BD Mouse T-Cell Cytokine and Treg Kits and Templates

Analysis of Mouse Th1, Th2, Th17, and Treg Cells on the BD Accuri™ C6 Flow Cytometer

Features

- Preconfigured kits, protocols, and software templates
- Simplify the measurement of key mouse T-cell cytokines and Treg markers on the BD Accuri C6
- Support studies involving mouse Th1, Th2, Th17, and Treg cells
- Enable quick and easy setup and analysis using the BD Accuri C6

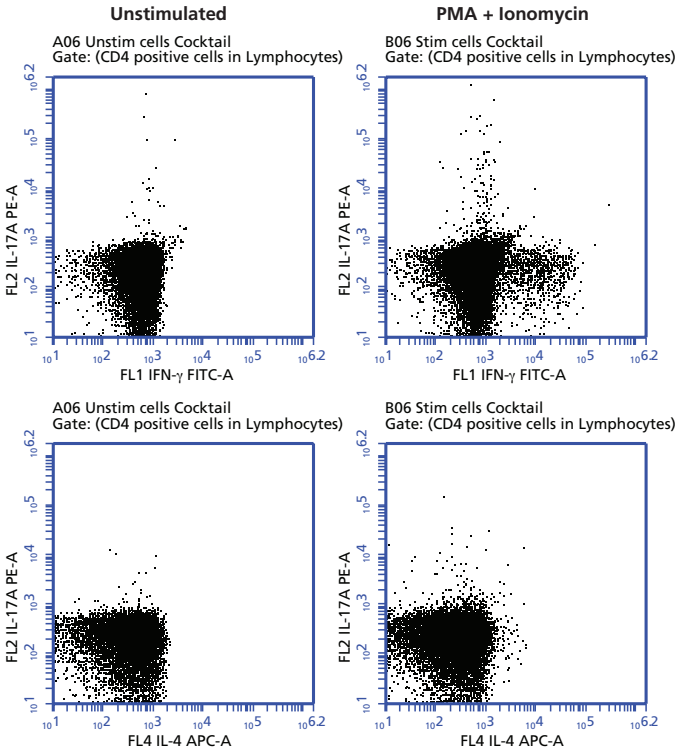

Figure 1. BD Pharmingen Mouse Th1/Th2/Th17 Phenotyping Kit (Cat. No. 560758) analysis on the BD Accuri C6

Mouse splenocytes were either unstimulated or activated with PMA (50 ng/mL) and Ionomycin (1 µg/mL) in the presence of monensin (BD GolgiStop™ protein transport inhibitor, included in the kit or Cat. No. 554724) for 5 hours at 37°C. Cells were then washed, fixed, and stained using the buffers in the kit (or alternately, Cat. No. 554714). Specifically, stimulated cells were incubated with BD Cytofix™ buffer for 10–20 minutes, pelleted, and incubated in BD Perm/Wash™ buffer for 15 minutes. After two washes with the Perm/Wash buffer, both stimulated and non-stimulated cells were stained according to the kit procedure, acquired on a BD Accuri C6 using the kit template, and analyzed using BD Accuri C6 software. **Results:** Density plots (gated on $CD4^+$ lymphocytes) show that stimulated cells (**right**) were more likely than unstimulated controls (**left**) to produce high levels of IFN-γ, IL-4, and IL-17A as they differentiate into Th1, Th2, and Th17 helper T cells, respectively.

BD mouse T-cell cytokine and Treg kits, protocols, and software templates for the BD Accuri™ C6 flow cytometer (Cat. No. 653118) simplify the detection of key cytokines, transcription factors, and cell surface markers using intracellular flow cytometry. BD offers two mouse T-cell kits—for studies involving Th1, Th2, Th17, and regulatory T cells (Tregs)—that include transport inhibitors, buffer systems, and fluorescent antibodies needed for acquisition and analysis. Free BD Accuri™ C6 software templates matched to each kit include predefined workspaces, markers, regions, gates, and parameter names for quick and easy setup and analysis.

The two kits are listed below. Figures 1 and 2 show data on the BD Accuri C6 using the preconfigured kits and software templates.

The **BD Pharmingen™ Mouse Th1/Th2/Th17 Phenotyping Kit** (Cat. No. 560758) is designed to assess the differentiation of naïve T cells into Th1, Th2, and Th17 cells, which secrete IFN-γ, IL-4, and IL-17A, respectively.

The **BD Pharmingen™ Mouse Th17/Treg Phenotyping Kit** (Cat. No. 560767) can identify both Th17 and Treg cells from a single sample, and includes optimized reagents necessary for successful intracellular staining. The panel is compatible with other markers to provide a base for Treg studies.

Intracellular flow cytometry offers distinct advantages over classical methods for the detection of cytokines secreted by T cells and other cells. It allows for the analysis of cytokines and other inflammatory mediators produced by multiple, phenotypically identified subpopulations within a heterogeneous sample. It can determine whether the cytokine production by an activated cell population is the result of a few cells producing large amounts of cytokine or a large cell population producing small quantities. Finally, it can easily measure multiple cytokines simultaneously for an individual cell.

