

PathScan® EGFR Signaling Antibody Array Kit (Chemiluminescent Readout)



✓ 1 Kit
(32 multiplexed assays)

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orders@cellsignal.com
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rev. 06/23/15

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

Species Cross-Reactivity: H

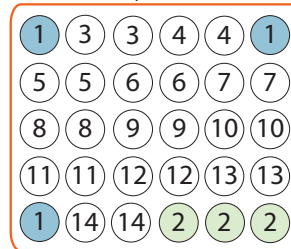
Description: The PathScan® EGFR Signaling Antibody Array Kit (Chemiluminescent Readout) uses glass slides as the planar surface and is based upon the sandwich immunoassay principle. The array kit allows for the simultaneous detection of phosphorylated EGFR, HER2, c-Met on distinct sites as well as a number of key signaling nodes found downstream of these receptor tyrosine kinases (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 HRP and LumiGLO® Reagent are then used to visualize the bound detection antibody by chemiluminescence. An image of the slide can be captured with either a digital imaging system or standard chemiluminescent film. The image can be analyzed visually or the spot intensities quantified using array analysis software.

Specificity/Sensitivity: PathScan® EGFR Signaling Antibody Array Kit (Chemiluminescent 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).

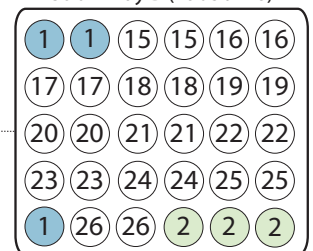
Products Included	Quantity	Cap Color
Array Slides	2 slides	
Multi-Well Gasket	2 gaskets	
Sealing Tape	2 sheets	
Chemiluminescent Development Folder	2 folders	
20X Array Wash Buffer	15 ml	White
Array Blocking Buffer	5 ml	Red
Array Diluent Buffer	15 ml	Blue
10X Detection Antibody Cocktail A	150 µl	White
10X Detection Antibody Cocktail B	150 µl	Green
10X HRP-linked Streptavidin	300 µl	Clear
20X LumiGLO® Reagent A #7003	5 ml	Brown
20X Peroxide Reagent B #7003	5 ml	Clear
*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).

Sub-Array A (Pads 1-8)



Sub-Array B (Pads 9-16)



EGFR Signaling

Target	Phosphorylation Site
1 Positive Control	N/A
2 Negative Control	N/A
3 EGFR	Total
4 EGFR	Thr669
5 EGFR	Tyr845
6 EGFR	Tyr998
7 EGFR	Tyr1068
8 EGFR - L858R Mutant	Total
9 EGFR - E746-A750 Deletion	Total†
10 MEK1	Total
11 MEK2	Total
12 MEK1/2	Ser221
13 MEK1/2	Ser217/221

Target	Phosphorylation Site
14 Stat3	Tyr705
15 HER2/ErbB2	Total
16 HER2/ErbB2	Tyr1196
17 HER2/ErbB2	Tyr1221/1222
18 Met	Total
19 Met	Tyr1349
20 Met	Tyr1234/1235
21 PLCγ1	Total
22 PLCγ1	Ser1248
23 Akt	Thr308
24 Akt	Ser473
25 Erk1/2	Thr202/Tyr204
26 PARP	Asp214 (Cleavage)

Figure 1. Target map of the PathScan® EGFR Signaling Antibody Array Kit (Chemiluminescent Readout) #12622. † A reduction in a signal associated with E746-A750 deletion mutant was observed after treatment of cells with the small molecule inhibitors gefitinib and erlotinib.

LumiGLO is a registered trademark of Kirkegaard & Perry Laboratories.

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.

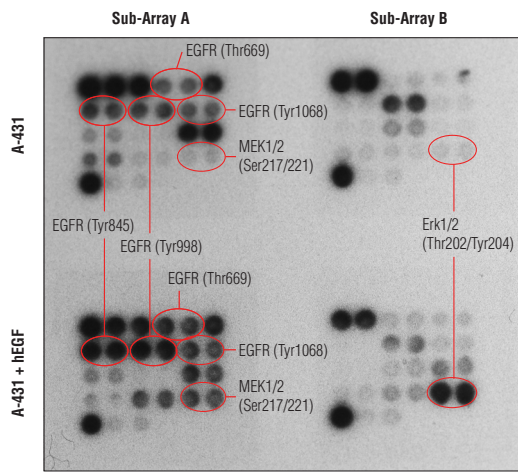


Figure 2. A-431 cells were grown to 90% confluency and then serum-starved overnight. Cells were stimulated with Human Epidermal Growth Factor (hEGF) #8916 (100 ng/ml, 5 min). Cell extracts were prepared and analyzed using the PathScan® EGFR Signaling Antibody Array Kit (Chemiluminescent Readout) #12622. Images were acquired by briefly exposing the slide to standard chemiluminescent film.

