BD Intracellular T-Cell Kits and Templates

Human Cytokine Analysis on the BD Accuri™ C6 Flow Cytometer

Features

- Preconfigured kits, protocols, and software templates to measure key human T-cell cytokines and surface markers on the BD Accuri C6
- Support studies involving production of human cytokines IFN-γ, IL4, and IL-17A
- Enable quick and easy setup and analysis using the BD Accuri C6

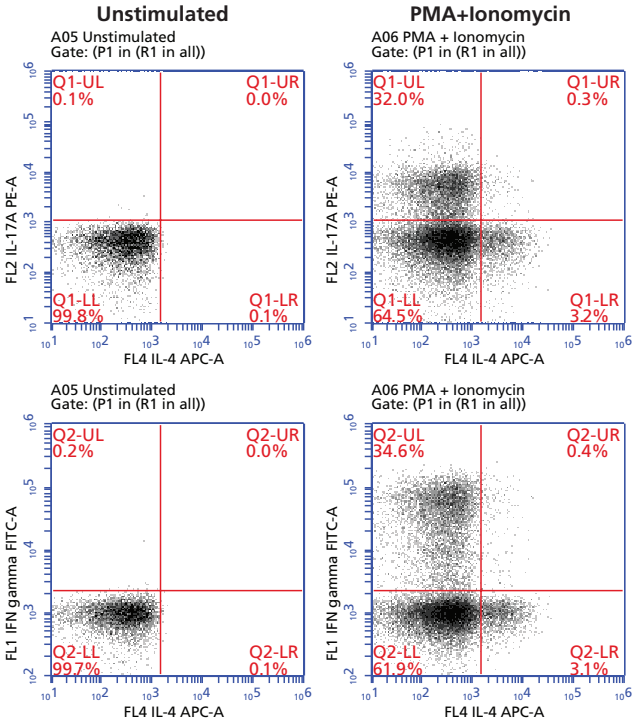

Figure 1. BD Pharmingen Human Th1/Th2/Th17 Phenotyping Kit (Cat. No. 560751) analysis on the BD Accuri C6.

Purified human peripheral blood mononuclear cells (PBMCs) were stimulated with PMA/Ionomycin (at 50 ng/mL and 1 µg/mL, respectively) in the presence of BD GolgiStop™ protein transport inhibitor (provided in the kit or Cat. No. 554724) for 5 hours at 37°C. Cells were stained according to the kit procedure, acquired on a BD Accuri C6 flow cytometer 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.

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

BD T-cell cytokine kits, protocols, and software templates for the BD Accuri™ C6 flow cytometer simplify the detection of cytokines and cell surface markers. BD offers three T-cell cytokine kits (for studies involving human Th1/Th2/Th17 cells, human IFN-γ/CD69/CD8/CD3, and human IFN-γ/IL-4) that include transport inhibitors, buffer systems, and fluorescent antibodies needed for acquisition and analysis. 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 three kits are listed below. Figures 1–3 show data on the BD Accuri C6 using the preconfigured kits and software templates.

The **BD Pharmingen™ Human Th1/Th2/Th17 Phenotyping Kit** (Cat. No. 560751) 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 FastImmune™ Human IFN-γ/CD69/CD8/CD3 Kit** (Cat. No. 346048) allows for the measurement of activated $CD8^+$ cytotoxic T cells' cytokine response to specific antigen stimulation in vitro, or to non-specific stimulation such as PMA+Ionomycin.

The **BD FastImmune™ Human IFN-γ/IL-4 Kit** (Cat. No. 340456) enables rapid characterization of activated cells expressing cytokines.

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. Once trapped, most cytokines are relatively accessible using the gentle BD Cytofix/Cytoperm™ fixation and permeabilization solution.

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. Compact design, fixed alignment, and pre-optimized detector settings result in a system that is simple to use, and 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.

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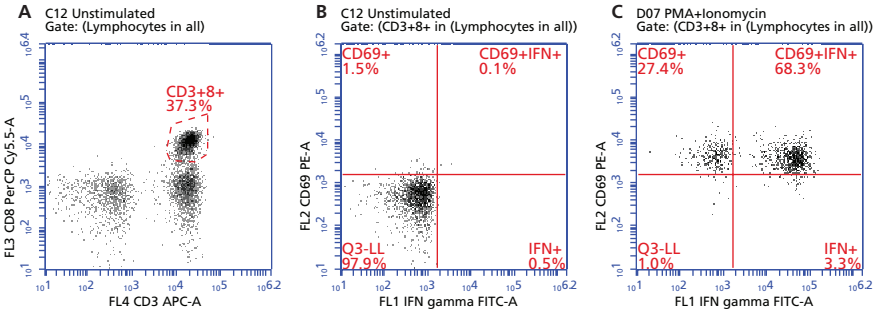

Figure 2. BD FastImmune IFN-γ/CD69/CD8/CD3 Kit (Cat. No. 346048) analysis on the BD Accuri C6.

