Protein Phosphorylation Analysis Using Flow Cytometry

Cell Signaling Applications on the BD Accuri™ C6

Features

- Flow cytometric method of measuring protein phosphorylation
- Provides complementary data to Western blot analysis with a simpler protocol
- Can assess phosphorylation in multiple subpopulations of a heterogeneous sample

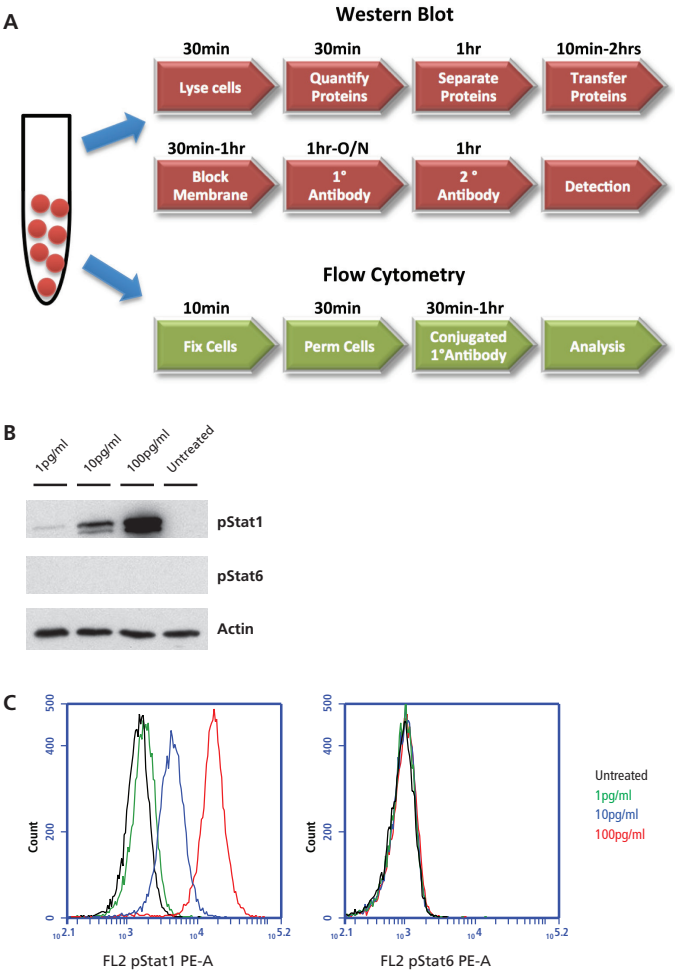

Figure 1. Phosphorylation analysis using Western blot and flow cytometry

U-937 cells (ATCC® CRL1593.2™, human histiocytic lymphoma) were stimulated with varying doses of BD Pharmingen™ Recombinant Human IFN-γ (1–100 pg/mL) for 15 minutes. **A.** Phosphorylation of Stat1 and Stat6 was assessed using Western blot (BD Transduction Laboratories™ antibodies) or flow cytometry (BD Phosflow™ antibodies) on the BD Accuri C6 according to the protocols shown. For flow cytometry analysis, cells were fixed with BD Phosflow™ Fix Buffer I and permeabilized with BD Phosflow™ Perm Buffer III. Results were consistent using both Western blot (**B**) and flow cytometry (**C**), showing a dose-dependent phosphorylation of Stat1 but not Stat6. Compared to Western blot, the flow cytometry protocol was simpler and took less time.

Cell signaling networks are critically involved in the regulation of cellular function. Cell survival, growth, and differentiation are tightly regulated through phosphorylation and dephosphorylation of key proteins in signaling cascades.

Flow cytometry provides a fast, accurate, and powerful method for studying cell signaling, and can complement the traditional use of Western blot. Using BD Phosflow™ technology on the BD Accuri™ C6 personal flow cytometer offers several advantages for cell signaling studies. The protein phosphorylation data provided by this method demonstrates equivalent specificity to Western blot. Yet the protocol is simpler, shorter, and less time-consuming (Figure 1), and can provide additional insights that supplement what is learned from Western blot.