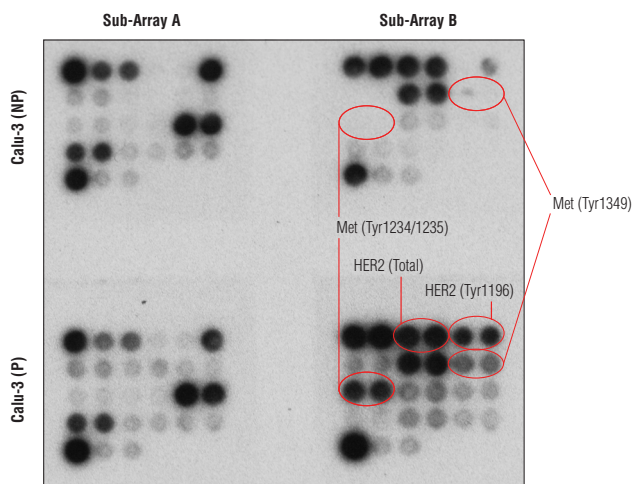


Figure 3. Calu-3 cells were grown to 90% confluency and then lysed using a buffer containing (P) or devoid of (NP) phosphatase inhibitors. Cell extracts were prepared and analyzed using the PathScan® EGFR Signaling Antibody Array Kit (Chemiluminescent Readout) #12622. Images were acquired by briefly exposing the slide to standard chemiluminescent film.

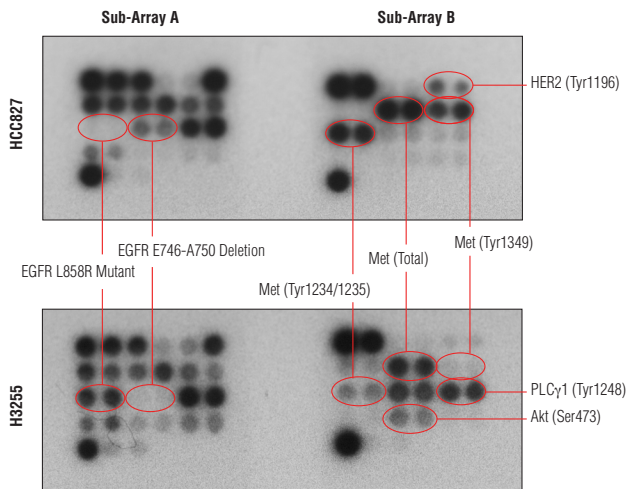


Figure 4. HCC827 and H3255 are two non-small cell lung cancer (NSCLC) cell lines carrying two different gefitinib-sensitive mutants of EGFR: E746-A750 deletion in exon 19 and L858R point mutation, respectively. Cell extracts were prepared and analyzed using the PathScan® EGFR Signaling Antibody Array Kit (Chemiluminescent Readout) #12622. Images were acquired by briefly exposing the slide to standard chemiluminescent film.

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.

PLC γ is a phosphoinositide-specific phospholipase. EGFR can activate PLC γ 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 caspase-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.
- (8) Hudelist, G. et al. (2003) *Breast Cancer Res Treat* 80, 353-61.
- (9) Engelman, J.A. et al. (2007) *Science* 316, 1039-43.
- (10) Comoglio, P.M. (2001) *Nat Cell Biol* 3, E161-2.
- (11) Benedettini, E. et al. (2010) *Am J Pathol* 177, 415-23.
- (12) Guo, A. et al. (2008) *Proc Natl Acad Sci USA* 105, 692-7.
- (13) Klein, S. and Levitzki, A. (2009) *Curr Opin Cell Biol* 21, 185-93.

PathScan® EGFR Signaling Antibody Array Kit (Chemiluminescent Readout) Protocol

A Preparing Cell Lysates

1. Thaw 10X Cell Lysis Buffer #9803 and mix thoroughly. Supplement Cell Lysis Buffer with phenylmethylsulfonyl fluoride (PMSF #8553) to a final concentration of 1 mM, or a cocktail of protease inhibitors (#5871, #5872). Prepare 1X Cell Lysis Buffer by diluting 10X Cell Lysis Buffer in deionized water. 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 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.

