Store at 4°C

#12923

PathScan® Stress and Apoptosis Signaling Antibody Array Kit (Fluorescent Readout)

Cell Signaling TECHNOLOGY®

☑ 1 Kit
(32 multiplexed assays)

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
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rev. 01/22/15

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

Species Cross-Reactivity: H

Description: The PathScan® Stress and Apoptosis 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 stress response and apoptosis. 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 lysate is incubated on the slide followed by a biotinylated detection antibody cocktail. Streptavidin-conjugated DyLight® 680 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® Stress and Apoptosis 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).

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).

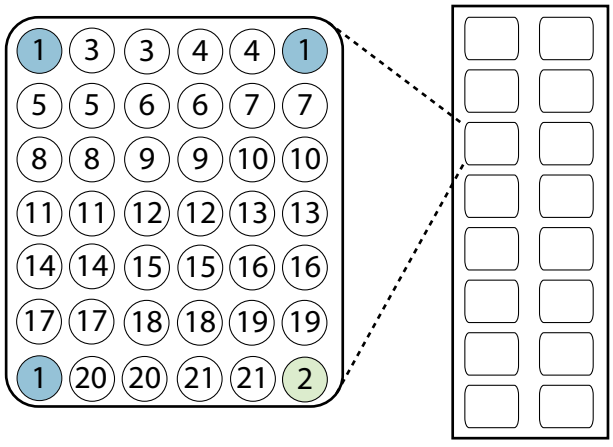

Figure 1. Target map of the PathScan® Stress and Apoptosis Signaling Antibody Array Kit (Fluorescent Readout) #12923.

Stress and Apoptosis 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	Bad	Ser136	Phosphorylation
6	HSP27	Ser82	Phosphorylation
7	Smad2	Ser465/467	Phosphorylation
8	p53	Ser15	Phosphorylation
9	p38 MAPK	Thr180/Tyr182	Phosphorylation
10	SAPK/JNK	Thr183/Tyr185	Phosphorylation
11	PARP	Asp214	Cleavage
12	Caspase-3	Asp175	Cleavage
13	Caspase-7	Asp198	Cleavage
14	IκBα	Total	N/A
15	Chk1	Ser345	Phosphorylation
16	Chk2	Thr68	Phosphorylation
17	IκBα	Ser32/36	Phosphorylation
18	eIF2α	Ser51	Phosphorylation
19	TAK1	Ser412	Phosphorylation
20	Survivin	Total	N/A
21	α-Tubulin	Total	N/A

Background: Cell death can occur due to a variety of circumstances including nutrient deprivation, inability to generate or store the energy required for metabolic functions, or deleterious environment that causes irreparable damage. Cells integrate multiple signals from a variety of sources before following either pro- or anti-apoptotic pathways. These signals can often carry conflicting information. Assessing the net effect of these processes in cell populations can be achieved by monitoring changes in a number of key signaling components. The caspase-3 and caspase-7 proteases exert a pro-apoptotic function through cleavage of multiple cellular targets. Caspase-3 and caspase-7 are activated by cleavage at Asp175 and Asp198, respectively. PARP is a DNA repair and apoptosis enzyme that is inactivated by cleavage at Asp214 by caspase-3 or caspase-7. HSP27 is a mediator of cell stress that confers resistance to adverse environmental conditions. HSP27 is activated by phosphorylation at Ser82. Chk1 and Chk2 kinases act downstream of ATM/ATR and play an important role in DNA damage checkpoint control. Activation of Chk1 and Chk2 involve phosphorylation at Ser345 and Thr68, respectively. Tumor suppressor p53 plays an important role in cellular response to DNA damage. p53 is phosphorylated at Ser15 by ATM/ATR or DNA-PK leading to its accumulation. Smad2 is a key mediator of TGF-β signaling. Stimulation by TGF-β leads to Smad2 phosphorylation at Ser465/467 and translocation of Smad2 into the nucleus. The outcome of TGF-β signaling is context dependent and can either induce apoptosis or contribute to tumor cell metastasis. Activation of NF-κB/Rel occurs through a proteasome-mediated degradation of IκBα. The inhibitor IκBα is targeted to the proteasome via phosphorylation of IκBα at Ser32 and Ser36. NF-κB activation is triggered by a diverse group of extracellular signals promoted by inflammatory cytokines, growth factors, and chemokines. TAK1 is a kinase that can be activated by TGF-β, bone morphogenetic proteins and other cytokines. Activated TAK1 phosphorylates MKK4, MKK3/6, and NIK. Phosphorylation of TAK1 at Ser412 is one of the mechanisms that regulate the levels of its activation. Cellular stress such as viral infection, endoplasmic reticulum stress, and amino acid deprivation leads to phosphorylation of eIF2α. Phosphorylation of eIF2α at Ser51 in response to cellular stress leads to a reduction of protein synthesis. The ERK1 and ERK2 MAP kinases are major signaling nodes that have many substrates and primarily transmit growth and proliferation signals. The ERK MAP kinase is activated by a dual phosphorylation of Thr202 and Tyr204. p38 MAPK and SAPK/JNK MAP kinases are activated through a similar dual phosphorylation mechanism in response to pro-inflammatory cytokines and genotoxic stress. Akt is activated by stimulation of growth-factor receptors and primarily promotes anabolic growth and survival signals via targeting its broad array of substrates. Akt phosphorylates Bad at Ser136 and inhibits its ability to induce apoptosis. Survivin is an anti-apoptotic protein that is highly expressed during fetal development and cancer cell malignancy. Survivin binds and inhibits caspase-3, controlling the cell cycle by inhibiting apoptosis and promoting cell division. α-tubulin is a building block of microtubules that are present in all eukaryotic cells. The levels of the globular α-tubulin are considered to remain relatively constant. Therefore, assessing the relative levels of α-tubulin may assist with signal normalization between the various samples.