In the basic protocol, cells of interest are stimulated to induce a signaling response, and fixed to preserve their phosphorylation states. The cells are permeabilized using BD Phosflow™ permeabilization buffers, stained with antibodies to the phosphorylated protein of interest, and analyzed on the BD Accuri C6. For higher throughput, cells can be stimulated, fixed, permeabilized, and stained in 96-well plates, and acquired and analyzed using the BD CSampler™ automation accessory. Figure 1 shows the comparative analysis of Stat1 (pY701) and Stat6 (pY641) phosphorylation in stimulated U-937 cells by Western blot and flow cytometry.

Unlike lysate-based approaches, flow cytometry facilitates the detection and analysis of heterogeneous signaling responses in a mixed cell population such as in whole blood. Thus, it is possible to distinguish between a robust protein phosphorylation response within a small population of cells vs a smaller but more homogeneous response. With the addition of fluorescent antibodies to specific cell subset markers, signaling responses mediated by protein phosphorylation can be detected at the single-cell level within distinct cell populations. In contrast, a Western blot analysis of a heterogeneous sample would measure only the *average* expression of the protein of interest among all the different subsets of cells.

As shown in Figure 2, the BD Phosflow™ Human Monocyte/NK Cell Activation Kit (Cat. No. 562089) was used to assess signaling responses to IL-6 stimulation of monocytes and B, T, and NK cells. The kit uses fluorochrome-labeled antibodies to surface markers, all excited by the blue laser and detected in the FL1, FL2, and FL3 channels of the BD Accuri C6, to differentiate among cell types. A fluorescent antibody to Stat3 (pY705), excited by the red laser and detected in the FL4 channel, assesses the signaling responses of all four cell types. Results showed that, upon stimulation with IL-6, phosphorylation of Stat3 occurred only in monocytes and a discrete subset of T cells.

Easy to use, simple to maintain, and affordable, the BD Accuri C6 personal flow cytometer is equipped with a blue laser, a red laser, two light scatter detectors, and four fluorescence detectors. A compact design, fixed alignment, and pre-optimized detector settings result in a system that is simple to use. A nonpressurized fluidics system enables kinetic measurements in real time. For walkaway convenience, the optional BD CSampler accessory offers automated sampling from 24-tube racks or multiwell plates.

