

# BD Human Regulatory T Cell Sorting Kit

## Simplify identification and isolation of viable Tregs

### Features

Contains a three-color reagent in an easy-to-use, one-step preoptimized kit

Enhances enrichment of CD4<sup>+</sup>CD25<sup>+</sup>CD127<sup>low</sup> viable, expandable Treg populations

Contains CD45RA as an optimized drop-in for the study of Treg subsets

The BD Pharmingen™ Human Regulatory T Cell Kit is a three-color reagent that provides an optimized, reproducible method for the analysis and isolation of CD4<sup>+</sup>CD25<sup>int/high</sup>CD127<sup>low</sup> viable natural regulatory T-cell (Treg) populations. The reagent kit includes anti-human CD4 PerCP-Cy™5.5 (clone L200), anti-human CD25 PE (clone 2A3), and anti-human CD127 Alexa Fluor® 647 (clone 40131). The one-step premixed cocktail simplifies Treg identification and significantly enhances enrichment of viable Treg populations by two to four times compared to gating on CD25<sup>high</sup> alone.

This kit also contains a vial of anti-human FITC-CD45RA (clone HI100) for further characterization of Treg subsets.

### A flexible and effective surface marker combination

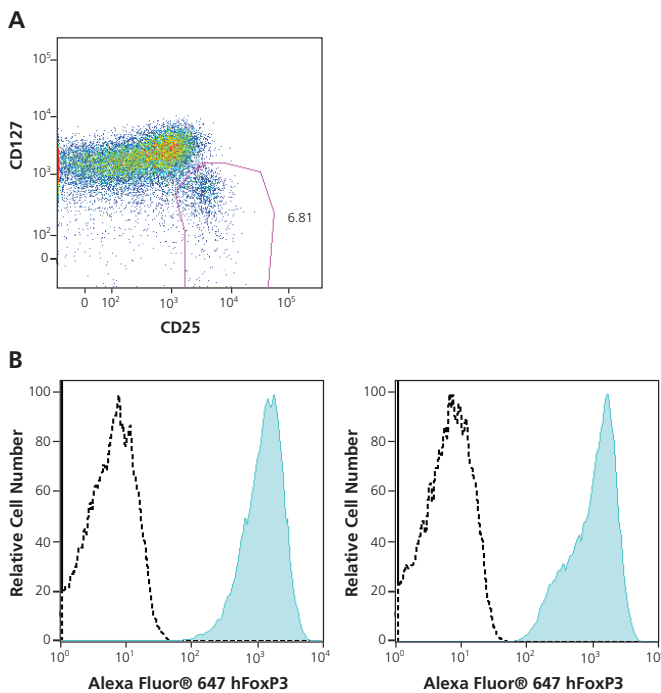
A common approach to the identification and isolation of Treg cells is the use of CD4<sup>+</sup> and CD25<sup>+</sup>. The Treg cell population represents 2–10% of all CD4<sup>+</sup> cells. Only a subset of CD25<sup>+</sup>, mostly cells with the highest levels of expression, is considered to be Tregs.<sup>1</sup> The capture and analysis of these cells with multiple markers increases the resolution for interrogating Treg fractions.

Lack of consensus regarding the definition of high and low levels of CD25 expression by Tregs has affected the ability to obtain consistent amounts of viable human Tregs via flow cytometric cell sorting. Ambiguity in this area led researchers to select only cells expressing the highest level of CD25, resulting in a reduction of total Treg recovery and requiring large quantities of samples and reagents. FoxP3, a transcription factor, is currently considered to be the most accepted marker of Tregs when expression level is high. However, detection of FoxP3 requires permeabilizing the cell membrane, preventing the use of these cells for expansion and downstream applications.