Since cytokines typically are secreted proteins, they must first be trapped inside the cell using a protein transport inhibitor. The best choice of transport inhibitor varies by cytokine and by species.

Naïve $CD4^+$ T cells can differentiate into either Th17 effector/memory cells or inducible Tregs (iTregs), depending upon the balance of local cytokines, costimulatory molecules, antigen levels, and genetic factors. Tregs, which suppress the function of other T cells, play an important role in maintaining immune system homeostasis. The transcription factor FoxP3 is the classic marker for Tregs. Plasticity between iTregs and other T-cell subtypes, such as Th1 and Th17, could undermine their eventual therapeutic value.

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 (Cat. No. 653124) offers automated sampling from 24-tube racks or multiwell plates.

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

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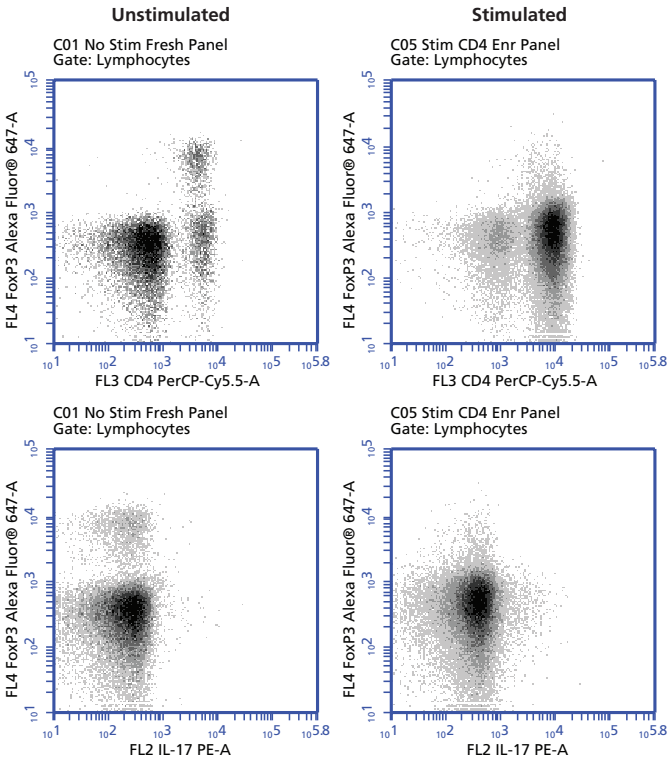

Figure 2. BD Pharmingen Mouse Th17/Treg Phenotyping Kit (Cat. No. 560767) analysis on the BD Accuri C6

Mouse splenocytes were either unstimulated or CD4-enriched, polarized, and stimulated with PMA (50 ng/mL) and Ionomycin (1 µg/mL) in culture for 4 hours to induce IL-17A production in the presence of monensin (BD GolgiStop™ protein transport inhibitor, included in the kit or Cat. No. 554724). The cells were then fixed, permeabilized using the mouse FoxP3 buffer set (included in the kit or Cat. No. 560409), and stained with the antibody cocktail according to the kit procedure. Samples were collected on the BD Accuri C6 using the kit template and analyzed using BD Accuri C6 software. Lymphocytes were identified and gated by light scatter profile (data not shown). **Results:** Compared to unstimulated cells (**left**), stimulated cells (**right**) expressed less FoxP3 (**top**) and more IL-17A (**bottom**) as they differentiated away from Tregs and toward a Th17 phenotype.

Ordering Information

All kits and their associated software templates are available at **bdbiosciences.com/go/templates**.

Description	Clone	Quantity	Number of Tests	Cat. No.
BD Pharmingen™ Mouse Th1/Th2/Th17 Phenotyping Kit, containing:				
Mouse CD4 PerCP-Cy™5.5	RM4-5	1 mL	50 tests	**560758**
Mouse IL-17A PE	TC11-18H10.1			
Mouse IFN-γ FITC	XMG1.2			
Mouse IL-4 APC	11B11			
BD Cytofix™ Fixation Buffer		100 mL		
BD Perm/Wash™ Buffer		25 mL		
BD GolgiStop™ Protein Transport Inhibitor (containing monensin)		0.7 mL		

BD Pharmingen™ Mouse Th17/Treg Phenotyping Kit, containing:				
Mouse CD4 PerCP-Cy™5.5	RM4-5	1 mL	50 tests	**560767**
Mouse IL-17A PE	TC11-18H10.1			
Mouse FoxP3 Alexa Fluor® 647	MF23			
Mouse FoxP3 Fixation Concentrate (20X)		10 mL		
Mouse FoxP3 Permeabilization Concentrate (5X)		80 mL		
BD GolgiStop™ Protein Transport Inhibitor (containing monensin)		0.7 mL		

Related Products

Description	Cat. No.
BD Pharmingen™ Human Th1/Th2/Th17 Phenotyping Kit	**560751**
BD Pharmingen™ Human Th17/Treg Phenotyping Kit	**560762**
BD Accuri™ C6 Flow Cytometer System	**653118**
BD CSampler™ Automated Sampling System	**653124**

Class 1 Laser Product.

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

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