BD Regulatory T-Cell Kits and Templates

Treg Identification and Analysis on the BD Accuri™ C6 Flow Cytometer

Features

- Preconfigured kits, protocols, and software templates to identify Tregs on the BD Accuri C6 flow cytometer
- Support studies involving human CD4, CD25, CD127, and/or FoxP3
- Enable quick and easy setup and analysis using the BD Accuri C6

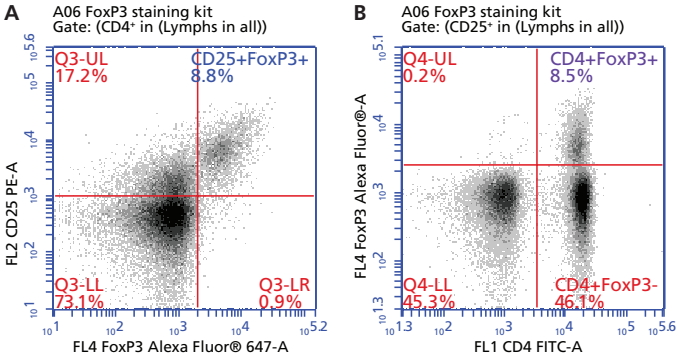

Figure 1. BD Pharmingen FoxP3 Staining Kit (Cat. No. 560132) analysis on the BD Accuri C6.

Human peripheral blood mononuclear cells (PBMCs) were stained with CD4 FITC and CD25 PE, fixed, permeabilized, and stained for intracellular content with FoxP3 Alexa Fluor® 647 according to the kit procedure. Samples were collected on the BD Accuri C6 flow cytometer using the kit template and analyzed using BD Accuri C6 software. **Results: A.** Gating on $CD4^+$ lymphocytes, Tregs are $CD25^+FoxP3^+$. **B.** Gating on $CD25^+$ lymphocytes, Tregs are $CD4^+FoxP3^+$. Both plots depict equivalent percentages of Treg cells.

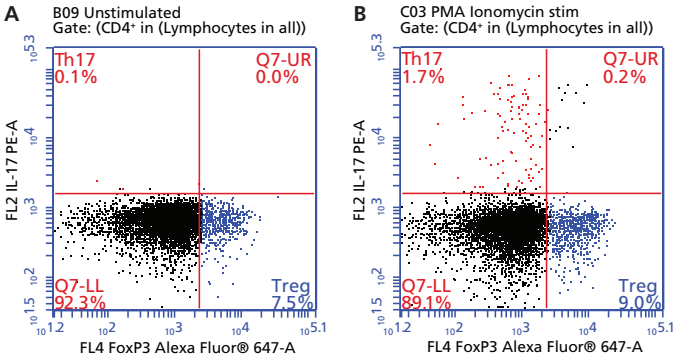

Figure 2. BD Pharmingen Human Th17/Treg Phenotyping Kit (Cat. No. 560762) analysis on the BD Accuri C6.

Human PBMCs were either unstimulated or stimulated with PMA and Ionomycin for 4 hours in the presence of BD GolgiStop™ protein transport inhibitor (included in the kit or Cat. No. 554724). The cells were fixed, permeabilized, and stained with the antibody cocktail according to the kit procedure. Samples were collected on the BD Accuri C6 flow cytometer using the kit template and analyzed using BD Accuri C6 software. $CD4^+$ lymphocytes were identified and gated by light scatter profile and fluorescence (data not shown). **Results:** Compared to unstimulated cells **(A)**, more PBMCs stimulated with PMA and Ionomycin **(B)** expressed IL-17, and some expressed FoxP3 as well.

BD regulatory T-cell (Treg) kits, protocols, and software templates for the BD Accuri™ C6 flow cytometer simplify the detection and characterization of Tregs using intracellular and surface markers. BD offers three Treg kits (for studies involving human CD4, CD25, CD127, and/or FoxP3) that include buffer systems and fluorescent antibodies needed for acquisition and analysis. The panels are compatible with other markers to provide a base for Treg studies. 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™ FoxP3 Staining Kit** (Cat. No. 560132) provides two different analysis strategies for identifying Treg populations, using FoxP3 expression in combination with CD4 and CD25.

The **BD Pharmingen™ Human Th17/Treg Phenotyping Kit** (Cat. No. 560762) can identify both Th17 and Tregs from a single sample, and includes optimized reagents necessary for successful intracellular staining.

The **BD Pharmingen™ Human Regulatory T-Cell Cocktail** (Cat. No. 560249) is a one-step, premixed cocktail for convenient, optimized analysis of Treg populations using surface markers, without the need to permeabilize cells.

Regulatory T cells, which suppress the function of other T cells, play an important role in maintaining immune homeostasis. The transcription factor FoxP3 is the classic marker for Tregs. Because FoxP3 staining requires fixation and permeabilization of cells, the cells cannot be used for further experiments. However, the surface marker CD127 is negatively correlated with FoxP3 and, when combined with CD4 and CD25 (as in the BD Pharmingen Human Regulatory T-Cell Cocktail), enables the identification of Tregs without permeabilization.