Selected References:

(1) Boatright, K.M. and Salvesen, G.S. (2003) *Curr Opin Cell Biol* 15, 725-31.

(2) Cohen, G.M. (1997) *Biochem J* 326 (Pt 1), 1-16.

(3) Bratton, S.B. and Cohen, G.M. (2001) *Trends Pharmacol Sci* 22, 306-15.

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(5) Basu, S. and Kolesnick, R. (1998) *Oncogene* 17, 3277-85.

(6) Jeremias, I. and Debatin, K.M. (1998) *Eur Cytokine Netw* 9, 687-8.

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(8) Bates, S. and Vousden, K.H. (1999) *Cell Mol Life Sci* 55, 28-37.

(9) Heyninck, K. and Beyaert, R. (2001) *Mol Cell Biol Res Commun* 4, 259-65.

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(11) Rath, P.C. and Aggarwal, B.B. (1999) *J Clin Immunol* 19, 350-64.

(12) Holoch, P.A. and Griffith, T.S. (2009) *Eur J Pharmacol* 625, 63-72.

(13) Gjertsen, B.T. and Døskeland, S.O. (1995) *Biochim Biophys Acta* 1269, 187-99.

(14) Clemens, M.J. (2001) *Prog Mol Subcell Biol* 27, 57-89.

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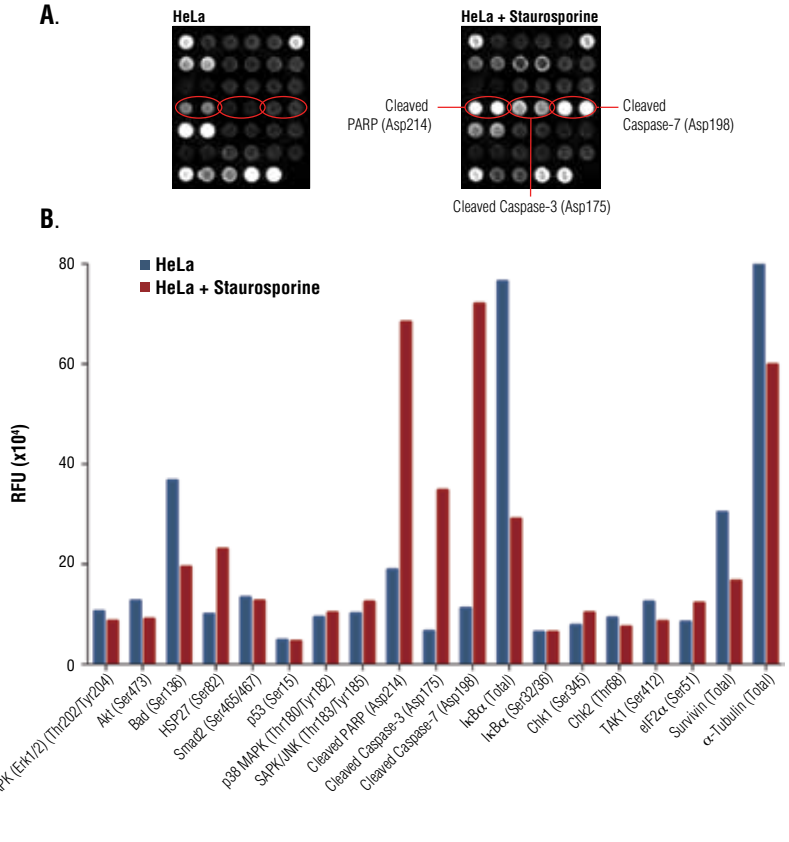


