

PathScan® Th1/Th2/Th17 Cytokine Antibody Array Kit (Fluorescent Readout)



Cell Signaling
TECHNOLOGY®

✓ 1 Kit
(32 multiplexed assays)

New 10/13

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

Species Cross-Reactivity: H

Description: The PathScan® Th1/Th2/Th17 Cytokine Antibody Array Kit (Fluorescent Readout) uses glass slides as the planar surface and is based upon the sandwich immunoassay principle. This array kit allows for the simultaneous detection of 12 unique extracellular signaling molecules. Target-specific capture antibodies have been spotted in duplicate onto nitrocellulose-coated glass slides. Each kit contains two slides allowing the user to test up to 32 different samples and generate 384 data points in a single experiment. Cell supernatant 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® Th1/Th2/Th17 Cytokine Antibody Array Kit (Fluorescent Readout) detects the target proteins as specified on the Array Target Map (figure 1). No significant cross reactivity has been observed between targets. This kit is optimized for cell culture supernatants. Recommended starting cell culture supernatant range is 20-75 µl. All antibodies have been validated for human-derived cell culture supernatants.

Background: Cytokines are secreted intercellular signaling molecules that regulate many biological processes including inflammation, host defense, and cell differentiation. Cytokine profiles may provide insight into the molecular mechanisms

Products Included	Quantity	Cap Color
Array Slides	2 slides	
16-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

*Kit should be stored at 4°C.

that distinguish between healthy and diseased states. The PathScan® Th1/Th2/Th17 Cytokine Antibody Array Kit offers an antibody panel against a broad array of cytokines to enable measurement of their relative changes in cell culture supernatants.

Upon activation, naive CD4+ helper T cells differentiate into distinct functional subsets. The development of these subsets is driven, in part, by the cytokine milieu. Type 1 (Th1) cells help drive cellular immunity against intracellular pathogens. IL-12 and IFN-γ induce Th1 cell development. Th1 cells produce IFN-γ and IL-2, which provide a positive feedback loop to enhance Th1 cell differentiation and NK cell and CD8+ T cell cytolytic activity.

Th2 cells play a crucial role in the humoral immune response against extracellular pathogens. IL-4 drives development of Th2 cells, which subsequently produce IL-4, IL-5, and IL-13. These cytokines induce B cell proliferation, antibody production, IgE class switching, and activate eosinophils respectively.

Another distinct helper T cell lineage, Th17, is important for mucosal immunity. Dysregulation of Th17 may significantly contribute to the development of autoimmunity. IL-17 produced by Th17 cells induce secretion of pro-inflammatory cytokines IL-6, IL-8, GM-CSF, and TNF-α. Many of these molecules link innate and adaptive immunity through the recruitment and activation of innate immune cells.

Effective immune responses require finely tuned coordination between pro and anti-inflammatory signals. Pro-inflammatory molecules play important roles in activating key immune players to fight infection. IL-8 induces granulocyte migration and activates neutrophil phagocytic activity. GM-CSF mobilizes monocytes into infected tissue and activates macrophage and neutrophils. TNF-α is a multifunctional pro-inflammatory cytokine involved with a number of processes including cell proliferation, differentiation, and apoptosis.

Uncontrolled inflammation may damage surrounding host tissue. IL-10 is a prototypical anti-inflammatory cytokine that serves to terminate the acute inflammatory response by inhibiting Th1 cell function and pro-inflammatory cytokine production.

Background References:

- (1) Romagnani, S. (1999) *Inflamm Bowel Dis* 5, 285-94.
- (2) Bradley, L.M. et al. (2000) *Immunol Res* 21, 149-58.
- (3) O'Garra, A. and Arai, N. (2000) *Trends Cell Biol* 10, 542-50.
- (4) Harrington, L.E. et al. (2005) *Nat Immunol* 6, 1123-32.
- (5) Stockinger, B. and Veldhoen, M. (2007) *Curr Opin Immunol* 19, 281-6.
- (6) Köhldai, L. and Csaba, G. (1998) *Cytokine* 10, 481-6.
- (7) Carey, A.J. et al. (2012) *JAKSTAT* 1, 159-167.

