**BB515 Mouse Anti-Human CD28**

### Product Information

- **Material Number**: 564492
- **Alternate Name**: CD28 antigen; T44; Tp44; TP44
- **Size**: 100 Tests
- **Vol. per Test**: 5 µl
- **Clone**: CD28.2
- **Immunogen**: Human CD28 Transfected Cell Line
- **Isotype**: Mouse (C3H x BALB/c) IgG1, κ
- **Reactivity**: QC Testing: Human
- **Workshop**: V 5T CD28.05
- **Storage Buffer**: Aqueous buffered solution containing ≤0.09% sodium azide.

### Description

The CD28.2 monoclonal antibody specifically binds to CD28, a 44 kDa homodimeric transmembrane glycoprotein present on most mature T cells, thymocytes and plasma cells. CD28 is a costimulatory receptor that binds CD80 and CD86 as ligands and plays a very important role in T cell-B cell interactions. It has been suggested that CD28 initiates and regulates a separate and distinct signal transduction pathway from those stimulated by the TCR complex. Additionally, it has been reported that CD28 antibody clones vary in their ability to stimulate T cells to produce IL-2 and increase intracellular Ca2+ concentration. This finding suggests the existence of functionally distinct subregions on the CD28 molecule. CD28.2 has been demonstrated to bind to the same molecule as clone L293, another CD28 mAb, and has been reported to induce Ca2+ influx in Jurkat T cells.

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 (eg, 530/30-nm filter). This dye provides a much brighter alternative to FITC with less spillover into the PE detector.

### Flow Cytometric Analysis

Flow cytometric analysis of CD28 expression on human peripheral blood lymphocytes. Whole blood was stained with either BD Horizon™ BB515 Mouse IgG1, κ Isotype Control (Cat. No. 564416; dashed line histogram) or BD Horizon BB515 Mouse Anti-Human CD28 antibody (Cat. No. 564492/564493; solid line histogram). Erythrocytes were lysed with BD FACS Lysing Solution (Cat. No. 349202). The fluorescence histograms were derived from events with the forward and side light-scatter characteristics of intact lymphocytes. 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 was removed.

### Application Notes

**Application**

| Flow cytometry | Routinely Tested |

**Recommended Assay Procedure:**

For optimal results, it is recommended to perform 2 washes after staining with antibodies. Cells may be prepared, stained with antibodies and washed twice with wash buffer per established protocols for immunofluorescent staining, prior to acquisition on a flow cytometer. Performing fewer than the recommended wash steps may lead to increased spread of the negative population.
Suggested Companion Products

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>554656</td>
<td>Stain Buffer (FBS)</td>
<td>500 mL</td>
<td>(none)</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>564493</td>
<td>BB515 Mouse Anti-Human CD28</td>
<td>25 Tests</td>
<td>CD28.2</td>
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<tr>
<td>349202</td>
<td>BD FACSTM Lysing Solution</td>
<td>100 mL</td>
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</tr>
<tr>
<td>555899</td>
<td>Lysing Buffer</td>
<td>100 mL</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 $1 \times 10^6$ 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. 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.
4. The manufacture, 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.
5. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
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References