Store at 4°C

#12785

PathScan® EGFR Signaling Antibody Array Kit (Fluorescent Readout)

Cell Signaling TECHNOLOGY®

☑ 1 Kit
(32 multiplexed assays)

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

rev. 01/22/15

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

Species Cross-Reactivity: H

Description: The PathScan® EGFR 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 phosphorylated EGFR, HER2, c-Met on distinct sites as well as a number of key signaling nodes found downstream of these RTKs. Target-specific capture antibodies have been spotted in duplicate onto nitrocellulose-coated glass slides. Each kit contains two slides allowing for the interrogation of 16 different samples. To improve assay performance the content of this array is split between two sub-arrays. The pads on left-hand side of each slide belong to sub-array A while the pads on the right-hand side of each slide belong to sub-array B. Cell lysates are incubated on the slide followed by a biotinylated detection antibody cocktail A or cocktail B (each cocktail for the corresponding sub-array). 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® EGFR 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).

† Note: Detection in the EGFR (E746-A750) deletion mutant specific assay may be sensitive to EGFR inhibitors targeting the active site. (See Figure 5).

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 A	150 µl	White
10X Detection Antibody Cocktail B	150 µl	Green
10X DyLight™ 680-linked Streptavidin	300 µl	Brown
*Cell Lysis Buffer #9803	15 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).

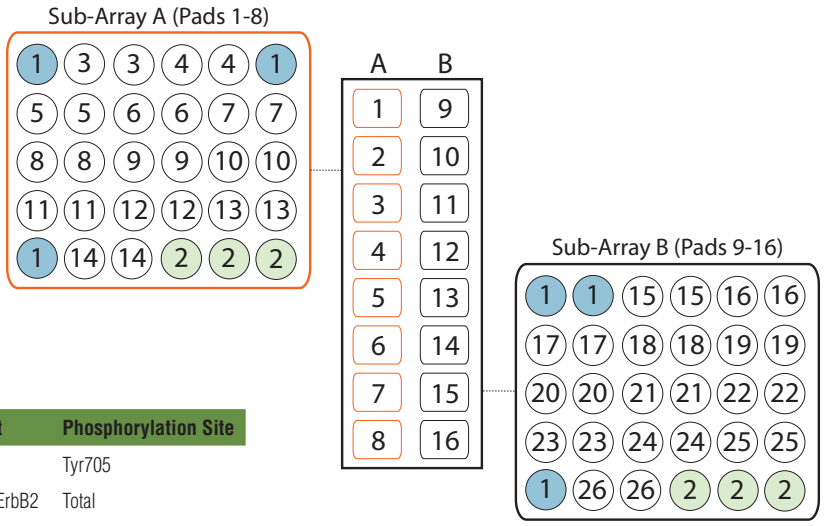

Figure 1. Target map of the PathScan® EGFR Signaling Antibody Array Kit (Fluorescent Readout) #12785.

EGFR Signaling

	Target	Phosphorylation Site		Target	Phosphorylation Site
1	Positive Control	N/A	14	Stat3	Tyr705
2	Negative Control	N/A	15	HER2/ErbB2	Total
3	EGFR	Total	16	HER2/ErbB2	Tyr1196
4	EGFR	Thr669	17	HER2/ErbB2	Tyr1221/1222
5	EGFR	Tyr845	18	Met	Total
6	EGFR	Tyr998	19	Met	Tyr1349
7	EGFR	Tyr1068	20	Met	Tyr1234/1235
8	EGFR - L858R Mutant	Total	21	PLCγ1	Total
9	EGFR - E746-A750 Deletion	Total †	22	PLCγ1	Ser1248
10	Mek1	Total	23	Akt	Thr308
11	Mek2	Total	24	Akt	Ser473
12	Mek1/2	Ser221	25	Erk1/2	Thr202/Tyr204
13	Mek1/2	Ser217/221	26	PARP	Asp214 (Cleaved)

U.S. Patent No. 5,675,063

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **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 **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.

