

PathScan® RTK Signaling Antibody Array Kit (Fluorescent Readout)

✓ 1 Kit
(16 multiplexed assays)



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rev. 03/24/15

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

Species Cross-Reactivity: H

Description: The PathScan® RTK Signaling Antibody Array Kit (Fluorescent Readout) is a slide-based antibody array product founded upon the sandwich immunoassay principle. The array kit allows for the simultaneous detection of 28 receptor tyrosine kinases and 11 important signaling nodes when phosphorylated at tyrosine or other residues. Target-specific capture antibodies, biotinylated protein (positive control) and nonspecific IgG (negative control) have been spotted in duplicate onto nitrocellulose-coated glass slides. Each kit contains two 8-pad slides, allowing the user to test up to 16 samples. 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: Cell Signaling Technology's PathScan® RTK Signaling Antibody Array Kit detects the indicated RTKs and signaling nodes only when phosphorylated at tyrosine or specified residues (see Array Target Map). No significant crossreactivity has been observed between targets, with the exception of some crossreactivity of the FLT3 antibody with phosphorylated EphB3. In addition, Stat1 (Tyr701) and Stat3 (Tyr705) may be detected when phosphorylated at other tyrosine sites within the proteins. This kit is optimized for cell lysates diluted to a total protein concentration between 0.2 and 1 mg/ml (see Figure 4). All capture antibodies have been validated for human targets. Although this kit has not been tested with mouse lysates, it is expected that many capture antibodies will crossreact in murine systems.

Background: Receptor Tyrosine Kinases (RTKs) are a family of cell surface receptors that signal primarily through tyrosine phosphorylation events (1). RTKs trigger a wide range of downstream signaling cascades, including the PI3K/Akt, MAPK and Jak/Stat pathways. These pathways control basic cellular functions such as division, growth, metabolism, differentiation, migration and survival. Dysregulation of RTK signaling has been implicated in a large number of cancers (2), making RTKs popular targets for pharmaceutical intervention.

Background References:

- Schlessinger, J. (2000) *Cell* 103, 211-25.
- Blume-Jensen, P. and Hunter, T. (2001) *Nature* 411, 355-65.

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.

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

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 #9803	15 ml	Clear

*Kit should be stored at 4°C with the exception of 10X Cell Lysis Buffer, which is stored at -20°C (packaged separately).

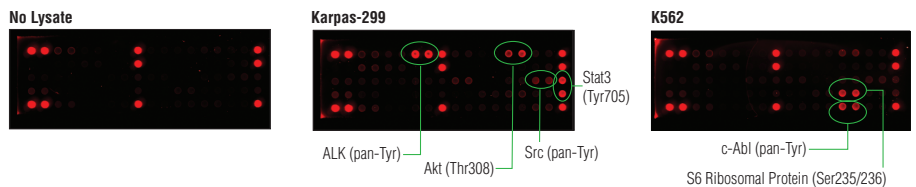
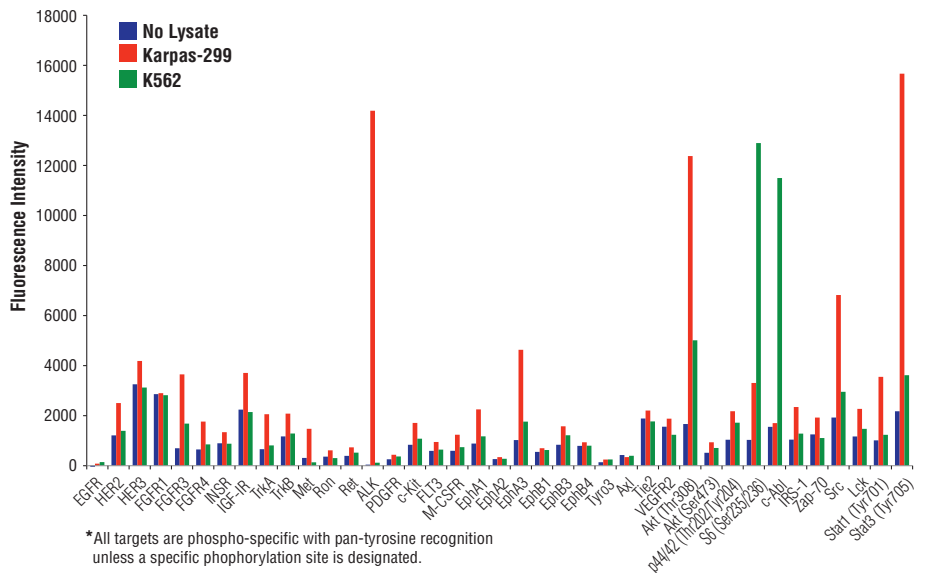


