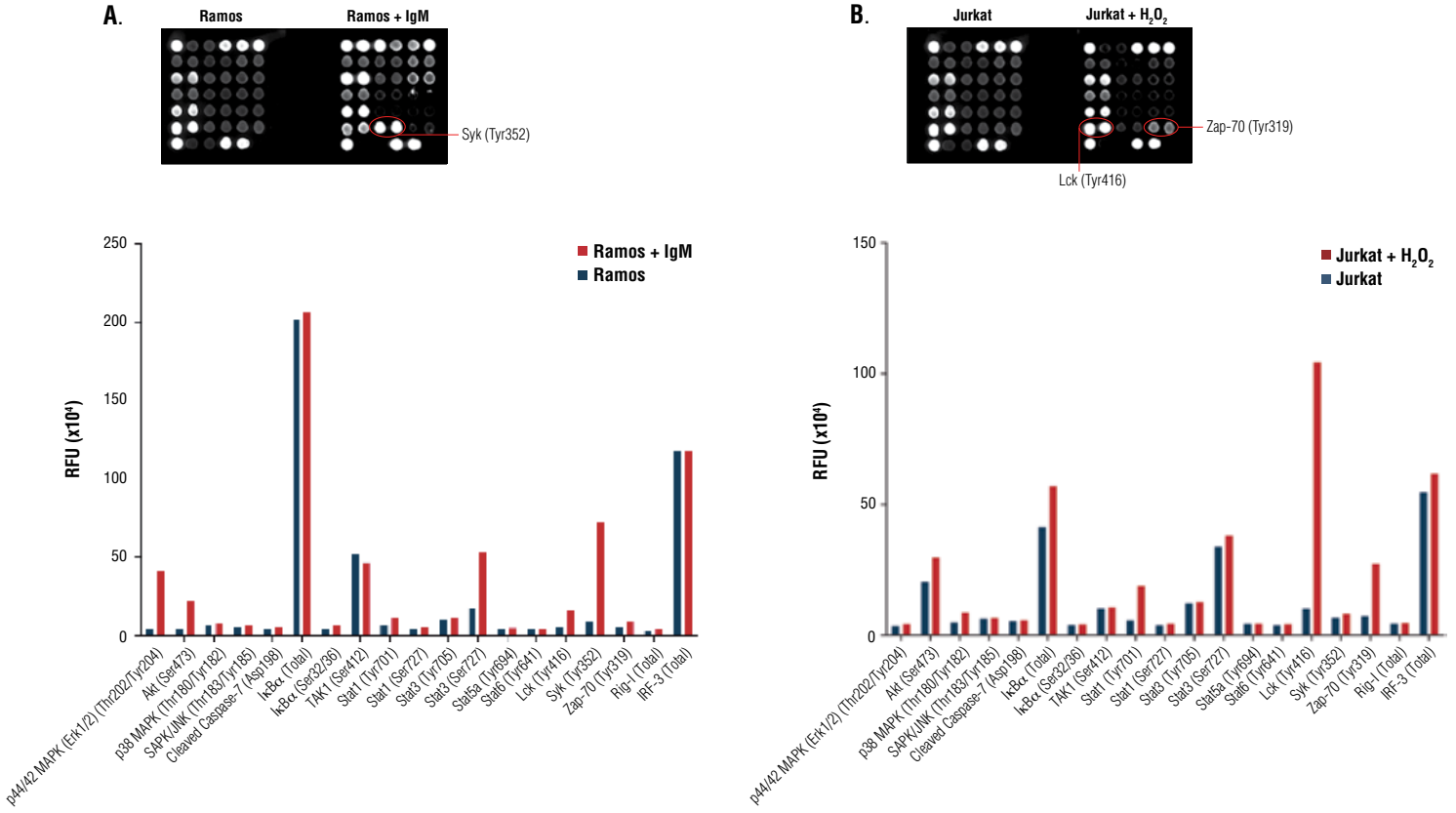
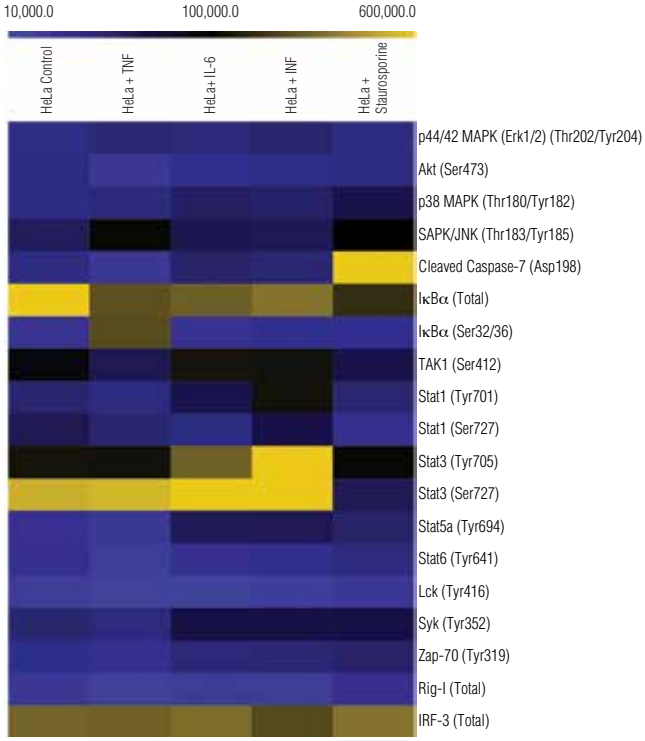


