

BD Phosflow Violet Fluorescent Cell Barcoding Kit

Increase throughput with fluorescent cell barcoding technology

Features

Increase sample throughput and productivity

Enable large-scale experimental design

Minimize staining variability between samples

Multiplex experimental conditions for studies of cell signaling

Optimize reagent consumption and generate up to 800 test results per kit

This kit includes two violet laser excitable barcoding dyes, CBD450 and CBD500, reserving other fluorescence channels for other markers

Today's scientists increasingly need to perform assays in ways that provide higher sample throughput and decreased time to assay results, all while managing reagent consumption. Fluorescent cell barcoding technology, licensed from Stanford University (Krutzik and Nolan, *Nature Methods*, 2006) uses an innovative reagent system that enables scientists to increase their throughput capabilities without compromising the quality of results expected from multicolor flow cytometry experiments. With the BD Phosflow™ Violet Fluorescent Cell Barcoding Kit, individual samples are covalently labeled (barcoded) with either no, low, medium, or high concentrations of cell barcoding dye CBD450 and/or CBD500. This creates a unique fluorescent staining pattern for up to 16 samples.

Increase sample throughput and productivity

Fluorescent cell barcoding technology increases sample throughput and productivity by bulk processing samples in several procedural steps. Once barcoded, samples are ready to be pooled and stained for multicolor flow cytometry. See Figure 1.

Enable large-scale experiments

Large-scale, complex experiments are often logistically impossible to execute. Consolidated staining and acquisition make sample handling more manageable, saving time and allowing more experimental variables such as multiple donors, cell lines, or cell treatments (ie, activators or inhibitors) to be included in the same experiment.

Minimize staining variability by simultaneously labeling samples in a single tube

Because multiple samples are stained in the same tube, staining variability between samples is eliminated. This provides researchers with confidence that differences in staining intensity observed between cell populations are a function of the experimental model, contributing to a robust experimental design.

Multiplex experimental conditions for studies of cell signaling

Normal cell signaling is critical for healthy regulation of cell survival, growth, and differentiation. During signaling, cell-associated proteins are phosphorylated and dephosphorylated by kinases and phosphatases. The phosphorylation status of these proteins can be detected using phosphospecific antibody reagents. Signaling dysregulation results in disease states that can be detected by observing phosphoprotein profiles in resting and perturbed (activated) cells. Users can apply fluorescent cell barcoding to simultaneously evaluate multiple signaling pathways, in multiple cell types, stimulated by multiple activating agents. See Figure 2.