Figure 1. Screening of cell lines using the PathScan® RTK Signaling Antibody Array (Fluorescent Readout) #7949 reveals various phosphorylated RTKs and signaling nodes. Karpas-299 and K562 cells were lysed without starvation or treatment. The fluorescent image (lower panel) and the quantification of that image (upper panel) are shown.

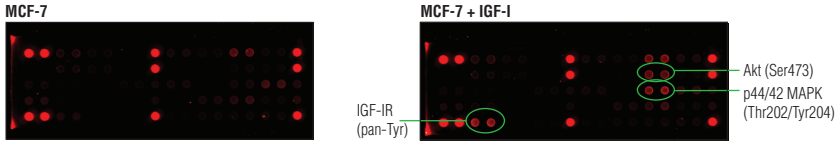
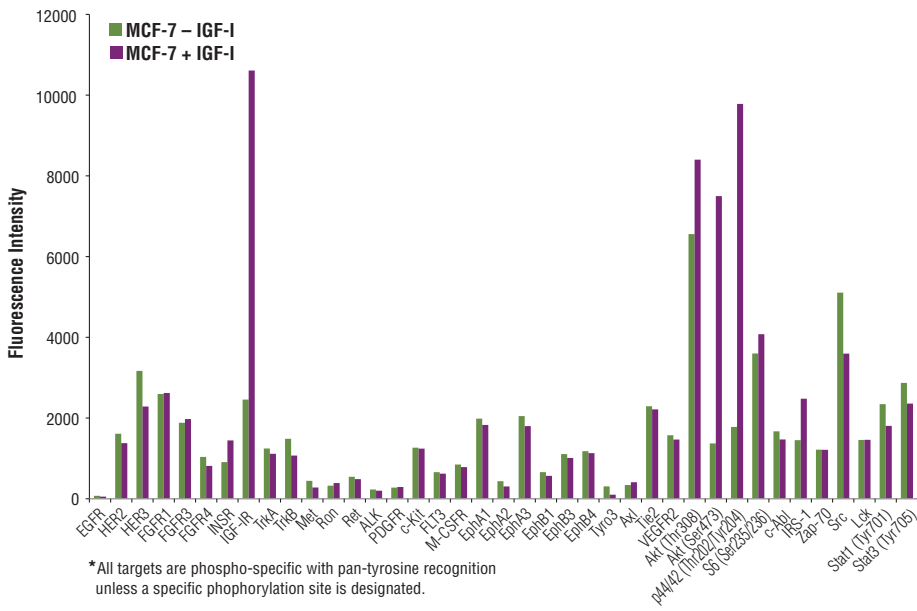


Figure 2. Treatment of MCF-7 cells with IGF-I stimulates phosphorylation of IGF-IR at tyrosine residues, Akt at Ser473 and p44/42 MAPK at Thr202/Tyr204 as detected by the PathScan® RTK Signaling Antibody Array Kit (Fluorescent Readout) #7949. MCF-7 cells were starved for 24 hours, then treated with 100 ng/ml IGF-I #3093 for 5 minutes at 37°C. The fluorescent image (upper panel) and the quantification of that image (lower panel) are shown.