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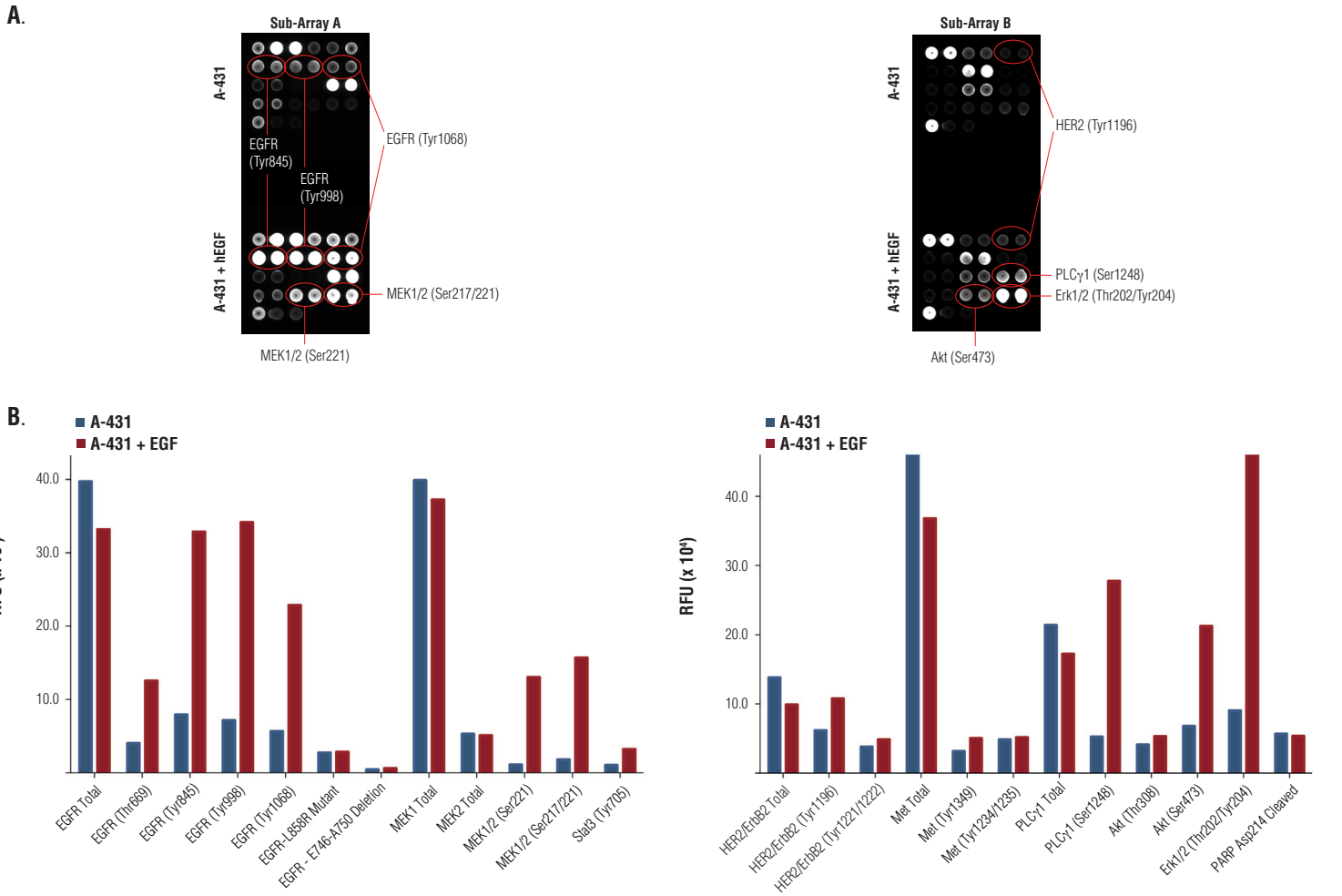

Figure 2. A-431 cells were grown to 90% confluency and then serum-starved overnight. Cells were stimulated with Human Epidermal Growth Factor (hEGF) #8916 (100 ng/ml, 5 min). Cell extracts were prepared and analyzed using the PathScan® EGFR Signaling Antibody Array Kit (Fluorescent Readout) #12785. Panel A shows images that were acquired using the LI-COR® Biosciences Odyssey® imaging system. Panel B shows raw values of quantified fluorescence intensity. Pixel intensity was quantified using the LI-COR® Image Studio v2.0 array analysis software.