Figure 2. HeLa cells were grown to 90% confluency and then either untreated (A, left panel) or treated with Staurosporine #9953 (1 µM, 3.5 hr; A, right panel). Cell extracts were prepared and analyzed using the PathScan® Stress and Apoptosis Signaling Antibody Array Kit (Fluorescent Readout) #12923. Panel A shows images that were acquired using the LI-COR® Biosciences Odyssey® imaging system. Panel B shows quantified fluorescence intensity (RFU) for each target in the presence or absence of staurosporine. Relative fluorescent intensities were quantified as pixel intensities using the LI-COR® Image Studio v2.0 array analysis software.

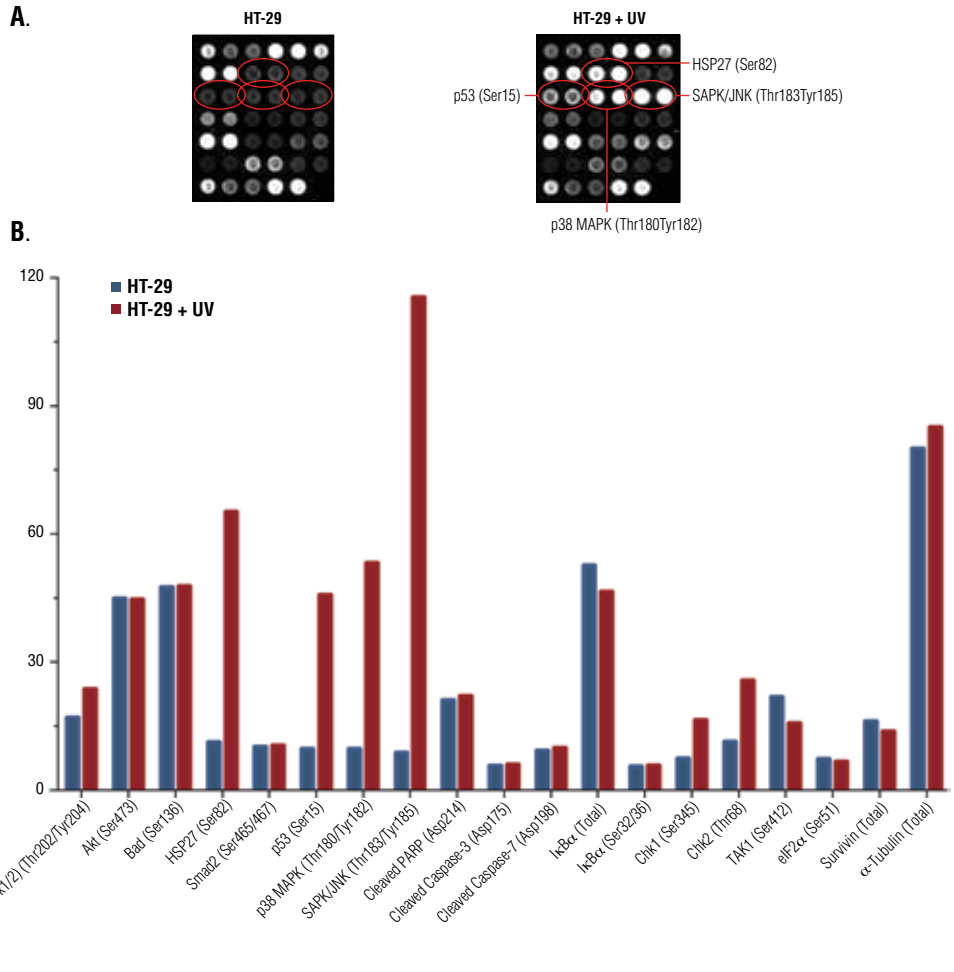


Figure 3. HT-29 cells were grown to 80% confluency and then either untreated (A, left panel) or UV-irradiated and allowed to recover for 60 minutes (A, right panel). Cell extracts were prepared and analyzed using the PathScan® Stress and Apoptosis Signaling Antibody Array Kit (Fluorescent Readout) #12923. Panel B shows quantified fluorescence intensity (RFU) for each target in the presence or absence of UV-irradiation. Relative fluorescent intensities were quantified as pixel intensities using the LI-COR® Image Studio v2.0 array analysis software.