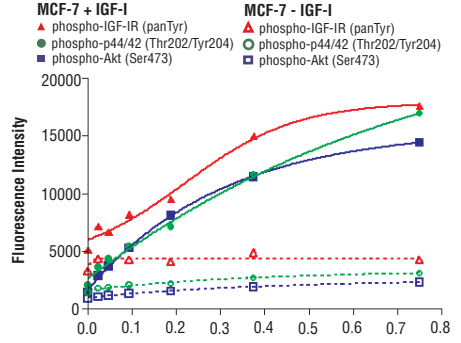


Figure 4. The relationship between lysate protein concentration from untreated and IGF-I treated MCF-7 cells and the relative fluorescence of phospho-IGF-IR (panTyr) and phospho-p44/42 (Thr202/Tyr204) is shown. MCF-7 cells were starved for 24 hours, then treated with 100 ng/ml IGF-I #3093 for 5 minutes at 37°C.

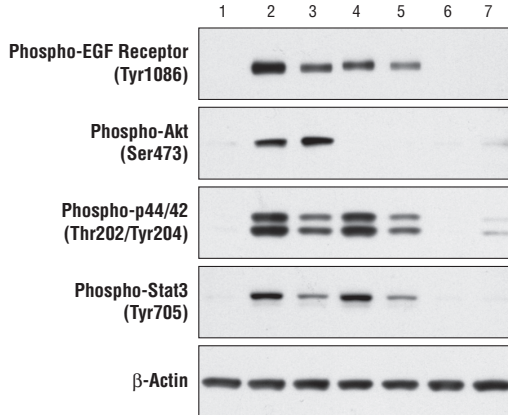
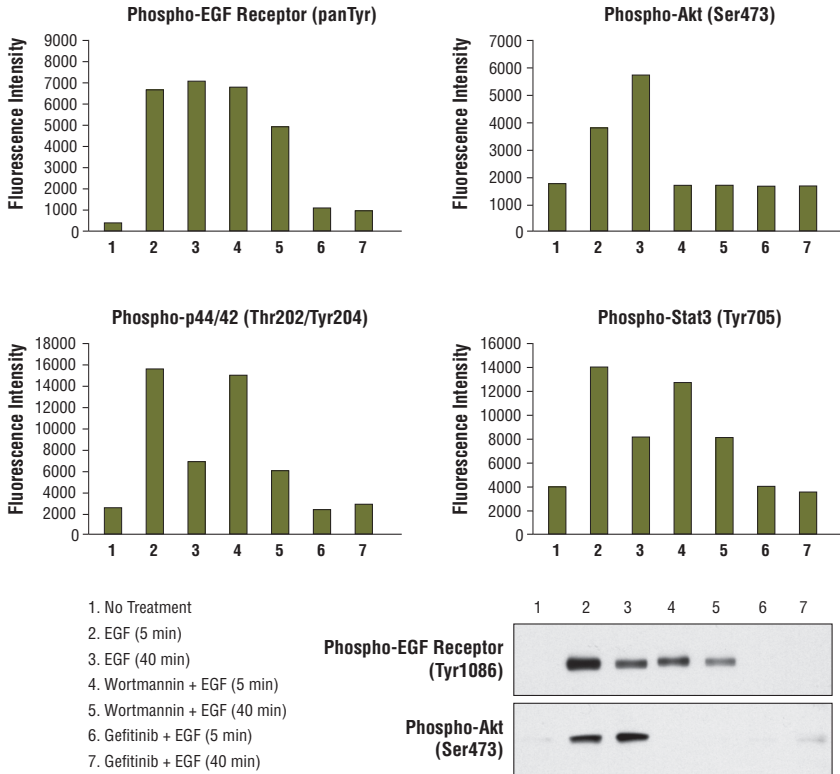


Figure 3. Treatment of A431 cells with EGF stimulates phosphorylation of EGFR, Akt, p44/42 MAPK and Stat3 as detected by the PathScan® RTK Signaling Antibody Array Kit (Fluorescent Readout) #7949. A431 cells were starved for 24 hours and treated with 100 ng/ml hEGF #8916 for 5 or 40 minutes. In some cases, cells were treated with either 1 μM wortmannin #9951 for 1 hour or 1 μM gefitinib for 2 hours before EGF stimulation. Fluorescence intensities obtained from the array are shown in the top panel, while western blots are shown in the bottom panel.