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

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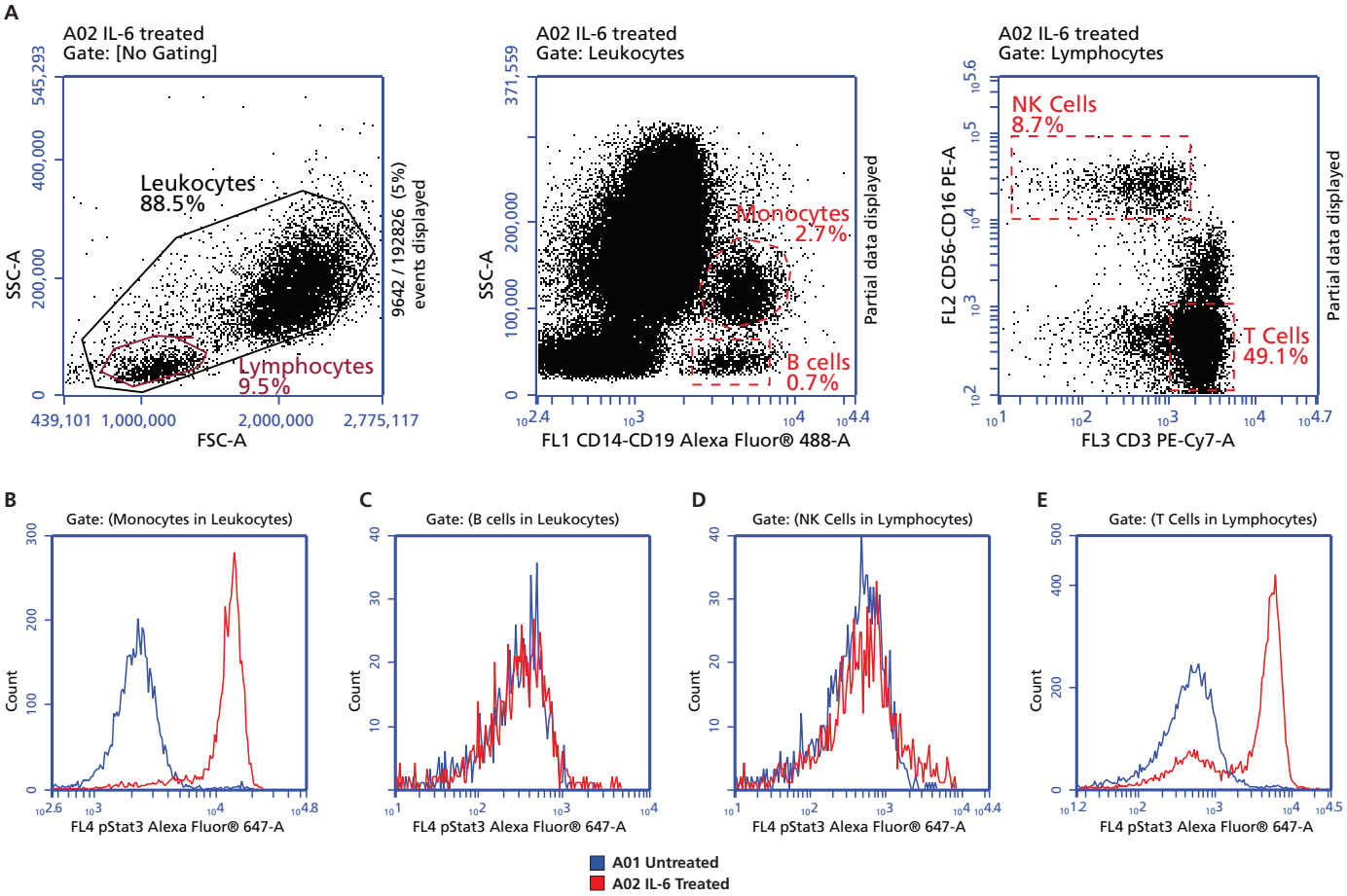

Figure 2. Multiparametric phosphorylation analysis

Human whole blood was stimulated with BD Pharmingen™ Recombinant Human IL-6 for 15 minutes or left untreated as a control. Using the BD Phosflow Human Monocyte/NK Cell Activation Kit, cells were fixed, permeabilized, and stained according to the kit protocol, and acquired and analyzed for Stat3 (pY705) phosphorylation on a BD Accuri C6. **Results: A.** Light scatter and CD3, CD14, CD16, CD56, and CD19 expression were used to identify T cells, monocytes, NK, and B cells. **B–E.** Each population was interrogated for expression of phosphorylated Stat3 after treatment with IL-6 (red histograms) vs untreated controls (blue histograms). Stat3 phosphorylation was observed in monocytes (**B**) and a subset of T cells (**E**) in response to IL-6 stimulation, but not in B cells (**C**) or NK cells (**D**).

Ordering information

Description	Clone	Cat. No.
BD Accuri™ C6 Flow Cytometer System	–	**653118**
BD CSampler™ Automated Sampling System (optional)	–	**653124**
BD Phosflow™ Human Monocyte/NK Cell Activation Kit	–	**562089**
BD Phosflow™ PE Mouse Anti-Stat1 (pY701)	4a	**612564**
BD Phosflow™ PE Mouse Anti-Stat6 (pY641)	18/P-Stat6	**612701**
BD Transduction Laboratories™ Purified Mouse Anti-Stat1 (pY701)	4a	**612232**
BD Transduction Laboratories™ Purified Mouse Anti-Stat6 (pY641)	18/P-Stat6	**611566**
BD Phosflow™ Fix Buffer I	–	**557870**
BD Phosflow™ Perm Buffer III	–	**558050**
BD Pharmingen™ Recombinant Human IFN-γ	–	**554616**
BD Pharmingen™ Recombinant Human IL-6	–	**550071**

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