

BD Phosflow Human Monocyte/NK Cell Activation Kit

Features

Analysis of key phosphoproteins in monocytes and NK cells without prior isolation of cells from whole blood

Can also be used to look at phosphoprotein expression in B and T cells

Convenient, ready-to-use kit with antibody cocktail, fluorochrome-labeled antibodies to six key phosphoproteins, and optimized protocols and buffers

Lyophilized positive and negative control cells to increase confidence and standardization

	Expressed by	Activated by
Stat1	B Cells	IFN- α
	T Cells	IFN- α
	Monocytes	IFN- α , IFN- γ
	NK Cells	IFN- α
Stat3	B Cells	IFN- α
	T Cells	IFN- α , IL-6
	Monocytes	IFN- α , IL-6
	NK Cells	IFN- α
Stat5	B Cells	IFN- α , IL-4
	T Cells	IFN- α , IL-2
	Monocytes	IFN- α
	NK Cells	IFN- α , IL-2
Stat6	B Cells	IL-4
	T Cells	IL-4
	Monocytes	IFN- α , IL-4
	NK Cells	IL-4
Erk1/2	B Cells	PMA
	T Cells	PMA
	Monocytes	PMA, LPS
	NK Cells	PMA
p38	B Cells	PMA
	T Cells	PMA
	Monocytes	PMA, LPS
	NK Cells	unknown

Table 1. Summary of expression and activators for the phosphoproteins contained in the BD Phosflow Human Monocyte/NK Cell Activation Kit. This table presents representative information for the study of the indicated phosphoproteins in immune cells. Users should determine which activators and/or inhibitors are useful for their specific samples and experimental conditions.

The new BD Phosflow™ Human Monocyte/NK Cell Activation Kit is a complete system for the determination of the activation status of six different key phosphoproteins in B cells, monocytes, NK cells, and T cells using 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.¹

Protein phosphorylation through the JAK/STAT, ERK, and p38 stress/MAPK pathways is important for cell activation, signaling, and differentiation. Different types of stimuli such as exposure to pathogens or other environmental factors can lead to differential activation of these proteins across a variety of cell types, leading to the expression of important proteins such as cytokines.²⁻⁴ This kit contains optimized cocktails and reagents to identify monocytes, NK cells, B cells, and T cells, as well as monoclonal antibody reagents to six important phosphoproteins (summarized in Table 1) provided as Alexa Fluor® 647 conjugates.

CD markers included in the cocktail

CD Marker	Clone	Format	Used to Identify
CD14	M5E2	Alexa Fluor® 488	Monocytes
CD16	3G8	PE	NK cells
CD19	HIB19	Alexa Fluor® 488	B cells
CD56	B159	PE	NK cells
CD3*	SK7	PE-Cy™7	T cells

*Provided as a separate vial

Other kit components:

- **Compensation control reagents:** Alexa Fluor® 488 anti-Human CD4, PE anti-Human CD4
- **BD™ CompBead particles:** Anti-Mouse Ig, κ and negative control
- **Control cells:** Stimulated (positive) and unstimulated (negative)
- **Buffers:** BD Phosflow™ lyse/fix buffer, BD Phosflow™ perm buffer IV, BD Pharmingen™ stain buffer (FBS)

Visit bdbiosciences.com/phosflow for more information.



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Activation profiles monitored with the BD Phosflow Human Monocyte/NK Cell Activation Kit

