Human Regulatory T Cell Cocktail

**Product Information**

**Material Number:** 560249  
**Size:** 50 tests  
**Vol. per Test:** 20 ul  
**Storage Buffer:** Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

**Description**

Cocktail Component | Clone | Isotype
--- | --- | ---
FITC anti-Human CD4 | SK3 | IgG1, κ
PE-Cy7 anti-Human CD25 | 2A3 | IgG1, κ
Alexa Fluor® 647 anti-Human CD127 | HIL-7R-M21 | IgG1, κ

The Human Regulatory T cell cocktail is a three color reagent used to identify the natural T regulatory cell (nTreg) population. An expression pattern of CD4<sup>+</sup>CD25<sup>int/bright</sup>CD127<sup>dim</sup> closely correlates to the expression of the transcription factor Forkhead box P3 (FoxP3). FoxP3 is considered a specific marker to T regulatory cells (Tregs). The SK3 antibody recognizes CD4, an antigen expressed primarily on helper/inducer T lymphocytes, although this antigen is also present in low density on monocytes. The 2A3 antibody recognizes CD25, an antigen also known as the human low-affinity interleukin-2 receptor (IL-2R). The CD25 antigen is present on a subset of peripheral blood lymphocytes. Antigen density increases on phytohemagglutinin (PHA)-, concanavalin A (Con A)-, and CD3-activated T lymphocytes; T lymphocytes from mixed lymphocyte cultures; and human T-lymphocyte leukemia virus (HTLV)-infected T-lymphocyte leukemia lines. hIL-7R-M21 reacts with the 60 - 90 kDa glycoprotein, CD127. CD127 is also known as the IL-7 receptor alpha (IL-7R<sub>α</sub>). The receptor is a heterodimer composed of the CD127 and the common gamma chain, shared by other cytokine receptors (IL-2R, IL-4R, IL-9R, and IL-15R). CD127 is expressed on thymocytes, T- and B-cell progenitors, mature T cells, and some lymphoid and myeloid cells.

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. No. 557872/555909; data not shown) or Human Regulatory T Cell Cocktail (Cat. No. 560249). The PBMCs were then fixed, lysed and permeabilized using BD Pharmingen Human FoxP3 Buffer Set (Cat. No. 560098) and stained with PE conjugated anti-human FoxP3 monoclonal antibody (Cat. No.560082). During data analysis, lymphocytes were identified by light scatter profile and CD4 positive expression. The figure on the left represents the CD25 and CD127 expression profile of the CD4 positive cells. The panel on the right shows hFoxP3 expression on CD127<sup>dim</sup>CD25<sup>bright</sup> T regulatory cells (solid line) and other T cells (dashed line). Flow cytometry was performed on a BD FACSCanto™.
Preparation and Storage
The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography. The antibody was conjugated with R-PE under optimum conditions, and unconjugated antibody and free PE were removed. The antibody was conjugated with PE-Cy7 under optimum conditions, and unconjugated antibody and free PE-Cy7 were removed. The antibody was conjugated with FITC under optimum conditions, and unreacted FITC was removed. Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Flow cytometry Tested During Development

Recommended Assay Procedure:
For an Application Note on how to isolate and characterize regulatory T cells, please refer to the following link:

Suggested Companion Products

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<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
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<tr>
<td>555899</td>
<td>Lysing Buffer</td>
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<tr>
<td>557872</td>
<td>PE-Cy™7 Mouse IgG1 κ Isotype Control</td>
<td>100 tests</td>
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<tr>
<td>560098</td>
<td>Human FoxP3 Buffer Set</td>
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<tr>
<td>560082</td>
<td>PE Mouse anti-Human FoxP3</td>
<td>25 tests</td>
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Product Notices
1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
3. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
4. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,486,616; 5,569,587; 5,569,766; 5,627,027.
5. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
6. Warning: Some APC-Cy7 and PE-Cy7 conjugates show changes in their emission spectrum with prolonged exposure to formaldehyde. If you are unable to analyze fixed samples within four hours, we recommend that you use BD™ Stabilizing Fixative (Cat. No. 338036).
7. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
8. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References