Figure 3. Ramos cells were serum starved (3 hr) then stimulated with anti-human IgM (12 µg/ml, 5 min; Panel A). Jurkat cells were stimulated with H_2O_2 (10 mM, 3 min; Panel B). Cell lysates were analyzed using the PathScan® Immune Cell Signaling Antibody Array Kit (Fluorescent Readout) #13788. Array fluorescent images were acquired using the LI-COR® Biosciences Odyssey® imaging system (upper) and fluorescence intensities for each spot were quantified using the LI-COR® Image Studio v2.0 array analysis software and depicted as bar graphs (lower).



◀ *Figure 4. HeLa cells were untreated or treated with hTNF-α #8902 (100 ng/ml, 5 min), hIL-6 #8904 (100 ng/ml, 15 min), hIFN-α1 #8927 (100 ng/ml, 15 min), or Staurosporine #9953 (1 µM, 3.5 hr). Following treatments, cell lysates were analyzed using the PathScan® Immune Cell Signaling Antibody Array Kit (Fluorescent Readout) #13788. Array fluorescent images were acquired using the LI-COR® Biosciences Odyssey® imaging system and fluorescence intensities for each spot were quantified using the LI-COR® Image Studio v2.0 array analysis software. Duplicate spot intensities were averaged and analyzed using MultiExperiment Viewer (MeV) analysis software. The heatmap depicted was generated using the raw fluorescence intensity values after subtraction of background signal.*

#13788

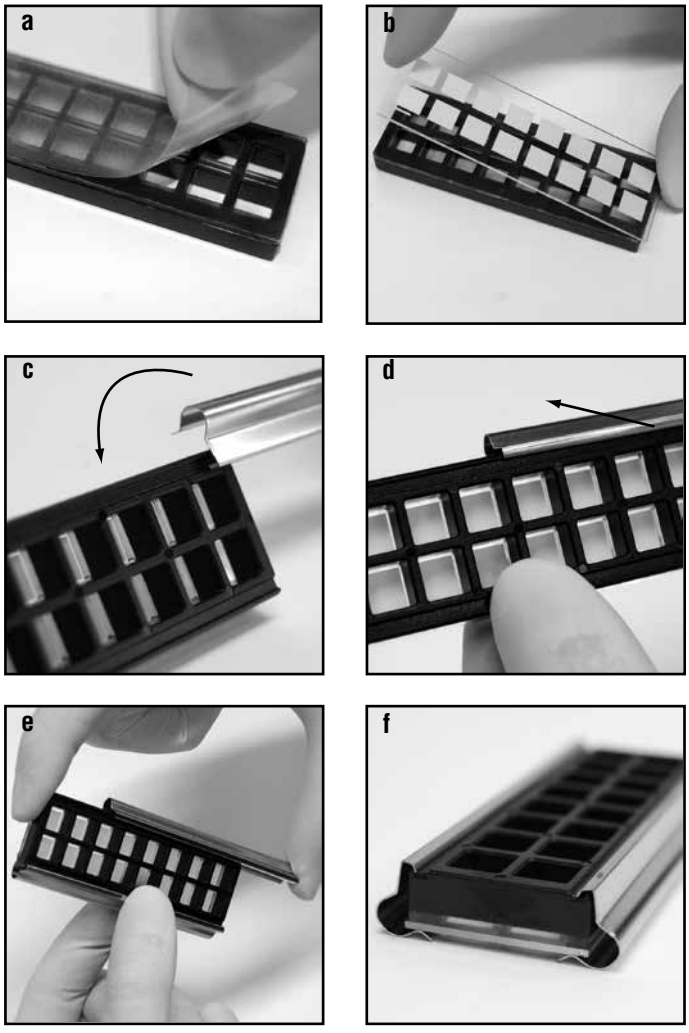
PathScan® Immune Cell Signaling Antibody Array Kit (Fluorescent readout)