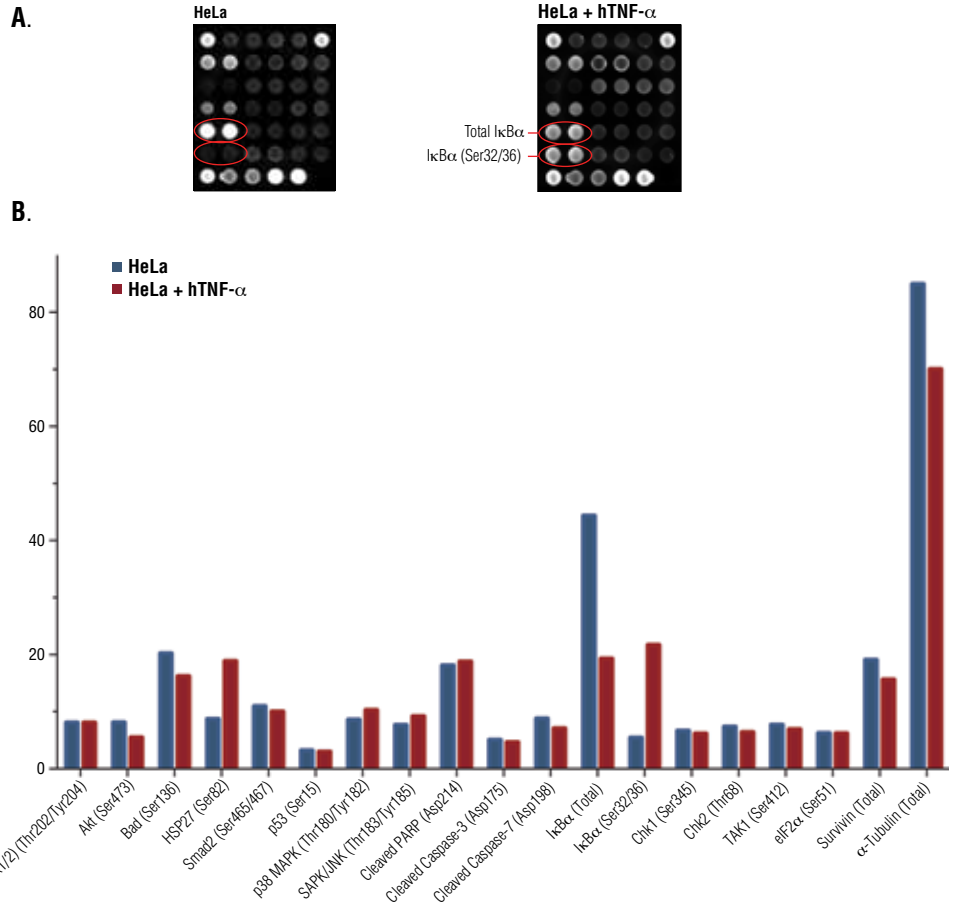
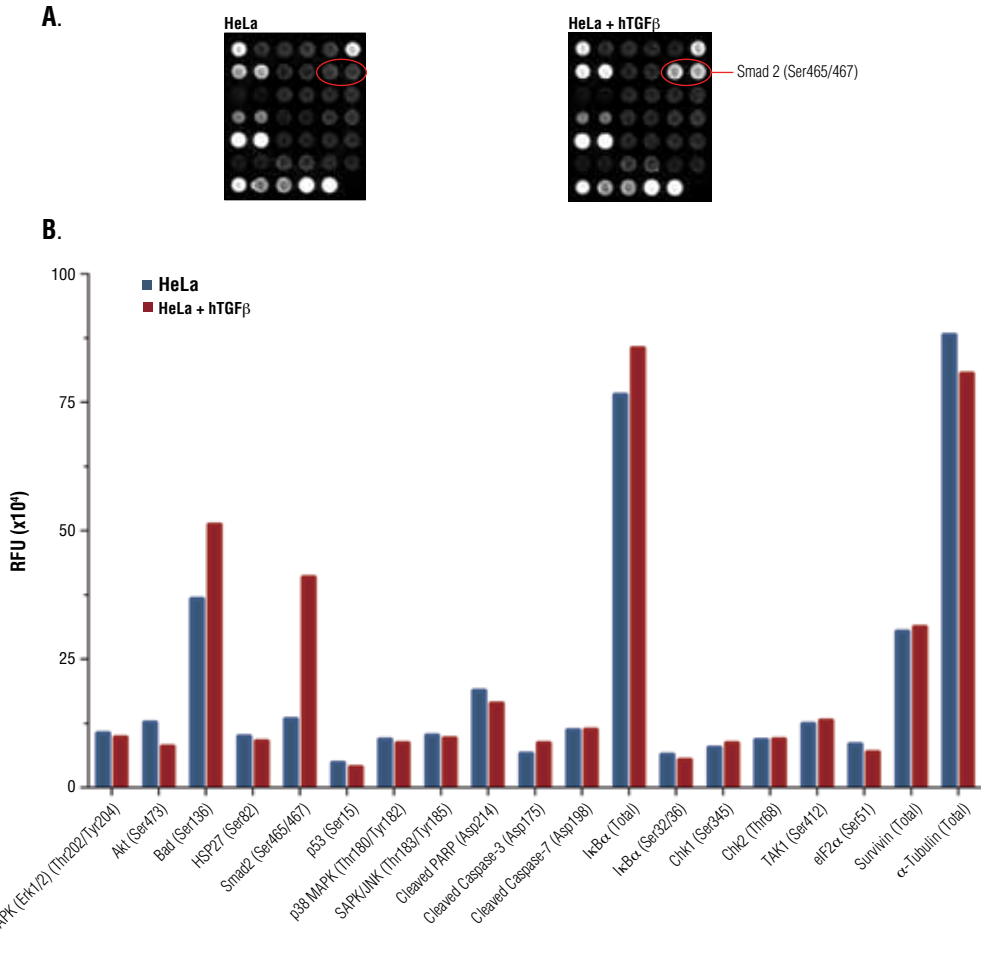
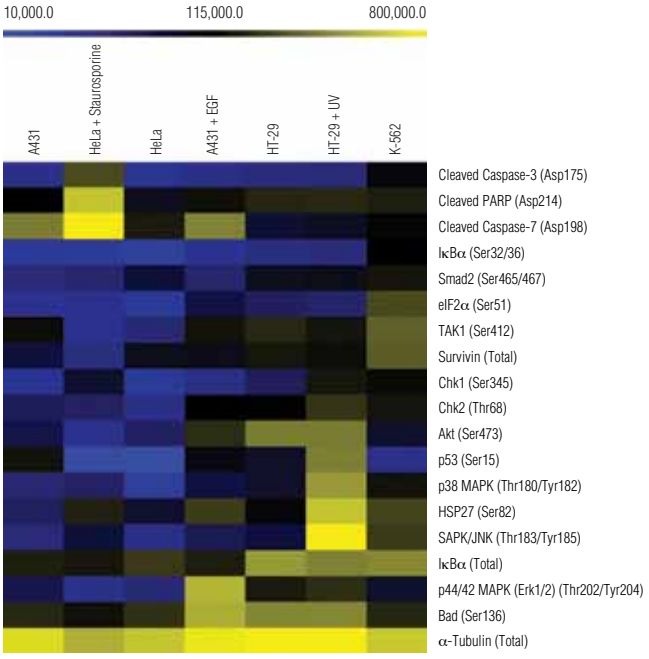


Figure 4. HeLa cells were grown to 90% confluency and then either untreated (A, left panel) or treated with Human Tumor Necrosis Factor-α (hTNF-α) #8902 (100 ng/ml, 20 min; A, right panel). Cell extracts were prepared and analyzed using the PathScan® Stress and Apoptosis Signaling Antibody Array Kit (Fluorescent Readout) #12923. Panel A shows images that were acquired using the LI-COR® Biosciences Odyssey® imaging system. Panel B shows quantified fluorescence intensity (RFU) for each target in the presence or absence of hTNF-α. Relative fluorescent intensities were quantified as pixel intensities using the LI-COR® Image Studio v2.0 array analysis software.



