

# BD Human Regulatory T Cell Cocktail (CD4/CD25/CD127)

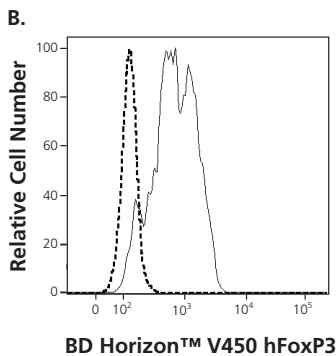
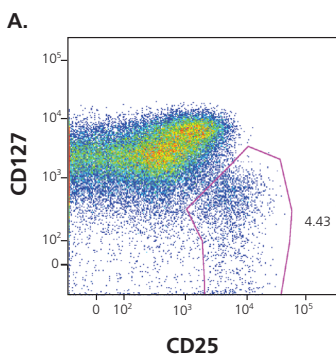
## Simplify identification and isolation of viable Tregs

### Features

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

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

Can be used in combination with other reagents on most BD FACSTM brand flow cytometers, including the BD FACSCaliburTM system



**Three-color analysis of the expression of CD4, CD25, and CD127 on peripheral blood mononuclear cells (PBMCs).** PBMCs were stained with either an Isotype Control (Cat. Nos. 557872/555909; data not shown) or Human Regulatory T Cell Cocktail (Cat. No. 560249). The PBMCs were then fixed and permeabilized using the BD Pharmingen Human FoxP3 Buffer Set (Cat. No. 560098), and stained with BD Horizon<sup>TM</sup> V450 conjugated anti-human FoxP3 monoclonal antibody (Cat. No. 560459).

During data analysis, lymphocytes were identified by light scatter profile and CD4 positive expression. **A)** Data representing the CD25 and CD127 expression profile of the CD4 positive cells. **B)** Data showing hFoxP3 expression on CD127<sup>low</sup>/CD25<sup>high</sup> Tregs (solid line) and other T cells (dashed line). Flow cytometry was performed on a BD<sup>TM</sup> LSR II system.

The BD Pharmingen<sup>TM</sup> Human Regulatory T Cell Cocktail 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> live natural regulatory T cell (Treg) populations. The reagent cocktail includes 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). The one-step premixed cocktail simplifies Treg identification and significantly enhances enrichment of live Treg populations by 2 to 4 times compared to gating on CD25<sup>high</sup> alone.

### A flexible and effective surface marker combination

Tregs are a rare cell population representing 5–10% of all CD4<sup>+</sup> cells. The capture and analysis of these cells requires multiple markers. A common approach to the identification and isolation of Treg cells is the use of CD4<sup>+</sup> and CD25<sup>+</sup>. Only a subset of CD25<sup>+</sup>, mostly those with the highest levels of expression, are Tregs.

In the past, the 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 the cells expressing the highest level of CD25, resulting in reduction of total Treg recovery while using large quantities of samples and reagents.

FoxP3, a transcription factor, is considered to be a currently accepted marker of Tregs. However, detection of FoxP3 requires permeabilizing the cell membrane, preventing the use of these cells in downstream applications.

### Maximizing recovery of viable Tregs

In 2006, CD127 was identified as a suitable marker for Treg isolation. When used during flow cytometric sorting experiments, a gating strategy relying on CD4<sup>+</sup>CD25<sup>int/high</sup>CD127<sup>low</sup> cells enriches the recovery of FoxP3 Tregs 2 to 4 times compared to gating on CD25<sup>high</sup> cells alone. A simple-to-use reagent, the BD Pharmingen brand Human Regulatory T Cell Cocktail streamlines discovery while maximizing the isolation and recovery of viable Tregs.

Visit [bdbiosciences.com/treg](http://bdbiosciences.com/treg) for more information.



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## References

1. 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(7):1693-1700.
2. Liu W, Putnam AL, Xu-Yu Z, et al. CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4<sup>+</sup> Treg cells. *J Exp Med.* 2006;203(7):1701-1711.

## Ordering Information

| Product description   | Clone                    | Isotype  | Format  | Quantity | Cat. No.      |
|---|--------------------------|--|---|----------|---------------|
| <b>BD Pharmingen Human Regulatory T Cell Cocktail containing CD4<br/>CD25<br/>CD127</b> | SK3<br>2A3<br>hIL-7R-M21 | Mouse IgG <sub>1</sub> , κ<br>Mouse IgG <sub>1</sub> , κ<br>Mouse IgG <sub>1</sub> , κ | FITC<br>PE-Cy <sup>TM</sup> 7<br>Alexa Fluor® 647 | 50 Tests | <b>560249</b> |

**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 as there is a risk of signal spillover.

## Related Human Reagents

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

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Class I (1) laser product.

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