A Preparing Cell Lysates

1. Thaw 1X Cell Lysis Buffer #7018 and mix thoroughly. Supplement 1X Cell Lysis Buffer with a Protease Inhibitor Cocktail (100X) #5871. Keep lysis buffer on ice.
2. Remove media and wash cells once with ice-cold 1X PBS.
3. Remove PBS and add ice-cold 1X Cell Lysis Buffer. For adherent cells, use 0.5 ml 1X Cell Lysis Buffer #7018 for each plate (10 cm in diameter). Incubate on ice for 2 minutes.
4. Transfer lysates to a microcentrifuge tube and microcentrifuge at maximum speed for 2 minutes at 4°C.
5. Transfer the supernatant to a new tube. The supernatant is the cell lysate and may be used immediately or stored at –80°C in single-use aliquots.
6. Dilute lysates to 0.2 – 1.0 mg/ml in Array Diluent Buffer immediately before performing the assay. Set aside on ice.

B Assay Procedure

1. Bring glass slides and blocking buffer to room temperature before use.
2. Prepare 1X Array Wash Buffer by diluting 20X Array Wash Buffer in deionized water.
   Dilute 1 ml of 20X Array Wash Buffer with 19 ml of deionized water. Label as 1X Array Wash Buffer. Keep at room temperature.
3. Prepare 1X Detection Antibody Cocktail as follows:
   For running only 1 slide: Dilute 150 µl of 10X Detection Antibody Cocktail with 1350 µl of Array Diluent Buffer.
   For running 2 slides: Dilute 300 µl of 10X Detection Antibody Cocktail with 2700 µl of Array Diluent Buffer.
4. Prepare 1X DyLight™ 680-linked Streptavidin as follows:
   For running only 1 slide: Dilute 150 µl of 10X DyLight™ 680-linked Streptavidin with 1350 µl of Array Diluent Buffer.
   For running 2 slides: Dilute 300 µl 10X DyLight™ 680-linked Streptavidin with 2700 µl of Array Diluent Buffer.
   *Keep on ice and protect from light.
5. Affix the multi-well gasket to the glass slide (see figure at right):
   a. Place the multi-well gasket facedown on the bench top (the silicone layer should be facing up). Remove the protective plastic film.
   b. Carefully place the glass slide on top of the multi-well gasket with the nitrocellulose pads facing down while aligning the pads with the openings in the gasket. The orientation line should appear in the upper left hand corner when the slide is oriented vertically.
   c. Insert the metal clip into the groove in the gasket and rotate the clip into the locked position. Ensure that the clip is on the same side as the orientation line on the slide.
   Note: one of the clips has a small dot etched onto the upper rib to assist with pad designation (see slide assembly photos).
   d. Slide the clip into place.
   e. Snap the unmarked metal clip to the other side of the assembly in the same manner and slide into place.
   f. The assembled array is ready to use.
6. Add 100 µl Array Blocking Buffer to each well and cover with sealing tape. Incubate for 15 minutes at room temperature on an orbital shaker.
   Note: Do not allow the pads to dry out until after step 14.
7. Decant Array Blocking Buffer by gently flicking out the liquid into a sink or other appropriate waste receptacle. Add 75 µl of diluted lysate to each well and cover with sealing tape. Incubate for 2 hours at room temperature (or overnight at 4°C) on an orbital shaker.
8. Decant well contents by gently flicking out the liquid into a sink or other appropriate waste receptacle. Add 100 µl 1X Array Wash Buffer to each well and incubate for 5 minutes at room temperature on an orbital shaker. Repeat three more times. Decant well contents.
9. Add 75 µl 1X Detection Antibody Cocktail to each well and cover with sealing tape. Incubate for 1 hour at room temperature on an orbital shaker.
10. Wash 4 X 5 minutes with 100 µl 1X Array Wash Buffer as in step 8.
    Note: From this point on, keep slide protected from light.
11. Add 75 µl 1X DyLight™ 680-linked Streptavidin to each well and cover with sealing tape. Incubate for 30 minutes at room temperature on an orbital shaker.
12. Wash 4 X 5 minutes with 100 µl 1X Array Wash Buffer as in step 8.
13. Remove multi-well gasket by pulling the bottom of the metal clips away from the center of the slide, then peeling the slide and gasket apart.
14. Place the slide face up in a plastic dish (a clean pipette tip box cover works well). Wash once for 10 seconds with 10 ml deionized water.
15. Remove slide from plastic dish and allow to completely dry.
16. Capture an image of the slide using a fluorescent digital imaging system capable of exciting at 680 nm and detecting at 700 nm. Quantify spot intensities using commercially available array image analysis software.