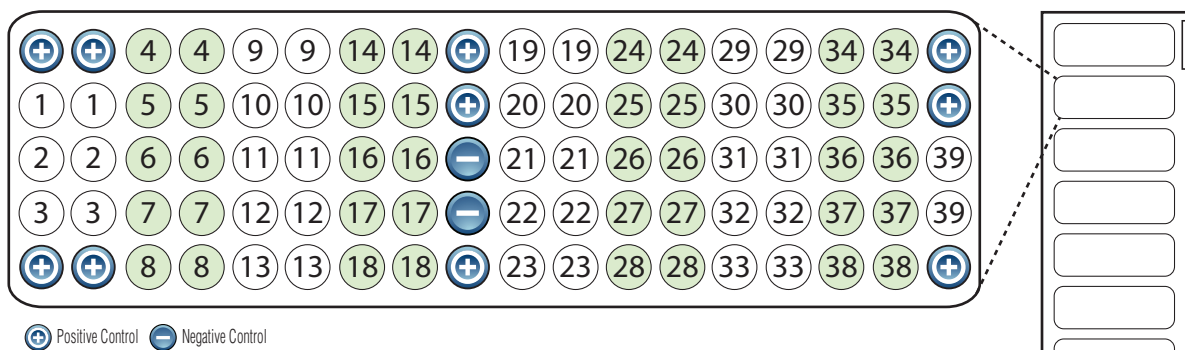


Figure 5. Target map of the PathScan® RTK Signaling Antibody Array Kit (Fluorescent Readout)

Receptor Tyrosine Kinases

Target	Phosphorylation Site	Family	
1	EGFR/ErbB1	pan-Tyr	EGFR
2	HER2/ErbB2	pan-Tyr	EGFR
3	HER3/ErbB3	pan-Tyr	EGFR
4	FGFR1	pan-Tyr	FGFR
5	FGFR3	pan-Tyr	FGFR
6	FGFR4	pan-Tyr	FGFR
7	InsR	pan-Tyr	Insulin R
8	IGF-IR	pan-Tyr	Insulin R
9	TrkA/NTRK1	pan-Tyr	NGFR
10	TrkB/NTRK2	pan-Tyr	NGFR
11	Met/HGFR	pan-Tyr	HGFR
12	Ron/MST1R	pan-Tyr	HGFR
13	Ret	pan-Tyr	Ret
14	ALK	pan-Tyr	LTK
15	PDGFR	pan-Tyr	PDGFR
16	c-Kit/SCFR	pan-Tyr	PDGFR
17	FLT3/Flk2	pan-Tyr	PDGFR
18	M-CSFR/CSF-1R	pan-Tyr	PDGFR
19	EphA1	pan-Tyr	EphR
20	EphA2	pan-Tyr	EphR
21	EphA3	pan-Tyr	EphR
22	EphB1	pan-Tyr	EphR
23	EphB3	pan-Tyr	EphR
24	EphB4	pan-Tyr	EphR
25	Tyro-3/Dtk	pan-Tyr	Axl
26	Axl	pan-Tyr	Axl
27	Tie2/TEK	pan-Tyr	Tie
28	VEGFR2/KDR	pan-Tyr	VEGFR

Signaling Nodes

Target	Phosphorylation Site	Family	
29	Akt/PKB/Rac	Thr308	Akt
30	Akt/PKB/Rac	Ser473	Akt
31	p44/42 MAPK (ERK1/2)	Thr202/Tyr204	MAPK
32	S6 Ribosomal Protein	Ser235/236	RSK
33	c-Abl	pan-Tyr	Abl
34	IRS-1	pan-Tyr	IRS
35	Zap-70	pan-Tyr	Zap-70
36	Src	pan-Tyr	Src
37	Lck	pan-Tyr	Src
38	Stat1	Tyr701	Stat
39	Stat3	Tyr705	Stat