### Maximizing recovery of viable Tregs

CD127 inversely correlates with FoxP3 expression.<sup>2</sup> This provides an extracellular marker for the isolation of the CD25 intermediate and bright population without relying on CD25<sup>high</sup> expression alone for obtaining cells with a FoxP3 phenotype. The CD127<sup>low</sup> phenotype might also be especially useful in experimental conditions for which it is known that CD25 expression is downregulated or CD25<sup>+</sup> T cells are ablated. Separate studies published by Miyara<sup>3,5</sup> and Hoffmann<sup>4</sup> have described the enrichment of nTreg cells in the CD45RA<sup>+</sup> subpopulation, suggesting that CD45RA is a useful marker for the identification and isolation of subpopulations of Tregs.

The Human Regulatory T Cell Kit allows the fractionation of Treg populations according to CD45RA expression, to maximize flexibility for the user. In studies performed by BD, samples from healthy donors were separated into CD45RA<sup>+</sup> and CD45RA<sup>-</sup>



**Figure 1. Four-color analysis of the expression of CD4, CD25, CD127, and CD45RA on sorted peripheral blood mononuclear cells (PBMCs).** PBMCs were stained with the Human Regulatory T Cell Sorting Kit (Cat. No. 560753) and then sorted on a BD FACSAria™ cell sorter. Lymphocytes were identified by light scatter profile and CD4 positive expression and sorted for CD4 Treg profile (panel A). The CD45RA negative and positive fractions (data not shown) were sorted, then separately expanded. Fractions were fixed and permeabilized using the BD Pharmingen Human FoxP3 Buffer Set (Cat. No. 560098), and stained with isotype control (Cat. No. 557732) and conjugated anti-human FoxP3 monoclonal antibody (Cat. No. 560132). A) Data representing the CD25 and CD127 expression profile of the CD4 positive cells prior to gating on CD45RA populations for sorting. B) Data showing hFoxP3 expression on sorted CD25<sup>high</sup> CD127<sup>low</sup> Tregs (blue solid histogram) and isotype control (dashed line) for the CD45RA<sup>+</sup> and CD45RA<sup>-</sup> fractions respectively. Acquisition and analysis were performed on a BD™ LSR II system.

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populations and expanded in culture for 14 days. These fractions yielded the highest percentage of FoxP3 cells possible, with only minor differences in FoxP3 intensity and percent positive (Figure 1). This data suggests a high FoxP3<sup>+</sup> expression in the CD4<sup>+</sup>CD25<sup>+</sup>CD127<sup>low</sup> populations was maintained post expansion.

### Two Treg products available to suit your research needs

In addition to the Human Regulatory T Cell Sorting Kit, BD also carries the Human Regulatory T Cell Cocktail (Cat. No. 560249) composed of anti-human CD4 FITC (clone SK3), anti-human CD25 PE-Cy<sup>TM</sup>7 (clone 2A3), and anti-human CD127 Alexa Fluor® 647 (clone hIL-7R-M21) for convenient, optimized analysis of Treg populations without the need for permeabilizing cells.

### References

- Seddiki N, Santner-Nanan B, Martinson J, et al. Expression of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and activated T cells. *J Exp Med*. 2006;203:1693-1700.
- Liu W, Putnam AL, Xu-Yu Z, et al. CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4<sup>+</sup> T reg cells. *J Exp Med*. 2006; 203:1701-1711.
- Miyara M, Wing K, Sakaguchi S. Therapeutic approaches to allergy and autoimmunity based on FoxP3<sup>+</sup> regulatory T-cell activation and expansion. *J Allergy Clin Immunol*. 2009;123:749-755.
- Hoffmann P, Boeld TJ, Eder R, et al. Loss of FOXP3 expression in natural human CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells upon repetitive in vitro stimulation. *Eur J Immunol*. 2009;39:1088-1097.
- Miyara M, Yoshioka Y, Kitoh A, et al. Functional delineation and differentiation dynamics of human CD4<sup>+</sup> T cells expressing the FoxP3 transcription factor. *Immunity*. 2009;30:899-911.