Background: The Epidermal Growth Factor Receptor (EGFR) is a receptor tyrosine kinase (RTK) that constitutes an important disease driver, as well as a validated drug target. The potency of EGFR in driving tumorigenesis can be attributed to its pleiotropic intracellular signaling. Activated EGFR initiates a wide range of signaling modules and switches such as the Ras - Erk/MAP kinase, Akt, Src, Stat, and PKC. Two of the most common EGFR mutations occurring in lung cancer are the E746-A750 deletion and L858R point mutation. This array utilizes unique antibodies made by Cell Signaling Technology that are sensitive to each of these EGFR mutants, allowing specific target detection in cell extracts.

EGFR can interact and heterodimerize with other RTKs. HER2 (also known as ErbB2) is an oncogenic RTK belonging to the EGFR/HER family of RTKs and is an important heterodimerization partner of all HER family members. Another prominent heterodimerization partner of EGFR is c-Met. c-Met is an RTK serving as a receptor for the hepatocyte growth factor (HGF). c-Met can induce cell scattering, migration, and invasion. It has been shown that c-Met is responsible for some cases of tumor resistance to EGFR-targeted therapies and is a contributing factor to tumor metastasis.

PLCγ is a phosphoinositide-specific phospholipase. EGFR can activate PLCγ that, in turn, hydrolyzes phosphoinositide phospholipids residing within the inner leaflet of the plasma membrane. This hydrolysis generates two important second messengers: inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG). IP3 causes calcium mobilization from intracellular storage pools, while DAG (together with calcium) activates PKC. MEK1 is a dual-specificity protein kinase and serves as the MAP kinase kinase for Erk1 and Erk2. Upon EGFR activation, MEK1 is phosphorylated by Raf and, in turn, phosphorylates the Erk kinases at Thr202 and Tyr204, leading to their activation. Activated Erk MAP kinase is a major signaling node with a multitude of substrates and primarily transmits growth and proliferation signals. Akt is another important protein kinase downstream of EGFR. Akt is activated by many RTKs and has a large number of intracellular substrates. Akt generates anabolic growth and survival signals. Stat3 is activated in response to EGFR stimulation, as well as in response to activation of a variety of cytokine receptors. Stat3 is a well-established oncogene that is also a transcription factor.

The oncogenic signals generated by activated EGFR are a focus of intense drug discovery efforts. It has become clear that in many cases a single agent inhibiting only one target is unable to cause tumor cell death *in vivo*. To monitor the blockade of EGFR signals alongside markers of cell death, cleaved PARP is included in this array. PARP is an enzyme involved in DNA repair. As a part of the apoptotic process, PARP is irreversibly inactivated by endoproteolytic cleavage executed by activated cell death proteases, such as caspase-3 and caspasae-7.

Background References:

(1) Yarden, Y. (2001) *Eur J Cancer* 37 Suppl 4, S3-8.

(2) Zwick, E. et al. (1999) *Trends Pharmacol Sci* 20, 408-12.

(3) Hackel, P.O. et al. (1999) *Curr Opin Cell Biol* 11, 184-9.

(4) Avraham, R. and Yarden, Y. (2011) *Nat Rev Mol Cell Biol* 12, 104-17.

(5) Levitzki, A. (2003) *Lung Cancer* 41 Suppl 1, S9-14.

(6) Sharma, S.V. and Settleman, J. (2009) *Exp Cell Res* 315, 557-71.

(7) Knowles, L.M. et al. (2009) *Clin Cancer Res* 15, 3740-50.

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(14) Saeed, A.I. et al. (2003) *Biotechniques* 34, 374-8.