PathScan® Antibody Array Kit (Fluorescent Readout) Protocol

A Preparing Cell Lysates

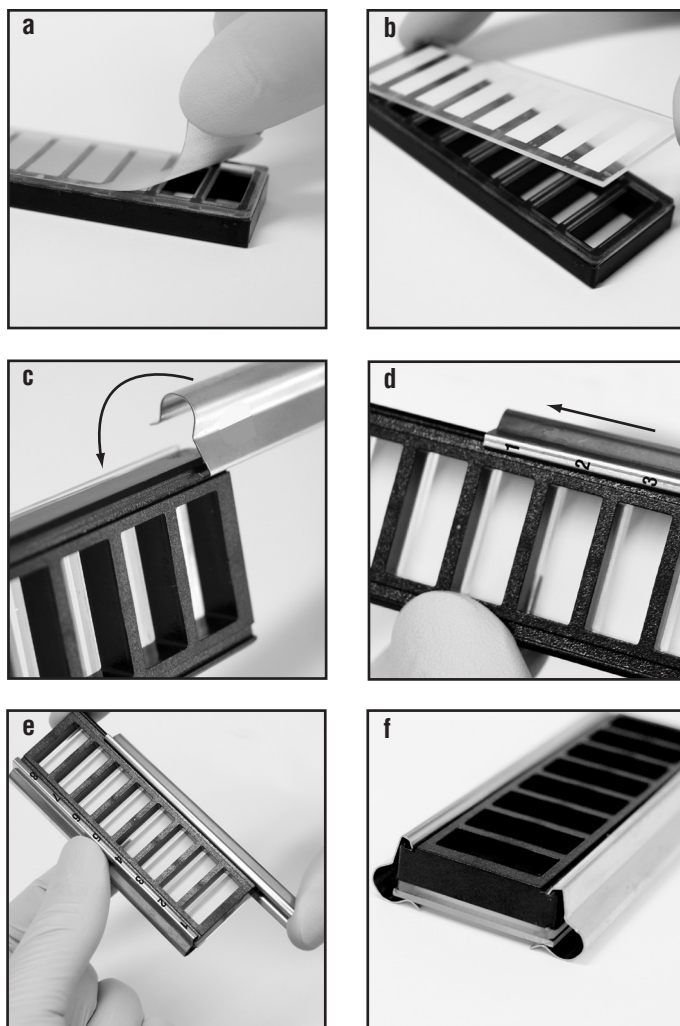
1. Thaw 10X Cell Lysis Buffer #9803 and mix thoroughly. Prepare 1X Cell Lysis Buffer by diluting 10X Cell Lysis Buffer in deionized water. Supplement 1X Cell Lysis Buffer with phenylmethylsulfonyl fluoride (PMSF) to a final concentration of 1 mM. Keep on ice.
2. Remove media and wash cells once with 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 for each plate (10 cm in diameter). Incubate on ice for 5 minutes.
4. If using adherent cells, dislodge the cells using a cell scraper. Transfer lysed cells to an appropriate tube. Keep on ice.
5. Microcentrifuge at maximum speed for 10 minutes at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. 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 1 mL of 20X Array Wash Buffer with 19 ml of deionized water. Label as 1X Array Wash Buffer.
3. Prepare 1X Detection Antibody Cocktail as follow:
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 follow:
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 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 numbered 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.
 - d. Slide the clip into place. The number "1" on the metal clip will now be in the same corner of the assembly as the orientation line.
 - 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 150 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 150 µl diluted lysate to each well and cover with sealing tape. Incubate for 2 hours at room temp (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 200 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 150 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 200 µl 1X Array Wash Buffer as in step 8.
Note: From this point on, keep slide protected from light.
11. Add 150 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 200 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 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™ is a registered trademark of Thermo Fisher Scientific Inc. and its subsidiaries.