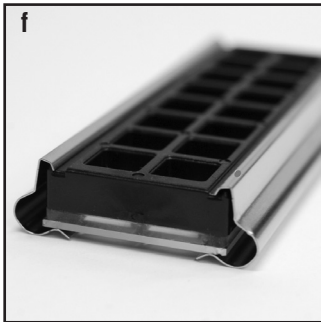
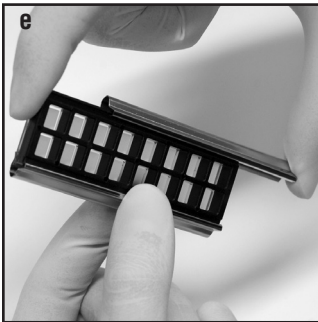
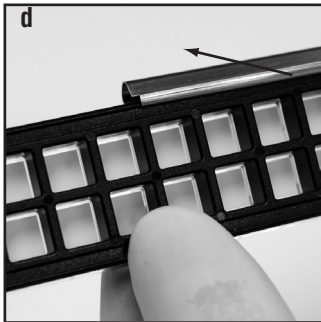
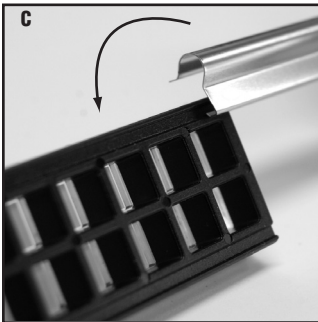
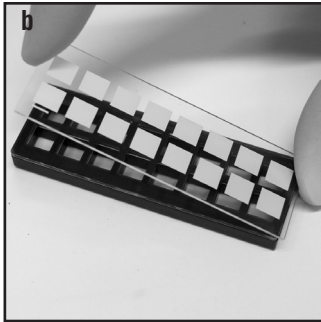
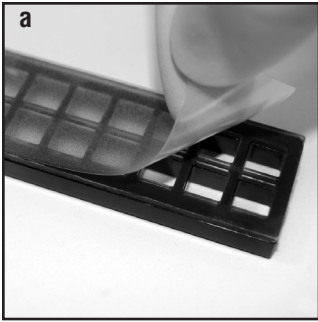
B Assay Procedure

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 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 HRP-linked Streptavidin as follows:
For running only **1 slides**:
Dilute 150 µl of 10X HRP-linked Streptavidin with 1350 µl of Array Diluent Buffer into an appropriately labeled tube. Keep the 1X HRP-linked Streptavidin on ice.
For running **2 slides**:
Dilute 300 µl of 10X HRP linked Streptavidin with 2700 µl of Array Diluent Buffer into an appropriately labeled tube. Keep the 1X HRP-linked Streptavidin on ice.
5. Affix the multi-well gasket to the glass slide (see figure on next page):
 - 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 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.
11. Add 75 µl 1X HRP-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 briefly with 10 ml 1X Array Wash Buffer.
15. Dilute and combine LumiGLO® and Peroxide reagents immediately before use (to make 10 ml of a 1X solution, combine 9 ml deionized water with 0.5 ml of 20X LumiGLO® and 0.5 ml of 20X Peroxide).
Note for Kodak® BioMax® film users: This dilution of LumiGLO®/Peroxide may necessitate very short exposure times (2-3 sec) for some targets. For more convenient exposure times (20-30 sec) add 20 ml of deionized water to the 10 ml LumiGLO®/Peroxide mix to make a 3 fold more diluted chemiluminescent reagent.
16. Decant Array Wash Buffer and cover slide with LumiGLO®/Peroxide reagent.
17. Transfer slide to chemiluminescent development folder, ensuring that it is still covered by LumiGLO®/ Peroxide reagent (add a small amount on top of the slide).
18. Immediately capture an image of the slide using a digital imaging system capable of detecting chemiluminescent signals. If desired, quantify spot intensities using commercially available array image analysis software. Alternatively, chemiluminescent film may be used. Expose film for 2-30 seconds using even and light pressure on the top of the development cassette (do not fasten the cassette clamps) to avoid squeezing out the LumiGLO®/ Peroxide reagent. Develop the film using an automated film developer.
Note: If both slides are being used, it is not recommended to expose them simultaneously in the same development cassette. In this case, leave the second slide in the wash buffer (step 12) while proceeding with steps 13-18 using the first slide. After the first slide is finished, proceed with steps 13-18 using the second slide and freshly diluted LumiGLO®/Peroxide reagent.

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Kodak® and BioMax® are trademarks of Eastman Kodak Company.

#12622



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



I. Identification:

Product name: PathScan® Antibody Array Kit
Product Catalog: 7744, 7323, 12785, 12622 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.