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Cytokine Targets

Target

- | | |
|----|------------------|
| 1 | Positive Control |
| 2 | Negative Control |
| 3 | IL-2 |
| 4 | IL-4 |
| 5 | IL-5 |
| 6 | IL-6 |
| 7 | IL-8 |
| 8 | IL-10 |
| 9 | IL-12 p70 |
| 10 | IL-13 |
| 11 | IL-17A |
| 12 | TNF-α |
| 13 | GM-CSF |
| 14 | IFN-γ |

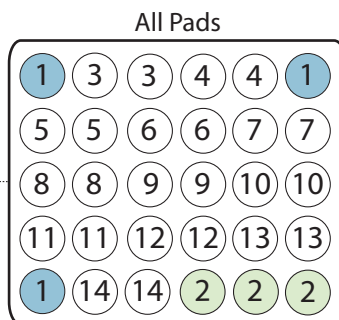
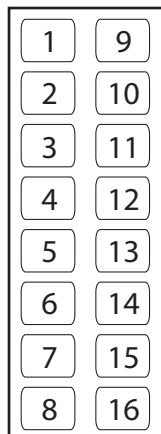


Figure 1. Target map of the PathScan® Th1/Th2/Th17 Cytokine Antibody Array Kit (Fluorescent Readout) #13124.

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.

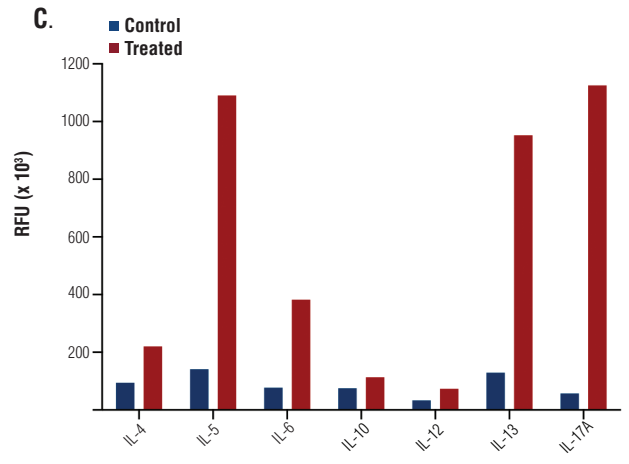
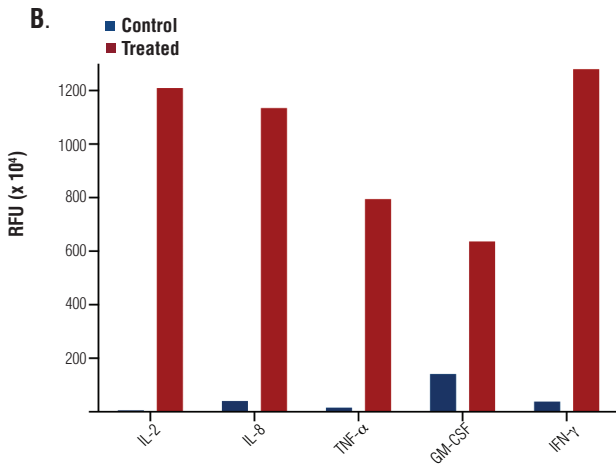
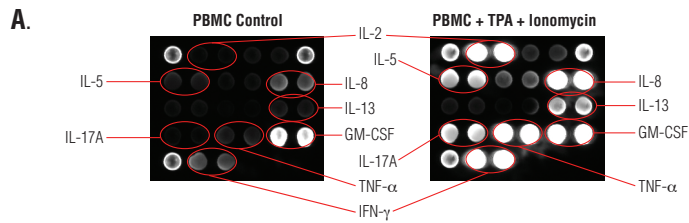