Human whole blood was drawn into heparinized tubes and stimulated with PMA (10 ng/mL) and Ionomycin (1 µg/mL) for 5 hours at 37°C in the presence of 10 ng/mL of Brefeldin A protein transport inhibitor (BD GolgiPlug™, Cat. No. 555029). Samples were harvested, fixed, lysed, permeabilized, and stained according to the kit procedure. They were acquired on a BD Accuri C6 flow cytometer using the kit template and analyzed using BD Accuri C6 software, gating on lymphocytes using light scatter and then on $CD3^+CD8^+$ cytotoxic T cells **(A)**. **Results:** Compared to unstimulated controls **(B)**, stimulated cells **(C)** were more likely to produce high levels of the activation marker CD69. Most of these $CD69^+$ cells expressed IFN-γ as well. Data is representative of five donors.

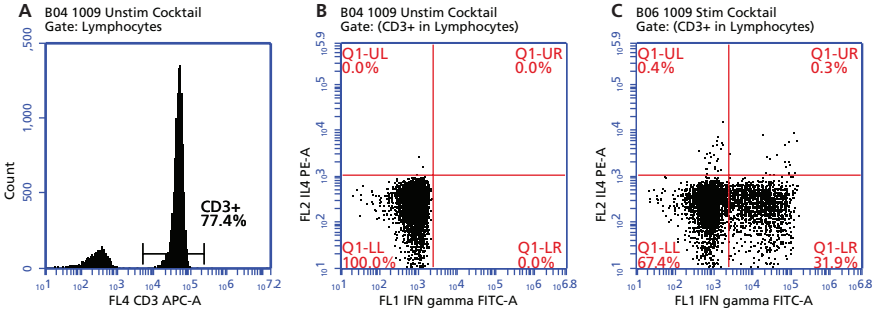

Figure 3. BD FastImmune IFN-γ/IL-4 Kit (Cat. No. 340456) analysis on the BD Accuri C6.

Human lysed whole blood was activated with PMA/Ionomycin (at 50 ng/mL and 1 µg/mL, respectively) in the presence of Brefeldin A protein transport inhibitor (BD GolgiPlug, Cat. No. 555029) for 4 hours at 37°C. Cells were washed, fixed, and permeabilized using the BD Cytofix/Cytoperm fixation/permeabilization solution kit procedure (Cat. No. 554714). After two washes with BD Perm/Wash™ buffer, the cells were stained according to the kit procedure and with CD3 APC (Cat. No. 555335) to identify T cells. They were acquired on a BD Accuri C6 flow cytometer using the kit template and analyzed using BD Accuri C6 software, gating on lymphocytes using light scatter and then on $CD3^+$ T cells **(A)**. **Results:** Compared to unstimulated controls **(B)**, stimulated cells **(C)** were more likely to produce high levels of IFN-γ (lower-right quadrant). Production of high levels of IL-4 was rare (upper-left and upper-right quadrants), but was also more common in stimulated than in unstimulated cells.

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 Human Th1/Th2/Th17 Phenotyping Kit containing:				
Human CD4 PerCP-Cy™5.5	SK3	1 mL	50 tests	**560751**
Human IL-17A PE	N49-653			
Human IFN-γ FITC	B27			
Human IL-4 APC	MP4-25D2			
BD Cytofix™ Fixation Buffer		100 mL		
BD Perm/Wash Buffer		25 mL		
BD GolgiStop Protein Transport Inhibitor		0.7 mL		

BD FastImmune Human IFN-γ/CD69/CD8/CD3 Kit containing:				
Human IFN-γ FITC	25723.11	1 mL	50 tests	**346048**
Human CD69 PE	L78			
Human CD8 PerCP-Cy5.5	SK1			
Human CD3 APC	SK7			

BD FastImmune Human IFN-γ/IL-4 Kit containing:				
Human IFN-γ FITC	25723.11	1 mL	50 tests	**340456**
Human IL-4 PE	3010.211	1 mL		

Related Kits

Description	Cat. No.
BD Pharmingen™ Human Th17/Treg Phenotyping Kit	**560762**
BD Pharmingen™ Mouse Th1/Th2/Th17 Phenotyping Kit	**560758**

Class 1 Laser Product.

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

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