The discovery of FoxP3 has led to the characterization of different types of Tregs. For example, natural Tregs (nTregs) emerge from the thymus "naturally" expressing high levels of FoxP3. In contrast, adaptive or inducible Tregs (iTregs) express FoxP3 only after antigenic stimulation in the presence of cognate antigen and specialized immunoregulatory cytokines. iTregs are reported to be more plastic, with the ability to convert to other T-cell subtypes such as Th1 and Th17, which could undermine their eventual therapeutic value. All three Treg kits can be used with antibodies to other markers such as CD45RA to study Treg plasticity.

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.

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

BD Regulatory T-Cell Kits and Templates

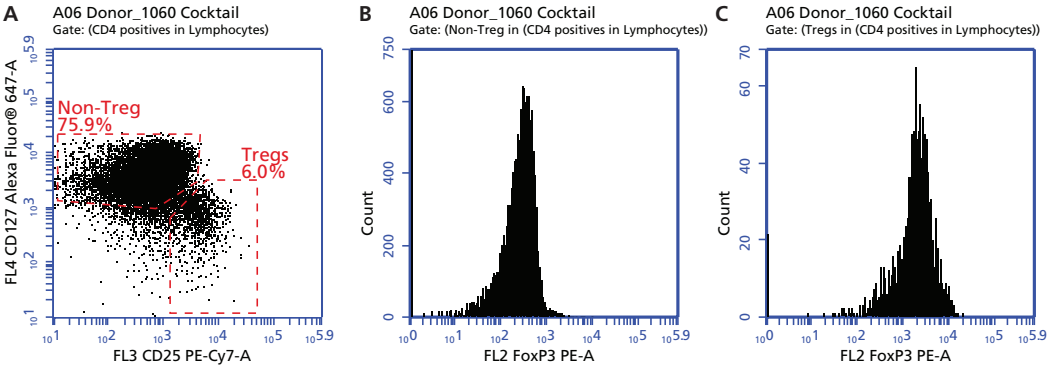

Figure 3. BD Pharmingen Human Regulatory T-Cell Cocktail (Cat. No. 560249) analysis on the BD Accuri C6.

Human PBMCs were stained according to the kit procedure. The cells were then fixed, lysed, and permeabilized using the BD Pharmingen™ Human Transcription Factor Buffer Set (Cat. No. 562574) and stained with PE-conjugated anti-human BD Pharmingen™ FoxP3 monoclonal antibody (Cat. No. 560082). Samples were collected on the BD Accuri C6 flow cytometer using the kit template and analyzed using BD Accuri C6 software. $CD4^+$ lymphocytes were identified and gated by light scatter profile and fluorescence (data not shown). **Results: A.** A CD25 vs CD127 plot was used to identify $CD25^{bright}CD127^{dim}$ Tregs and non-Treg $CD4^+$ cells. **B, C.** Tregs identified in Panel A were validated using FoxP3 staining. $CD4^+$ cells identified as Tregs **(C)** expressed higher levels of FoxP3 than did non-Tregs **(B)**.

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™ FoxP3 Staining Kit containing:				
Human FoxP3 Alexa Fluor® 647	259D/C7	1 vial	100 tests	**560132**
Human CD4 FITC	RPA-T4	1 vial		
Human CD25 PE	M-A251	1 vial		
Human FoxP3 Buffer A (10X)		25 mL		
Human FoxP3 Buffer A (50X)		1 vial		
BD Pharmingen™ Human Th17/Treg Phenotyping Kit containing:				
Human CD4 PerCP-Cy™5.5		1 mL	50 tests	**560762**
Human IL-17 PE				
Human FoxP3 Alexa Fluor® 647				
Human FoxP3 Buffer A (10X)		25 mL		
Human FoxP3 Buffer A (50X)		1 mL		
BD GolgiStop™ Protein Transport Inhibitor (containing monensin)		0.7 mL		
BD Pharmingen™ Regulatory T-Cell Cocktail containing:				
Human CD4 FITC	SK3	20 µL/test	50 tests	**560249**
Human CD25 PE-Cy™7	2A3			
Human CD127 Alexa Fluor® 647	hIL-7R-M21			

Related Kits

Description	Cat. No.
BD Pharmingen™ FoxP3 Staining Kit (APC, FITC, PE)	**560133**
BD Pharmingen™ FoxP3 Staining Kit (APC, Alexa Fluor® 488, PE)	**560131**
BD Pharmingen™ Human Th1/Th2/Th17 Phenotyping Kit	**560751**
BD Pharmingen™ Mouse Th17/Treg Phenotyping Kit	**560767**
BD Pharmingen™ Mouse Th1/Th2/Th17 Phenotyping Kit	**560758**

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