Figure 2. Human peripheral blood mononuclear cells (PBMCs) were isolated from whole blood and stimulated with immobilized anti-human CD3 antibody, soluble anti-human CD28 antibody (1 $\mu\text{g}/\text{ml}$), hIL-2 #8907 (2 ng/ml) and hIL-4 #8919 (1 ng/ml) for 2 days. Cells were washed and cultured for 3 days with hIL-2 (2 ng/ml) and hIL-4 (1 ng/ml). Cells were then washed and stimulated with TPA #4174 (10 ng/ml) and Ionomycin, Calcium Salt #9995 (1 $\mu\text{g}/\text{ml}$) for 24 hr before supernatant collection. Supernatants were prepared and analyzed using PathScan[®] Th1/Th2/Th17 Cytokine Antibody Array Kit (Fluorescent Readout) #13124. Panel A shows images that were acquired using LI-COR[®] Biosciences Odyssey[®] imaging system. Panels B and C show high and low end raw values of quantified fluorescence intensity. Pixel intensity was quantified using the LI-COR[®] Image Studio v2.0 array analysis software.

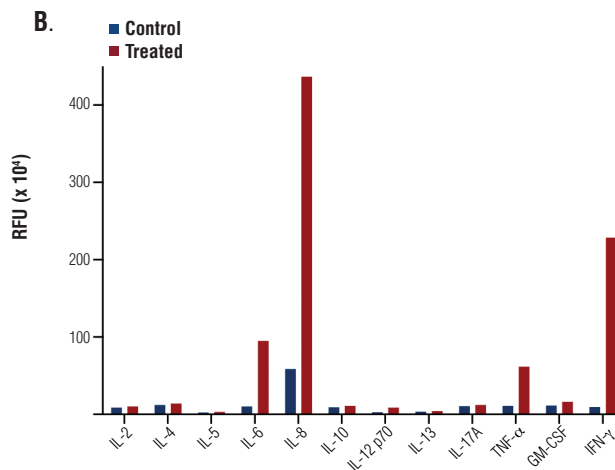
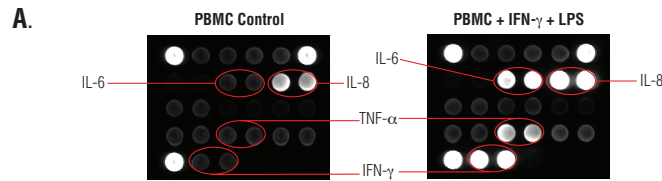


Figure 3. Human PBMCs were isolated from whole blood and pre-treated with hIFN- γ #8901 (10 ng/ml) for 2 hr. LPS was then added (1 $\mu\text{g}/\text{ml}$) and incubated for an additional 22 hr before supernatant collection. Supernatants were prepared and analyzed using the PathScan[®] Th1/Th2/Th17 Cytokine Antibody Array Kit (Fluorescent Readout) #13124. 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.