◀ *Figure 5. HeLa cells were grown to 90% confluency and serum starved overnight. Cells were then either untreated (A, left panel) or treated with Human Transforming Growth Factor β3 (hTGF-β3) #8425 (100 ng/ml, 20 min; A, right panel). Cell extracts were prepared and analyzed using the PathScan® Stress and Apoptosis Signaling Antibody Array Kit (Fluorescent Readout) #12923. Panel A shows images that were acquired using the LI-COR® Biosciences Odyssey® imaging system. Panel B shows quantified fluorescence intensity (RFU) for each target in the presence or absence of hTGF-β3. Relative fluorescent intensities were quantified as pixel intensities using the LI-COR® Image Studio v2.0 array analysis software.*



◀ *Figure 6. Various cell lysates indicated in the figure were analyzed using the PathScan® Stress and Apoptosis Signaling Antibody Array Kit (Fluorescent Readout) #12923. Images were acquired using the LI-COR® Biosciences Odyssey® imaging system. Fluorescence intensity was quantified using the LI-COR® Image Studio v2.0 array analysis software. Heatmap analysis was generated using MultiExperiment Viewer (MeV) analysis software using the raw fluorescence intensity values.*

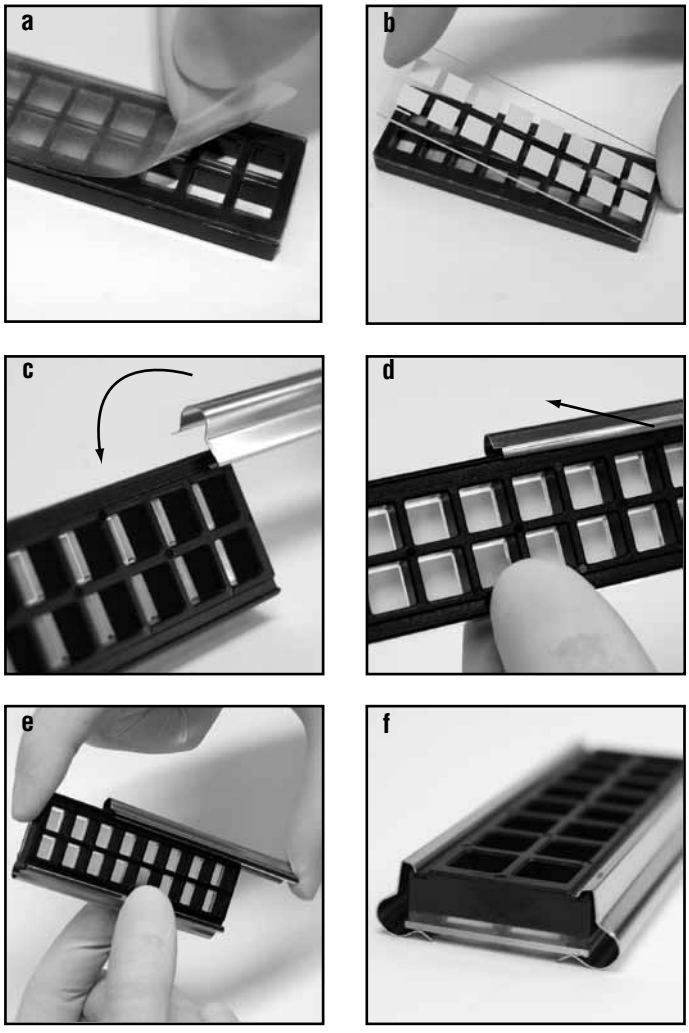
PathScan® Stress and Apoptosis Signaling Antibody Array Kit (Fluorescent Readout) Protocol

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

Cell Signaling TECHNOLOGY®

Product name: PathScan® Antibody Array Kit
Product Catalog: 12923 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 7323 only)
- DyLight 680®-linked Steptavidin MSDS (Kit 7744 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.