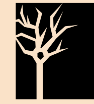
# PathScan® Th1/Th2/Th17 Cytokine Antibody Array Kit (Chemiluminescent Readout)

#13047 Store at 4°C



Cell Signaling TECHNOLOGY®

☑ 1 Kit
(32 multiplexed assays)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orders@cellsignal.com

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info@cellsignal.com

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

**Species Cross-Reactivity: H**

**Description:** The PathScan® Th1/Th2/Th17 Cytokine Antibody Array Kit (Chemiluminescent 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 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® Th1/Th2/Th17 Cytokine Antibody Array Kit (Chemiluminescent 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.

| Products Included | Quantity | Cap Color |
|---|---|---|
| Array Slides | 2 slides | |
| 16-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 | 300 µl | White |
| 10X HRP-linked Streptavidin | 300 µl | Clear |
| 20X LumiGLO® Reagent A #7003 | 5 ml | Brown |
| 20X Peroxide Reagent B #7003 | 5 ml | Clear |

*Kit should be stored at 4°C.

**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 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.

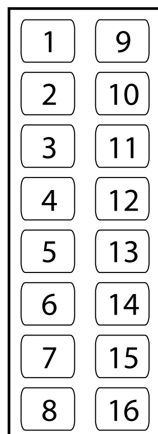
Another distinct helper T cell lineage, Th17, is important for mucosal immunity. Dysregulation of Th17 may significantly contribute to the development of autoimmunity. IL-17 produced by Th17 cells induces secretion of pro-inflammatory cytokines IL-6, IL-8, GM-CSF, and TNF-α. Many of these molecules link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

## 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 |



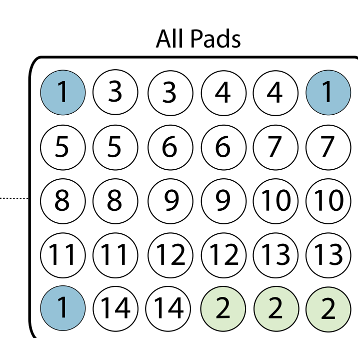




*Figure 1. Target map of the PathScan® Th1/Th2/Th17 Cytokine Antibody Array Kit (Chemiluminescent Readout) #13047.*

LumiGLO® is a registered trademark of Kirkegaard & Perry Laboratories.

**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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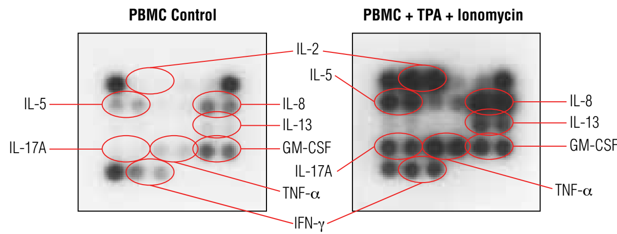


*Figure 2. Human peripheral blood mononuclear cells (PBMCs) were isolated from whole blood and stimulated with immobilized anti-human CD3 antibody, soluble anti-human CD28 antibody (1 μg/ml), hIL-2 #8907 (2 ng/ml) and hIL-4 #8919 (1 ng/ml) for 2 days. Cells were washed and cultured for 3 days with hIL-2 (2 ng/ml) and hIL-4 (1 ng/ml). Cells were washed and stimulated with TPA #4174 (10 ng/ml) and Ionomycin, Calcium Salt #9995 (1 μg/ml) for 24 hr before supernatant collection. Supernatants were prepared and analyzed using PathScan® Th1/Th2/Th17 Cytokine Antibody Array Kit (Chemiluminescent Readout) #13407. Images were acquired by briefly exposing the slide to standard chemiluminescent film.*