### Ordering Information

Description	Clone	Isotype	Format	Quantity	Cat. No.
<b>BD Pharmingen Human Regulatory T Cell Sorting Kit containing</b>					
CD4	L200	Mouse IgG <sub>1</sub> , κ	PerCP-Cy <sup>TM</sup> 5.5	10 Tests 1 Test = 1 x 10 <sup>6</sup> cells	560753
CD25	2A3	Mouse IgG <sub>1</sub> , κ	PE		
CD127	40131.111	Mouse IgG <sub>1</sub>	Alexa Fluor® 647		
Also includes CD45RA	HI100	Mouse IgG <sub>2b</sub> , κ	FITC		
<b>BD Pharmingen Human Regulatory T Cell Cocktail containing</b>					
CD4	SK3	Mouse IgG <sub>1</sub> , κ	FITC	50 Tests	560249
CD25	2A3	Mouse IgG <sub>1</sub> , κ	PE-Cy <sup>TM</sup> 7		
CD127	hIL-7R-M21	Mouse IgG <sub>1</sub> , κ	Alexa Fluor® 647		

**Note:** As with any tandems, there is a risk of degradation of the PE-Cy7 fluorophore. Users are advised against using PE to detect dim/weakly expressed antigens because there is a risk of signal spillover.

### Related Human Reagents

Description	Clone	Isotype	Format	Quantity	Cat. No.
CD39	TÜ66	Mouse IgG <sub>2b</sub> , κ	PE	100 Tests	555464
			APC	100 Tests	560239
CD45RA	HI100	Mouse IgG <sub>2b</sub> , κ	FITC	100 Tests	555488
			PE	100 Tests	555489
			APC	100 Tests	550855
			PE-Cy <sup>TM</sup> 5	100 Tests	555490
CD73	AD2	Mouse IgG <sub>1</sub> , κ	Purified	0.1 mg	550256
			PE	100 Tests	550257
CD127	hIL-7R-M21	Mouse IgG <sub>1</sub> , κ	Biotin	100 Tests	558633
			Alexa Fluor® 647	100 Tests	558598
			PE	100 Tests	557938
HLA-DR	L243 (G46-6)	Mouse IgG <sub>2b</sub> , κ	FITC	100 Tests	555811
			PE	100 Tests	555812
			PE-Cy <sup>TM</sup> 5	100 Tests	555813
			PE-Cy <sup>TM</sup> 7	100 Tests	335795
			APC-Cy <sup>TM</sup> 7	100 Tests	335796
			PerCP-Cy <sup>TM</sup> 5.5	50 Tests	339194
FoxP3	259D/C7	Mouse IgG <sub>1</sub>	PE	100 Tests	560046
			Alexa Fluor® 488	100 Tests	560047
			Alexa Fluor® 647	100 Tests	560045
			BD Horizon <sup>TM</sup> V450	120 Tests	560459
FoxP3 Staining Kit - Alexa Fluor® 488	259D/C7, RPA-T4, M-A251	Mouse IgG <sub>1</sub> , Mouse IgG <sub>1</sub> , κ, Mouse IgG <sub>1</sub> , κ	Alexa Fluor® 488, APC, PE	100 Tests	560131
FoxP3 Staining Kit - Alexa Fluor® 647	M-A251, 259D/C7, RPA-T4	Mouse IgG <sub>1</sub> , κ, Mouse IgG <sub>1</sub> , Mouse IgG <sub>1</sub> , κ	PE, Alexa Fluor® 647, FITC	100 Tests	560132
FoxP3 Staining Kit - PE	259D/C7, RPA-T4, M-A251	Mouse IgG <sub>1</sub> , Mouse IgG <sub>1</sub> , κ, Mouse IgG <sub>1</sub> , κ	PE, FITC, APC	100 Tests	560133
Human FoxP3 Buffer Set				100 Tests	560098



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