BD Phosflow™ T Cell Activation Kit (Human)

Features

- Analysis of key phosphoproteins in T cells without prior isolation from whole blood
- Convenient, ready-to-use kit containing an improved cocktail of fluorochrome-labeled antibodies to human CD3/CD4/CD8, fluorochrome-labeled antibodies to six key phosphoproteins, optimized protocol, and buffers
- Lyophilized positive and negative control cells to increase confidence and standardization
- Simplified data analysis and presentation of quality figures with Cytobank™ software

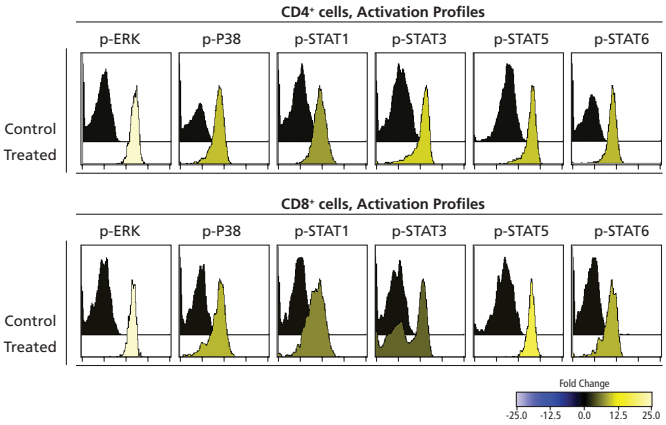

Figure 1. Activation profiles for CD4+ and CD8+ T cells monitored with the BD Phosflow T Cell Activation Kit. The phosphorylated proteins and their corresponding activators are shown in the following table.

Activator (response modifier)	Phosphorylation marker
PMA	ERK 1/2, p38MAPK
hIFN-α	STAT1
hIL-6	STAT3
hIL-2	STAT5
hIL-4	STAT6

CD4+ and CD8+ T cell signaling responses to treatment monitored using the BD Phosflow T Cell Activation Kit are plotted as histogram overlays using Cytobank software. These histograms show the signaling response of each phosphorylation marker induced by the corresponding activators.

The new BD Phosflow™ T Cell Activation Kit allows both experienced and novice researchers to obtain accurate and reproducible results from BD Phosflow experiments from human whole blood samples. BD Phosflow technology uses a combination of antibodies to cell surface markers and intracellular phosphorylated proteins in optimized fixation and permeabilization buffer systems to examine signal transduction pathways in subpopulations of cells by flow cytometry.

Protein phosphorylation through the JAK/STAT, ERK, and p38 MAPK pathways are important for T-cell activation, signaling, and differentiation. These pathways also play important and established roles in inflammation and disease states.

Kit components

- **BD Phosflow™ Alexa Fluor® 647 conjugated human antibodies:** p38 MAPK (pT180/Y182), ERK (pT202/204), Stat1 (pY701), Stat3 (pY705), Stat5 (pY694), and Stat6 (pY641)
- **BD Phosflow™ Human T-Cell (CD4/CD8) Antibody Cocktail:** Alexa Fluor® 488 anti-Human CD8 (RPA-T8), PE anti-Human CD3 (UCHT1), PerCP-Cy™5.5 anti-Human CD4 (SK3)
- **Compensation control reagents:** Alexa Fluor® 488 anti-Human CD8, PE anti-Human CD4, PerCP-Cy5.5 anti-Human CD3
- **BD™ CompBead Anti-Mouse Ig, κ and BD™ CompBead Negative control (FBS)**
- **BD Phosflow™ Lyophilized Control Cells:** Stimulated and unstimulated human T cells
- **Buffers:** BD Phosflow™ Lyse/Fix Buffer, BD Phosflow™ Perm Buffer III, BD Pharmingen™ Stain Buffer (FBS)

Rapid, accurate analysis by flow cytometry

Phosphorylation signaling events are typically transient by nature. Cell separation and other sample manipulation might lead to alterations of biological results. Flow cytometry allows for the analysis of T cells without prior isolation from whole blood, which can preserve these transient phosphorylation events. T cells are identified from whole blood using a cocktail of CD3, CD4, and CD8-conjugated antibodies, allowing the further subsetting of CD4 and CD8 subpopulations in combination with a choice of an antibody to the phosphoprotein of interest.

Visit **bdbiosciences.com/phosflow** for more information.

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A start-to-finish solution for BD Phosflow analysis

To support analysis of BD Phosflow data, Cytobank software, developed by leading flow cytometry researchers at Stanford University, now features templates for the analysis of data generated from use of the BD Phosflow T Cell Activation Kit. Cytobank software offers universal access web-based tools for BD Phosflow data analysis, data visualization, sharing, and collaboration. Even for large experiments consisting of multiple samples, stimulation conditions and markers, Cytobank simplifies analysis with workflow tools and overviews that guide users through typical analysis steps.

Backed by world-class service and support and the most advanced and flexible commercial flow cytometry instrumentation available, BD Phosflow solutions help researchers accelerate breakthrough discoveries that depend on complex systems analysis.

References

1. Rincón M, Davis RJ. Regulation of the immune response by stress-activated protein kinases. *Immunol Rev*. 2009;228:212-224.
2. Adamson AS, Collins K, Laurence A, O'Shea JJ. The Current STATus of lymphocyte signaling: new roles for old players. *Curr Opin Immunol*. 2009;21:161-166.
3. Yang XO, Panopoulos AD, Nurieva R, et al. STAT3 regulates cytokine-mediated generation of inflammatory helper T cells. *J Biol Chem*. 2007;282:9358-9363.
4. Kuida K, Boucher DM. Functions of MAP kinases: insights from gene-targeting studies. *J Biochem*. 2004;135:653-656.

Table 1. Summary of phosphoproteins studied using the BD Phosflow T Cell Activation Kit

	p38 (pT180/Y182)	ERK (pT202/204)	STAT1 (pY701)	STAT3 (pY705)	STAT5 (pY694)	STAT6 (pY641)
T cell subsets	Th1[1]	Th1/Th2	Th1	Th17[2,3]	Treg[2]	Th2/Th9
Activated by	Anti-CD3/CD28, PMA, pervanadate	Anti-CD3/CD28, PMA, pervanadate	IFN-α	IL6 IL-21	IL-2 IL-7 IL-15	IL-4
Result of signaling protein gene knockout in mouse T cells	Decreased numbers of Th1 and decrease in IFN-γ	Decrease in thymocyte maturation Decrease in activation of T cells[4]	—	Decrease in IL-17 and IL-21	No Tregs	Decrease in Th2-mediated immune response
Inhibitors	SB203580	PD-98059	JAK inhibitor I	JAK inhibitor I	JAK inhibitor I	JAK inhibitor I

This table features representative information for the study of these phosphoproteins in T cells. Users should determine which activators and/or inhibitors are useful for their specific samples and experimental conditions.

Ordering Information

Description	Size	Cat. No.
BD Phosflow T Cell Activation Kit	50 tests	**560750**

Related Products

Description	Size	Cat. No.
BD Phosflow Lyse/Fix Buffer	250 mL	**558049**
BD Phosflow Perm Buffer III	125 mL	**558050**
BD Pharmingen Stain Buffer (FBS)	500 mL	**554656**
BD Phosflow Stimulated and Unstimulated Human Control Cells – T cells (5 vials each of stimulated and unstimulated human T cells)	5 tests	**560760**

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