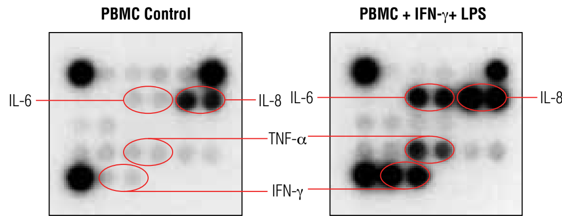


*Figure 3. Human PBMCs were isolated from whole blood and pre-treated with hIFN-γ #8901 (10 ng/ml) for 2 hr. LPS was then added (1 ug/ml) and incubated for an additional 22 hr before supernatant collection. Supernatants were prepared and analyzed using the PathScan® Th1/Th2/Th17 Cytokine Antibody Array Kit (Chemiluminescent Readout) #13047. Images were acquired by briefly exposing the slide to standard chemiluminescent film.*

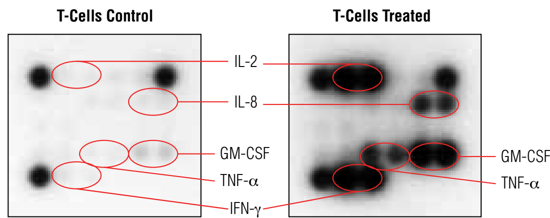


*Figure 4. Human PBMCs were isolated from whole blood and T cells separated by negative selection. T cells were cultured for 6 days with PHA (5 μg/ml) and hIL-2 #8907 (10 ng/ml). Cells were washed and incubated with PHA (5 μg/ml), TPA #4174 (10 ng/ml) and Ionomycin, Calcium Salt #9995 (1 μg/ml) for 24 hr before supernatant collection. Supernatants were prepared and analyzed using the PathScan® Th1/Th2/Th17 Cytokine Antibody Array Kit (Chemiluminescent Readout) #13047. Images were acquired by briefly exposing the slide to standard chemiluminescent film.*

**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) Köhidai, L. and Csaba, G. (1998) *Cytokine* 10, 481-6.

(7) Carey, A.J. et al. (2012) *JAKSTAT* 1, 159-167.


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# PathScan® Th1/Th2/Th17 Cytokine Antibody Array (Chemiluminescent 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 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. Keep 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. Keep 1X HRP-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 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 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 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.
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 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 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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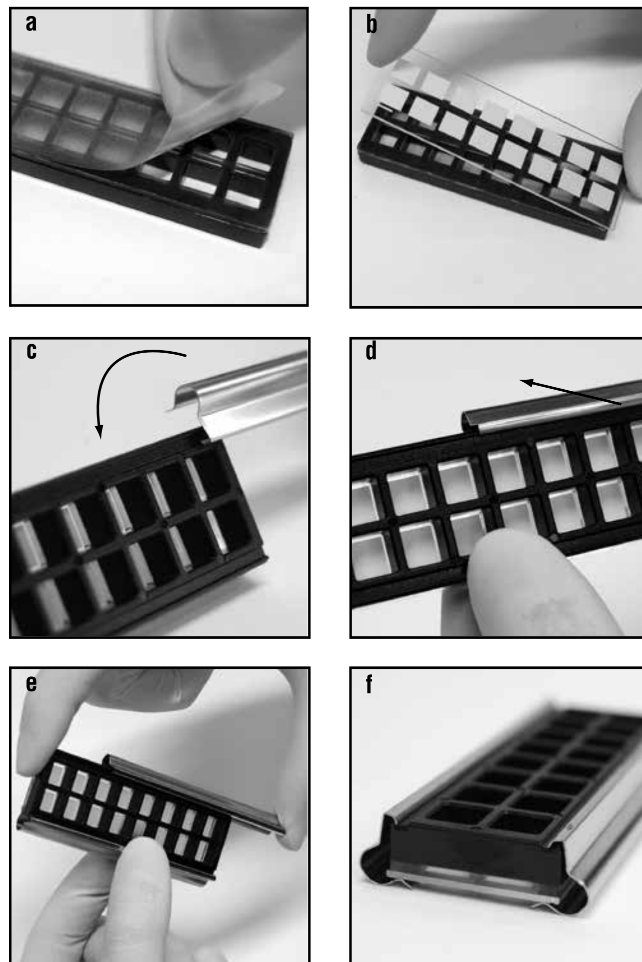


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MSDS

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

## I. Identification:

**Product name:** PathScan® Antibody Array Kit
**Product Catalog:** 13047, 13124 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
- 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.

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