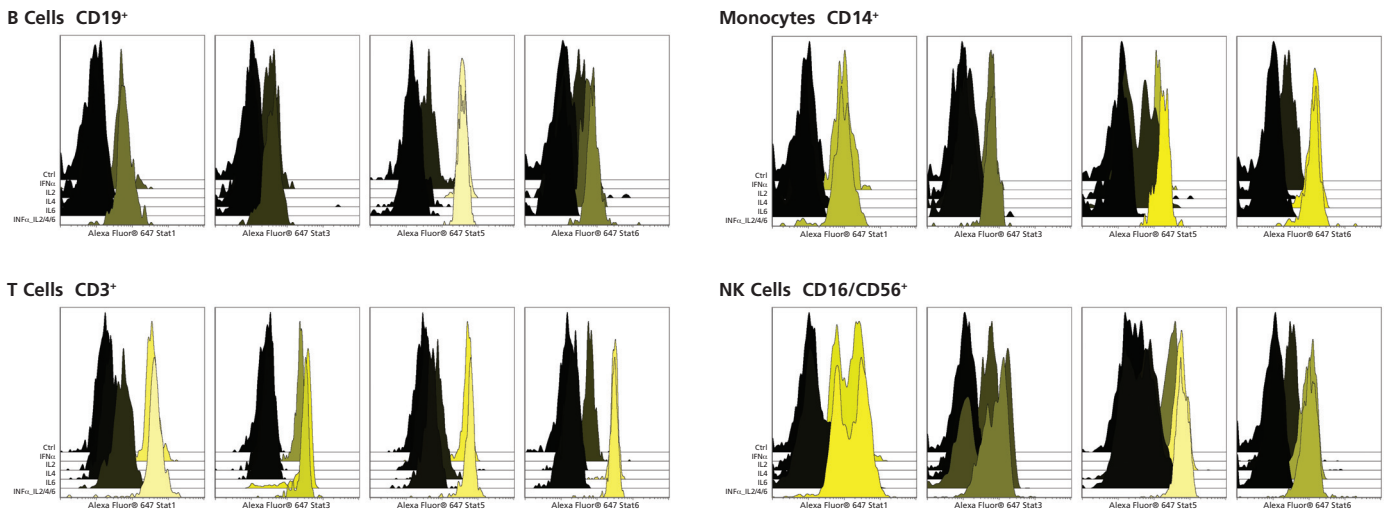


Figure 1. Activation profiles of phosphorylated Stat proteins for CD14⁺ monocytes, CD16/CD56⁺ NK cells, CD3⁺ T cells, and CD19⁺ B cells. Data were collected on a BD FACSCanto™ II flow cytometer and analyzed using Cytobank software. These histograms show the signaling response of each phosphorylation marker induced by the corresponding response modifier as listed in Table 1.

Two Kits Available for the Study of Phosphoproteins from Whole Blood

Phosphorylation and other signaling events are typically transient by nature. Cell separation and other manipulation of samples may lead to alterations of biological results. The BD Phosflow Human Monocyte/NK Cell Activation Kit allows the analysis of phosphoproteins in four key immune populations without prior enrichment of samples.

For researchers specifically interested in T cells, the BD Phosflow™ Human T Cell Activation Kit (Cat. No. 560750) contains antibodies to the same six phosphoproteins with a different cocktail to identify CD3, CD4, and CD8-positive T cells and control T cells.

To facilitate data analysis of BD Phosflow experiments, BD has partnered with Cytobank, a software application developed by leading flow cytometry researchers at Stanford University, to provide free analysis tools. Cytobank software offers easy-to-use web based tools for BD Phosflow data analysis, data visualization, and collaboration. Visit cytobank.org for more information.

References

- Perez OD, Mitchell D, Campos R, Gao GJ, Li L, Nolan GP. Multiparameter analysis of intracellular phosphoepitopes in immunophenotyped cell populations by flow cytometry. *Curr Protoc Cytom.* 2005; Chapter 6:Unit 6.20.
- Yu H, Pardoll D, Jove R. STATs in cancer inflammation and immunity: a leading role for STAT3. *Nat Rev Cancer.* 2009;9:798-809.
- Rincón M, Davis RJ. Regulation of the immune response by stress-activated protein kinases. *Immunol Rev.* 2009;228:212-224.
- 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.

Ordering Information

Description	Cat.No.
BD Phosflow Hu Monocyte/NK Cell Activation Kit	562089

Related Products

Description	Cat.No.
BD Phosflow Hu T Cell Activation Kit	560750

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