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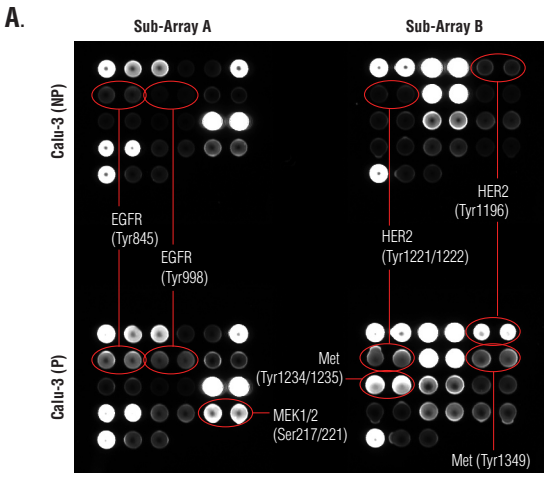


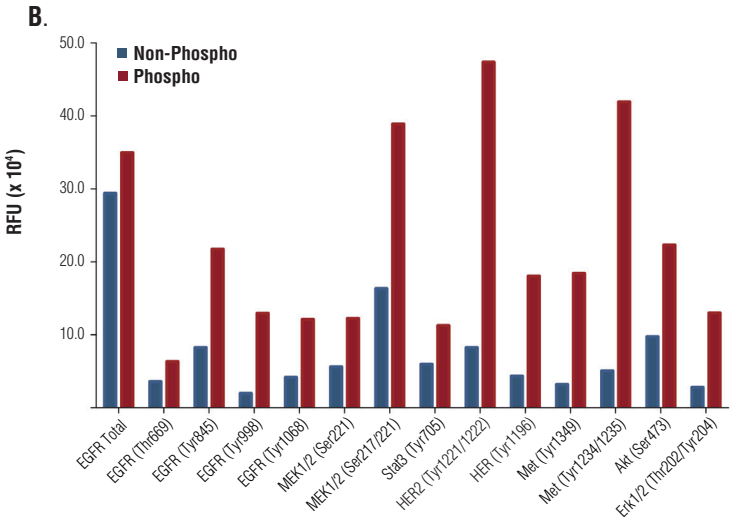
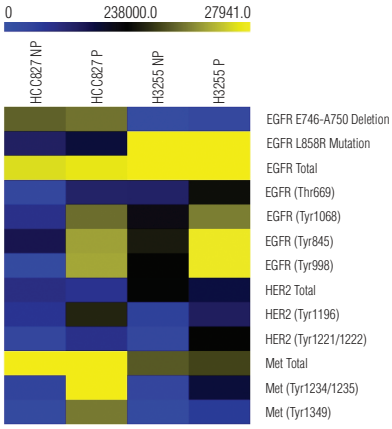
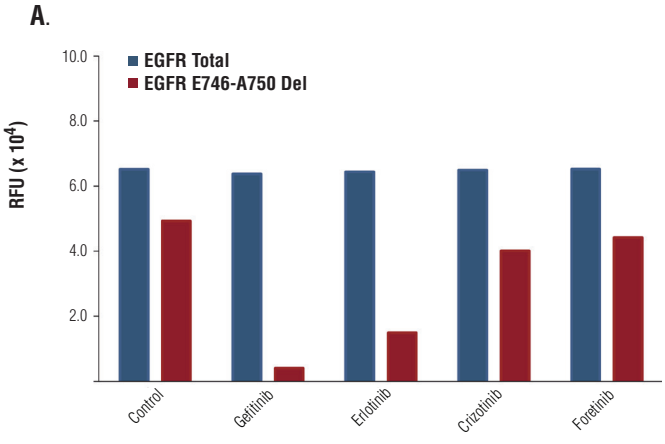
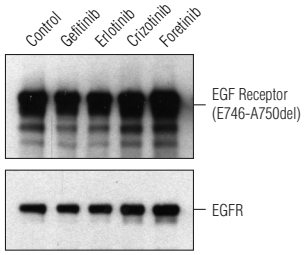


Figure 3. Calu-3 cells were grown to 90% confluency and lysed using a buffer containing (P) or devoid of (NP) phosphatase inhibitors. Cell extracts were prepared and analyzed using the PathScan® EGFR Signaling Antibody Array Kit (Fluorescent Readout) #12785. Panel A shows images that were acquired using the LI-COR® Biosciences Odyssey® imaging system. Panel B shows raw values of quantified fluorescence intensity for a selected set of targets. Pixel intensity was quantified using the LI-COR® Image Studio v2.0 array analysis software.