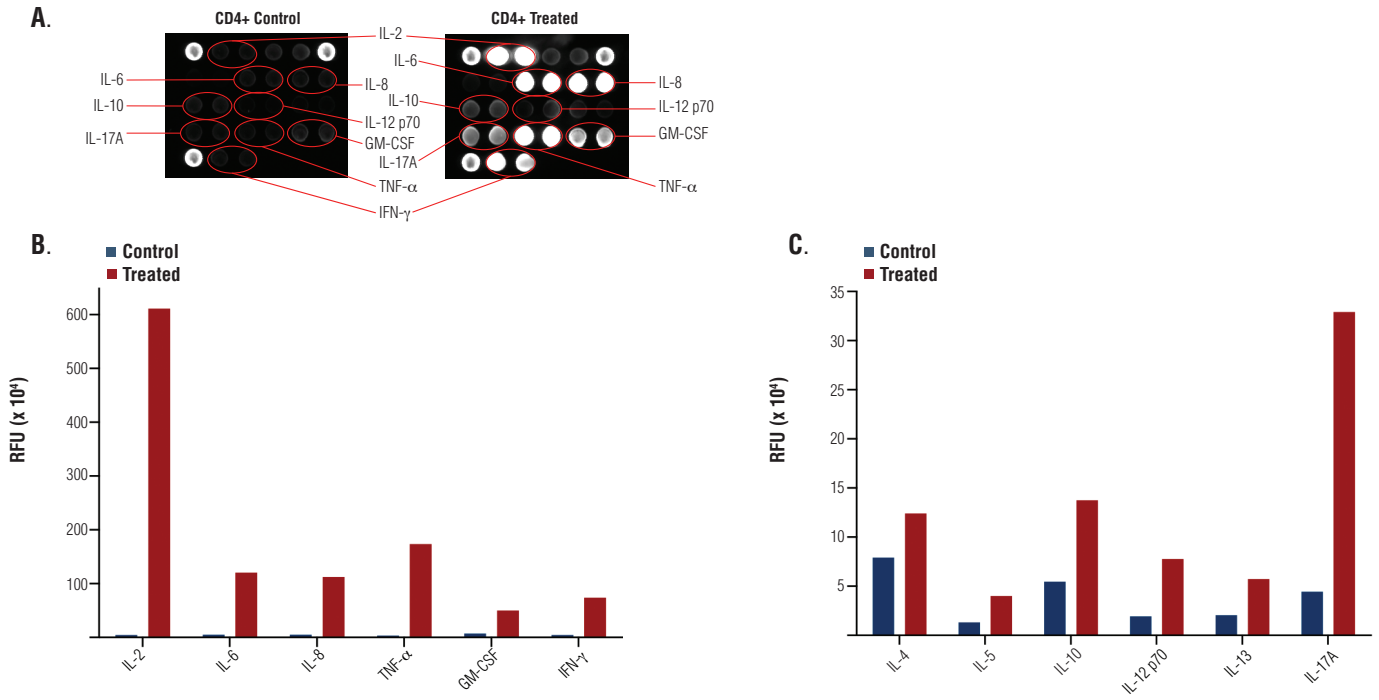


Figure 4. Human PBMCs were isolated from whole blood and CD4⁺ T cells separated by positive selection. The CD4⁺ T cells were stimulated with immobilized anti-human CD3 antibody, soluble anti-human CD28 antibody (1 μg/ml), hIL-23 (10 ng/ml), hTGF-β1 #8915 (1 ng/ml), hIL-6 #8904 (20 ng/ml), hTNF-α #8902 (10 ng/ml), hIL-1β #8900 (10 ng/ml), anti-human IL-4 antibody (10 μg/ml) and anti-human IFN-γ antibody (1 μg/ml) for 3 days before supernatant collection. Supernatants were prepared and analyzed using PathScan[®] Th1/Th2/Th17 Cytokine Antibody Array Kit (Fluorescent Readout) #13124. Panel A shows images that were acquired using LI-COR[®] Biosciences Odyssey[®] imaging system. Panels B and C show high and low end raw values of quantified fluorescence intensity. Pixel intensity was quantified using the LI-COR[®] Image Studio v2.0 array analysis software.

PathScan® Th1/Th2/Th17 Cytokine Antibody Array (Fluorescent Readout) Protocol

A Preparing Cell Culture Supernatants

1. Culture cells and perform the desired treatments.
2. Collect the conditioned medium/media and microcentrifuge. Transfer the supernatant to a new tube. Supernatant may be used immediately or stored at -80°C in single-use aliquots. Avoid multiple freezing and thawing.
3. Immediately before performing the assay, dilute the supernatant accordingly in Array Diluent Buffer. Alternatively, undiluted supernatants can be used.
4. Set aside on ice.