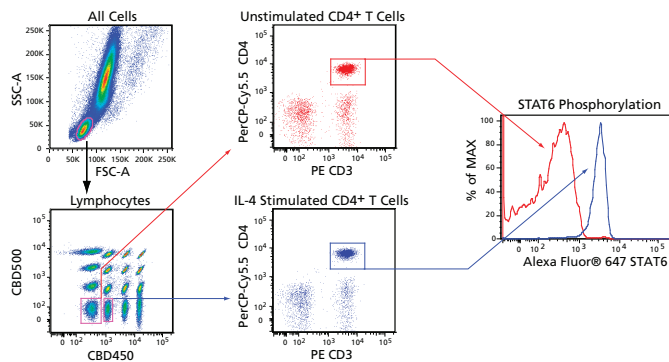


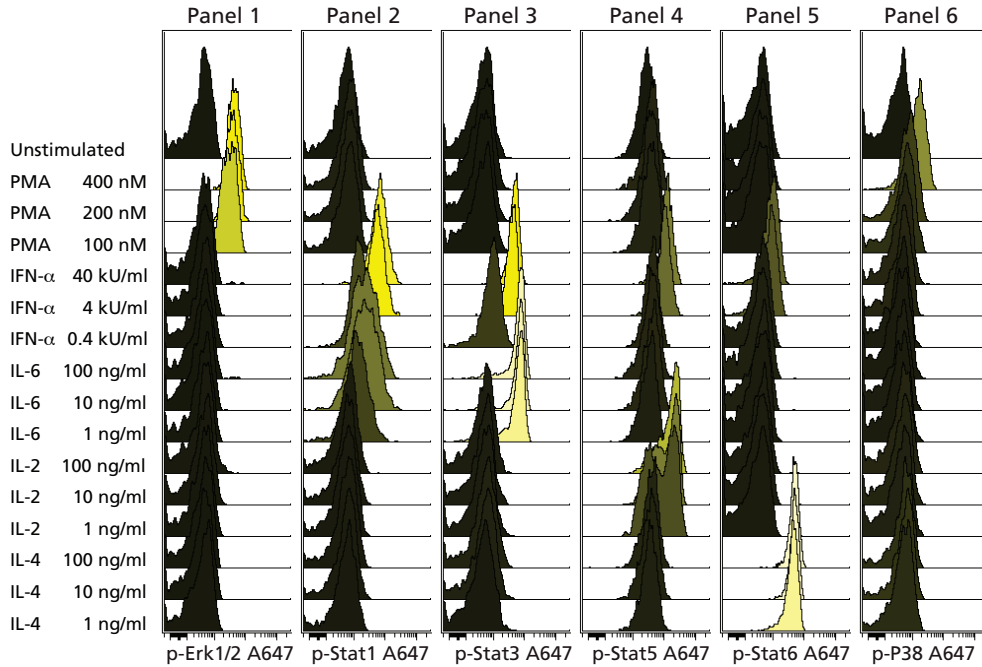
Figure 1. Gating strategy for activation profiles of CD4⁺ T cells using the BD Phosflow Violet Fluorescent Cell Barcoding Kit.

In this example, fresh whole blood was stimulated with IL-4 (blue) or unstimulated (red). After red cell lysis and leucocyte fixation and permeabilization, the cells were labeled with various concentrations of CBD450 and/or CBD500, pooled, and then stained with the fluorescently conjugated monoclonal antibodies listed in the related products table (see reverse side). This figure illustrates that it is possible to acquire and analyze data from pooled, barcoded unstimulated and stimulated samples simultaneously.

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A. Dose-Response Analysis in CD4⁺ T Cells



B. Heatmap: Responses in CD4 and CD8 T Cells

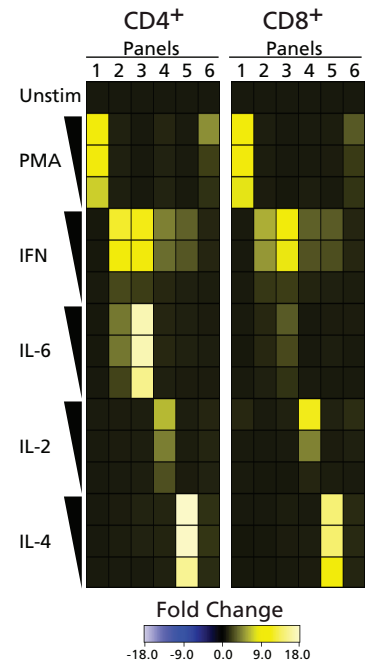


Figure 2. Multiparameter flow cytometric profiling of phosphorylated signaling protein expression induced in stimulated human peripheral blood T cells. The fluorescence histogram overlays were generated using Cytobank software and show phosphoprotein expression profiles in CD3⁺CD4⁺ T cells from 16 different stimulation conditions (96 histograms total). These data were generated from a single experiment of 6 tubes containing pooled barcoded samples that were acquired using a BD FACSCanto™ II flow cytometer and analyzed in Cytobank software.

Ordering Information

Description	Contents	Size	Cat.No.
BD Phosflow™ violet fluorescent cell barcoding kit	Cell barcoding dye 450 (CBD450), cell barcoding dye 500 (CBD500), fluorescent cell barcoding wash buffer 4X (barcoding wash buffer)	800 tests	561570
Choice of one:	Stat1 (pY701) Alexa Fluor® 647	50 tests	612597
	Stat3 (pY705) Alexa Fluor® 647	50 tests	557815
	Stat5 (pY694) Alexa Fluor® 647	50 tests	612599
	Stat6 (pY641) Alexa Fluor® 647	50 tests	612601
	ERK1/2 (pT202/pY204) Alexa Fluor® 647	50 tests	612593
	p38 MAPK (pT180/pY182) Alexa Fluor® 647	50 tests	612595

Related Products

Description	Size	Cat.No.
BD Phosflow™ lyse/fix buffer 5X	250 mL	558049
BD Phosflow™ perm buffer III	125 mL	558050
Fluorescent cell barcoding wash buffer 4X	500 mL	561550
CD3 (UCHT1) PE	100 tests	555333
CD4 (SK3) PerCP-Cy™5.5	50 tests	341654
CD8 (RPA-T8) Alexa Fluor® 488	50 tests	557704



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