MSDS

Material Safety Data Sheet (MSDS) for PathScan® Antibody Array Kit

Product name: PathScan® Antibody Array Kit
Product Catalog: 13788 Kits
Manufacturer Supplier: Cell Signaling Technology
3 Trask Lane
Danvers, MA 01923 USA
978-867-2300 TEL
978-867-2400 FAX
978-578-6737 EMERGENCY TEL

II. Composition/Information:

Substance Name: PathScan® Antibody Array Kit
CAS#: None
This product is For Research Use Only. According to 29 CFR 1910.1200(d), mixtures with hazardous ingredients at less than <1% and carcinogens at less than < 0.1% are considered non-hazardous. Please refer to the individual material safety data sheets for hazard information specific to kit components.

- Array Slides MSDS
- PathScan® Sandwich ELISA Lysis Buffer (1X) (CST#7018) MSDS
- Array Blocking Buffer MSDS
- Array Diluent Buffer MSDS
- Array Wash Buffer MSDS
- Detection Antibody Cocktail MSDS
- HRP-linked Streptavidin MSDS (Kit 13792 only)
- DyLight 680®-linked Steptavidin MSDS (Kit 13788 only)
- 20X LumiGLO & 20X Peroxide (CST#7003) MSDS

III. Hazard Identification:

Emergency Overview:
Not considered hazardous.
Not expected to produce significant adverse health effects when the recommended instructions for use are followed. No known significant effects or critical hazards.

IV. First Aid Measures:

Inhalation: Remove to fresh air. If breathing is difficult, get medical attention.
Ingestion: If person is conscious, wash out mouth with water. Get medical attention.
Skin exposure: Wash skin with soap and water. If irritation develops or persists, get medical attention.
Eye exposure: Immediately flush eyes water for at least 15 minutes. Get medical attention.

V. Fire Fighting Measures:

Flash Point: Not applicable.
Autoignition Temperature: Not applicable.
Explosion: Not applicable.
Fire extinguishing media: Water spray, dry chemical, alcohol foam, or carbon dioxide.
Firefighting: Wear protective clothing and self-contained breathing apparatus to prevent contact with skin and eyes.
Specific Hazard: None.

VI. Accidental Release Measures:

Wear appropriate personal protective equipment as indicated in Section VIII. Absorb liquid with an absorbent material. Transfer contaminated absorbent to a closed chemical waste container for disposal. Wash spill site after material has been picked up for disposal.

VII. Handling And Storage:

Storage: Store kit in tightly closed container at 4°C.

VIII - XIII.

Refer to individual MSDS for kit components for Sections 8-13 information: Exposure Controls/Personal Protection, Physical and Chemical Properties, Stability and Reactivity, Toxicological Information, Ecological information, Disposal Considerations.

XIV. Transport Information:

DOT: Proper Shipping Name: None.
This substance is considered Non-Hazardous for transport.

IATA: Proper Shipping Name: None.
This substance is considered Non-Hazardous for air transport.

XV. Regulatory Information:

EU Regulations/Classifications: Xi. Irritant.
Risk Phrases: Irritant. Irritating to eyes and skin. Harmful if swallowed.
Safety Phrases: In case of contact wash with water and seek medical attention.
US Regulatory Information: Irritant.

XVI. Other Information:

This product is not intended for use in humans. To the best of our knowledge, this document is accurate. It is intended to serve as a guide for safe use of this product in a laboratory setting by experienced personnel. The burden of safe use of this material rests entirely with the user. The above information is believed to be accurate but is not necessarily all-inclusive and shall be used only as a guide. Cell Signaling Technology, Inc., shall not be held liable for any damage resulting from the handling of or from contact with the above product.