B Assay Procedure

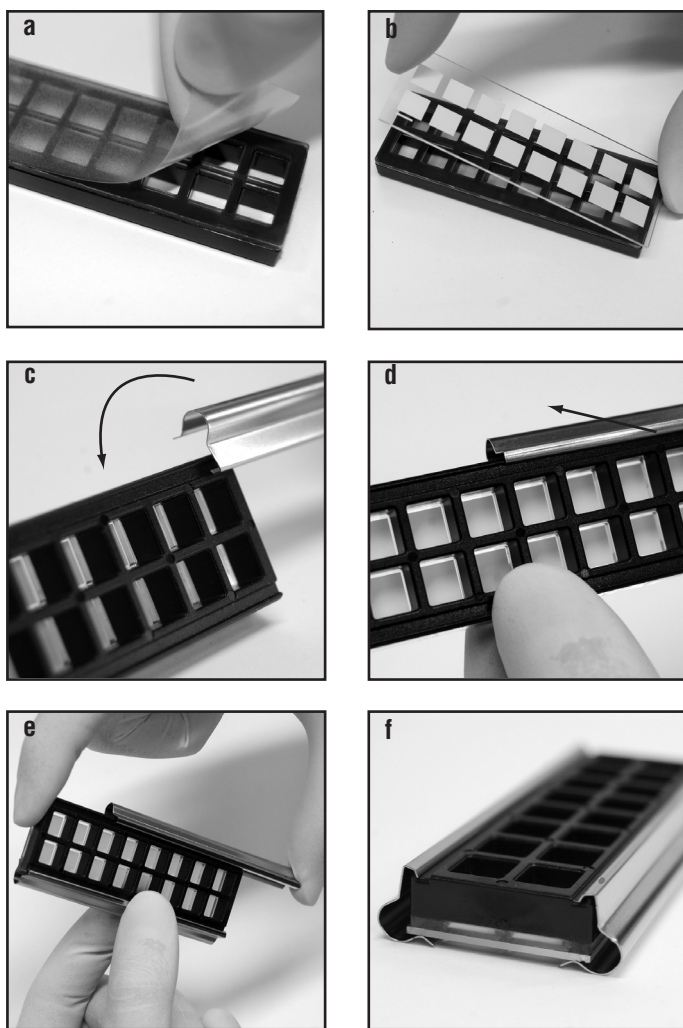
1. Bring glass slides and blocking buffer to room temperature before use.
2. Prepare 1X Array Wash Buffer by diluting 20X Array Wash Buffer in deionized water. Dilute 2.5 ml of 20X Array Wash Buffer with 47.5 ml of deionized water. Label as 1X Array Wash Buffer and keep at room temperature.
3. Prepare 1X Detection Antibody Cocktail as follows:
For running only **1 slide**:
Dilute 150 µl of 10X Detection Antibody Cocktail with 1350 µl of Array Diluent Buffer. Keep 1X Detection Antibody Cocktail on ice.
For running **2 slides**:
Dilute 300 µl of 10X Detection Antibody Cocktail with 2700 µl of Array Diluent Buffer. Keep 1X Detection Antibody Cocktail on ice.
4. Prepare 1X DyLight™ 680-linked Streptavidin as follows:
For running only **1 slides**:
Dilute 150 µl of 10X DyLight™ 680-linked Streptavidin with 1350 µl of Array Diluent Buffer. Keep 1X DyLight™ 680-linked Streptavidin on ice and protect from light.
For running **2 slides**:
Dilute 300 µl of 10X DyLight™ 680-linked Streptavidin with 2700 µl of Array Diluent Buffer. Keep 1X DyLight™ 680-linked Streptavidin 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 bench top, with 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 nitro-cellulose 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 of supernatant to each well and cover with sealing tape. Incubate for 2 hr 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 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 Cocktail to each well and cover with sealing tape. Incubate for 1 hr at room temperature on an orbital shaker.
10. Wash 4 times for 5 min each 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 min at room temperature on an orbital shaker.
12. Wash 4 times for 5 min each 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.

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



I. Identification:

Product name: PathScan® Antibody Array Kit
Product Catalog: 7744, 7323, 12785, 13124, 13047 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 13047 only)
- DyLight 680®-linked Steptavidin MSDS (Kit 13124 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.