◀ *Figure 4. HCC827 and H3255 are two non-small cell lung cancer (NSCLC) cell lines carrying two different Gefitinib-sensitive mutants of EGFR: E746-A750 deletion in exon 19 and L858R point mutation, respectively. Cells were grown to 90% confluency and then lysed using a buffer containing (P) or devoid of (NP) phosphatase inhibitors. Cell extracts were prepared and analyzed using the PathScan® EGFR Signaling Antibody Array Kit (Fluorescent Readout) #12785. 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 (14) using the raw fluorescence intensity values.*





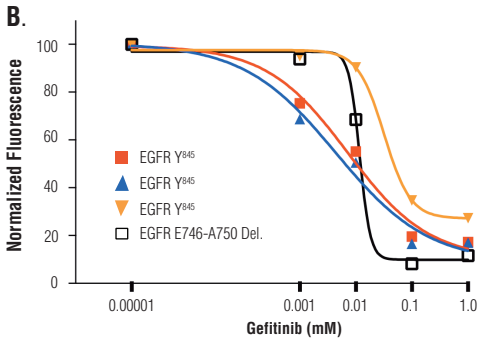


Figure 5. (A) HCC827 cells were treated with the indicated RTK inhibitors for 16 hours. The total amounts of EGFR (wt and mutant) or the EGFR (E746-A750) deletion mutant were quantified using the PathScan® EGFR Signaling Antibody Array Kit (Fluorescent Readout) (bar graph). The same samples were also analyzed by western blot using EGF Receptor (E746-A750del Specific) 6B6 XP® Rabbit mAb #2085 (upper) and EGF Receptor (15F8) Rabbit mAb #4405 (lower). ***Note: The western blot shows that sandwich immunoassay detection in the EGFR (E746-A750) deletion mutant specific assay (bar graph) may be sensitive to EGFR inhibitors targeting the active site.*** *(B) HCC827 cells were treated with increasing concentrations of Gefitinib #4765 for 2.5 hours. Tyrosine phosphorylation levels of the indicated sites in EGFR or the EGFR (E746-A750) deletion mutant were quantified using the PathScan® EGFR Signaling Antibody Array Kit (Fluorescent Readout) #12785.*

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PathScan® EGFR Signaling Array Kit (Fluorescent Readout) Protocol

