Technical Data Sheet

BB515 Mouse Anti-Human CD25

**Product Information**

- **Material Number:** 564467
- **Alternate Name:** IL-2R; IL2RA; IL-2Rα; TCGFR; TAC antigen; p55
- **Size:** 100 tests
- **Vol. per Test:** 5 µl
- **Clone:** 2A3
- **Immunogen:** Human Phytohemagglutinin-activated T Cells
- **Isotype:** Mouse (BALB/c) IgG1, κ
- **Reactivity:** QC Testing: Human
- **Workshop:** III A769,T153; IV A8
- **Storage Buffer:** Aqueous buffered solution containing ≤0.09% sodium azide.

**Description**

The 2A3 monoclonal antibody specifically binds to human CD25, the low-affinity alpha subunit of the Interleukin-2 Receptor (IL-2Rα). CD25 associates with CD122 (IL-2Rβ chain) and CD132 (common γ chain or γc) to form the high-affinity signal-transducing IL-2R complex. CD25 is expressed by subsets of thymocytes and peripheral blood lymphocytes including CD4+CD25+ regulatory T cells and memory T cells. CD25 antigen density increases on activated T cells including phytohemagglutinin (PHA)-, concanavalin A (Con A)-, and CD3-activated T lymphocytes. High levels of CD25 can be expressed by T lymphocytes from mixed lymphocyte cultures and by human T-lymphocyte leukemia virus (HTLV)-infected T-lymphocyte leukemia lines, for example, HUT-102. CD25 can also be expressed by activated B cells and macrophages. Recombinant IL-2 blocks the binding of the 2A3 antibody to PHA-activated T lymphocytes.

The antibody was conjugated to BD Horizon™ BB515 which was developed exclusively by BD Biosciences. With an excitation max of 490 nm and an emission max of 515 nm, BD Horizon BB515 can be excited by the 488 nm laser and detected in a standard FITC set (e.g. 530/30-nm filter). This dye provides a much brighter alternative to FITC with less spillover into the PE detector.

**Flow cytometric analysis of CD25 expression on unstimulated and stimulated human peripheral blood lymphocytes.**

- **Left and Middle Panels:** Human whole blood was stained with either BD Horizon™ BB515 Mouse IgG1, κ Isotype Control (Cat. No. 564416; Left Panel) or BD Horizon BB515 Mouse Anti-Human CD25 antibody (Cat. No. 564467/564468; Middle Panel). Erythrocytes were lysed with BD FACS Lysing Solution (Cat. No. 349202). Two-color flow cytometric contour plots showing the correlated expression patterns for Ig Isotype control staining (Left Panel) or CD25 expression (Middle Panel) versus Autofluorescence were generated for events with the forward and side light- scatter characteristics of intact lymphocytes.

- **Right Panel:** Human peripheral blood mononuclear cells were stimulated for 3 days with Phytohemagglutinin. The cells were stained with either BD Horizon™ BB515 Mouse IgG1, κ Isotype Control (dashed line histogram) or BD Horizon BB515 Mouse Anti-Human CD25 antibody (solid line histogram). The fluorescence histograms were derived from events with the forward and side light-scatter characteristics of viable lymphoblasts.

Flow cytometric analysis was performed using a BD™ LSR II Flow Cytometer System.

**Preparation and Storage**

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography. The antibody was conjugated with BD Horizon™ BB515 under optimum conditions, and unconjugated antibody and free BD Horizon BB515 were removed.
**Application Notes**

**Application**

Flow cytometry Routinely Tested

**Suggested Companion Products**

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
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<tbody>
<tr>
<td>554656</td>
<td>Stain Buffer (FBS)</td>
<td>500 ml</td>
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<tr>
<td>554657</td>
<td>Stain Buffer (BSA)</td>
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<tr>
<td>564416</td>
<td>BB515 Mouse IgG1, κ Isotype Control</td>
<td>100 µg</td>
<td>X40</td>
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<tr>
<td>564468</td>
<td>BB515 Mouse Anti-Human CD25</td>
<td>25 tests</td>
<td>2A3</td>
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<tr>
<td>349202</td>
<td>BD FACS™ Lysing Solution</td>
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<td>(none)</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).
2. An isotype control should be used at the same concentration as the antibody of interest.
3. The manufacture, use, sale, offer for sale, or import of this product is subject to one or more patents or pending applications. This product, and only in the amount purchased by buyer, may be used solely for buyer’s own internal research, in a manner consistent with the accompanying product literature. No other right to use, sell or otherwise transfer (a) this product, or (b) its components is hereby granted expressly, by implication or by estoppel. Diagnostic uses require a separate license.
4. 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.
5. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at wwwbdbiosciencescom/color.
6. Please refer to wwwbdbiosciencescom/pharmingen/protocols for technical protocols.

**References**


Leonard WJ, Desper JM, Uchiyama T, Smith KA, Waldmann TA, Greene WC. A monoclonal antibody that appears to recognize the receptor for human T-cell growth factor; partial characterization of the receptor. *Nature.* 1982; 300(5889):267-269. (Biology)


Robb RJ, Greene WC, Rusk CM. Low and high affinity cellular receptors for interleukin 2. Implications for the level of Tac antigen. *J Exp Med.* 1984; 160(4):1126-1146. (Biology)


Siegel JP, Sharon M, Smith PL, Leonard WJ. The IL-2 receptor beta chain (p70): role in mediating signals for LAK, NK, and proliferative activities. *Science.* 1987; 238(4823):75-78. (Biology)