Material Safety Data Sheet (MSDS) for 20X LumiGLO® and 20X Peroxide



I. Identification:

Product name: 20X LumiGLO® and 20X Peroxide
Product Catalog: 7003
CAS number: None
Manufacturer Supplier: Cell Signaling Technology
 3 Trask Lane
 Danvers, MA 01923 USA
 1-978-867-2300 TEL
 1-978-867-2400 FAX
 1-978-578-6737 Emergency TEL

II. Composition/Information on Ingredients:

Hazardous Reagent:	Percent	CAS#
Dimethyl sulfoxide	≤20%	67-68-5

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.

III. Hazard Identification:

CAUTION: This product is not for use in humans. It is intended for research purposes only. To the best of our knowledge, the chemical, physical, and toxicological properties of this material have not been established.

Emergency Overview: Irritant. Irritating to eyes, respiratory system, skin.

Potential Health Effects:

Inhalation: May be harmful if inhaled. May cause respiratory tract irritation.

Eye Contact: May cause eye irritation.

Skin Contact: May be harmful if absorbed through skin. Prolonged or repeated contact may cause skin irritation.

Ingestion: May be harmful if swallowed.

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: N/A

Autoignition Temperature: N/A

Explosion: N/A

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. May emit toxic fumes under fire conditions.

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:

Store at 4°C in tightly closed container.

Avoid inhalation of vapor or mist. Avoid contact with eyes, skin, and clothing. Wash thoroughly after handling. Avoid prolonged or repeated exposure.

VIII. Exposure Controls/Personal

Ventilation System: a system of local and/or general exhaust is recommended.

Skin Protection: wear compatible chemical resistant gloves and protective clothing.

Eye protection: wear protective safety glasses or chemical safety goggles. Maintain eye wash fountain and quick-drench facilities in work area.

IX. Physical And Chemical Properties:

Appearance:	clear faint yellow colored liquid
Odor:	data not available
pH:	data not available
Boiling Point:	>100°C/212°F (water)
Melting or Freezing Point:	<0°C/32°F (water)
Flash Point:	data not available
Volatile Organic Compounds (VOC):	data not available
Autoignition temp.	data not available
Solubility (water)	miscible in water

X. Stability and Reactivity:

Stability: Stable under normal conditions.

Conditions to avoid: strong oxidizing agents, strong acids, strong bases.

Hazardous Decomposition: carbon monoxide, carbon dioxide.

Hazardous polymerization: will not occur.

XI. Toxicological Information:

Acute toxicity: data not available. Chronic exposure: data not available

Potential Health Effects:

Inhalation: May be harmful if inhaled. Causes respiratory tract irritation.

Skin: May be harmful if absorbed through skin. Causes skin irritation.

Eyes: Causes eye irritation.

Ingestion: Harmful if swallowed.

Toxicity Data on Hazardous ingredient Dimethyl Sulfoxide, CAS#67-68-5

RTECS: P46210000

LD50 Oral rat 14,500 mg/kg

LC50 Inhalation rat 4 h 40250 ppm

LD50 Dermal rabbit > 5,000 mg/kg

XII. Ecological Information:

No data available.

XIII. Disposal Considerations: Dispose of in accordance with federal, state and local environmental regulations. Contact a licensed professional waste disposal service to dispose of this material.

XIV. Transport Information:

D.O.T. 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: Not classified

OSHA: Ingredient Dimethyl Sulfoxide, CAS#67-68-5: Combustible Liquid, Target Organ Effect Canadian DSL: Listed: Ingredient Dimethyl Sulfoxide, CAS#67-68-5

SARA 302, 313: Ingredients Not Listed.

SARA 311/312: Ingredient Dimethyl Sulfoxide, CAS#67-68-5: Fire Hazard, Chronic Health Hazard.

Massachusetts Right To Know: Ingredients Not Listed.

Pennsylvania Right To Know: Ingredient Dimethyl Sulfoxide, CAS#67-68-5

New Jersey Right To Know: Ingredient Dimethyl Sulfoxide, CAS#67-68-5

California Prop. 65: Ingredients Not Listed.

XVI. Other Information:

This product is for research use only and 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.