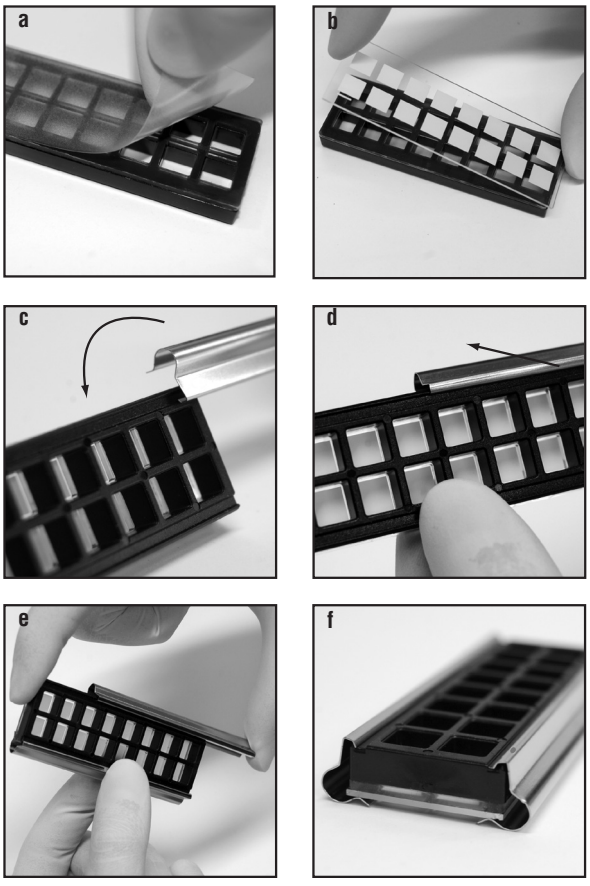
A Preparing Cell Lysates

1. Thaw 10X Cell Lysis Buffer #9803 and mix thoroughly. Prepare 1X Cell Lysis Buffer by diluting 10X Cell Lysis Buffer in deionized water. Supplement Cell Lysis Buffer with phenylmethylsulfonyl fluoride (PMSF #8553) to a final concentration of 1 mM, or a cocktail of protease inhibitors (# 5871,#5872). Keep lysis buffer on ice.
2. Remove media and wash cells once with ice-cold 1X PBS.
3. Remove PBS and add ice-cold Cell Lysis Buffer. For adherent cells, use 0.5 ml Cell Lysis Buffer for each plate (10 cm in diameter). Incubate on ice for 2 min.
4. Tilt the plate, scrape cells, and collect the lysate into a clean microcentrifuge tube.
5. Optional step: microcentrifuge the lysate at maximum speed for 3 min at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. This step is usually not required but can help remove any particles or large cell debris, if present. Lysate may be used immediately or stored at -80°C in single-use aliquots.
6. Immediately before performing the assay, dilute lysates to 0.2–1.0 mg/ml in Array Diluent Buffer. 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 2.5 ml of 20X Array Wash Buffer with 47.5 ml of deionized water. Label as 1X Array Wash Buffer and keep at room temperature.
3. Prepare both 1X Detection Antibody Cocktails A and B as follows:
   For running only **1 slide**:
   **NOTE: Cocktails A and B must be prepared in two separate tubes.
   Dilute 75 µl of 10X Detection Antibody Cocktails A and B each with 675 µl of Array Diluent Buffer into two separate appropriately labeled tubes. Keep 1X Detection Antibody Cocktails A and B on ice.
   For running **2 slides**:
   **NOTE: Cocktails A and B must be prepared in two separate tubes
   Dilute 150 µl of 10X Detection Antibody Cocktails A and B each with 1350 µl of Array Diluent Buffer into two separate appropriately labeled tubes. Keep 1X Detection Antibody Cocktails A and B on ice.
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 into an appropriately labeled tube. Keep the 1X DyLight 680®-linked Streptavidin on ice.
   For running **2 slides**:
   Dilute 300 µl of 10X DyLight 680™-linked Streptavidin with 2700 µl of Array Diluent Buffer into an appropriately labeled tube. Keep the 1X DyLight 680™-linked Streptavidin on ice.
5. Affix the multi-well gasket to the glass slide (see figure at right):
   a. Place the multi-well gasket face-down on the benchtop (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 second 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 min at room temperature on an orbital shaker.
   Note: Do not allow the pads to dry out at any time during the assay
7. Decant Array Blocking Buffer by gently flicking out the liquid into a sink or other appropriate waste receptacle. Add 50-75 µl diluted lysate making sure to add each individual sample to both sub-arrays A (left column pads) and sub-array B (right column pads). Cover array with sealing tape and incubate for 2 hr 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 min at room temperature on an orbital shaker. Repeat three more times. Decant well contents.
9. Add 75 µl 1X Detection Antibody Cocktails A, to sub-arrays A (left column pads), and B, to sub-arrays B (right column pads). Cover with sealing tape and incubate for 1 hr at room temperature on an orbital shaker.
10. Wash 4 times for 5 min with 100 µl 1X Array Wash Buffer as in step 8.
    Note: From this point on, keep slide protected from the light.
11. Add 75 µl 1X DyLight 680™-linked Streptavidin to each well and cover with sealing tape. Incubate for 30 min at room temperature on an orbital shaker.
12. Wash 4 times for 5 min 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 sec with 10 ml deionized water.
15. Remove slide from plastic dish and allow to dry completely.
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.

DyLight 680™ is a trademark of Thermo Fisher Scientific Inc. and its subsidiaries.



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MSDS

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

Cell Signaling TECHNOLOGY®

I. Identification:

Product name: PathScan® Antibody Array Kit
Product Catalog: 7744, 7323, 12785 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.

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