

**DESCRIPTION**

<b>Species Reactivity</b>	Mouse
<b>Specificity</b>	Detects mouse CD28 in ELISA.
<b>Source</b>	Monoclonal Rat IgG <sub>1</sub> Clone # 794716
<b>Purification</b>	Protein A or G purified from hybridoma culture supernatant
<b>Immunogen</b>	Mouse myeloma cell line NS0-derived recombinant mouse CD28 Accession # P31041
<b>Endotoxin Level</b>	<0.10 EU per 1 µg of the antibody by the LAL method.
<b>Formulation</b>	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 µm filtered solution in PBS.

**APPLICATIONS**

**Please Note:** Optimal dilutions should be determined by each laboratory for each application. *General Protocols* are available in the *Technical Information* section on our website.

	<b>Recommended Concentration</b>	<b>Sample</b>
<b>Agonist Activity</b>	0.07-0.42 µg/mL	See Below
<b>Flow Cytometry</b>	0.25 µg/10 <sup>6</sup> cells	See Below
<b>CyTOF-ready</b>	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

**DATA**

**Agonist Activity**

**Mouse CD28 Antibody Induces Proliferation in Mouse T Cells.** Rat Anti-Mouse CD28 Monoclonal Antibody (Catalog # MAB4832) induces proliferation in mouse T cells in the presence of 100 ng/mL Hamster Anti-Mouse CD3ε Monoclonal Antibody (Catalog # MAB484), in a dose dependent manner, as measured by Resazurin (Catalog # AR002). The ED<sub>50</sub> for this effect is typically 0.07-0.42 µg/mL.

**Flow Cytometry**

**Detection of CD28 in CD3<sup>+</sup> Mouse Splenocytes by Flow Cytometry.** CD3<sup>+</sup> mouse splenocytes were stained with Rat Anti-Mouse CD28 Monoclonal Antibody (Catalog # MAB4832, filled histogram) or isotype control antibody (Catalog # MAB005, open histogram), followed by Phycoerythrin-conjugated Anti-Rat IgG Secondary Antibody (Catalog # F0105B).

**PREPARATION AND STORAGE**

<b>Reconstitution</b>	Reconstitute at 0.5 mg/mL in sterile PBS.
<b>Shipping</b>	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
<b>Stability &amp; Storage</b>	<b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b> <ul style="list-style-type: none"> <li>• 12 months from date of receipt, -20 to -70 °C as supplied.</li> <li>• 1 month, 2 to 8 °C under sterile conditions after reconstitution.</li> <li>• 6 months, -20 to -70 °C under sterile conditions after reconstitution.</li> </ul>

**BACKGROUND**

CD28 and CTLA-4, together with their ligands B7-1 and B7-2, constitute one of the dominant costimulatory pathways that regulate T and B cell responses. CD28 and CTLA-4 are structurally homologous molecules that are members of the immunoglobulin (Ig) gene superfamily. Both CD28 and CTLA-4 are composed of a single Ig V-like extracellular domain, a transmembrane domain and an intracellular domain. CD28 and CTLA-4 are both expressed on the cell surface as disulfide-linked homodimers or as monomers. The genes encoding these two molecules are closely linked on human chromosome 2 and mouse chromosome 1. Mouse CD28 is expressed constitutively on virtually 100% of mouse T cells and on developing thymocytes. Cell surface expression of mouse CD28 is down-regulated upon ligation of CD28 in the presence of PMA or PHA. In contrast, CTLA-4 is not expressed constitutively but is upregulated rapidly following T cell activation and CD28 ligation. Cell surface expression of CTLA-4 peaks approximately 48 hours after activation. Although both CTLA-4 and CD28 can bind to the same ligands, CTLA-4 binds to B7-1 and B7-2 with a 20-100 fold higher affinity than CD28. CD28/B7 interaction has been shown to prevent apoptosis of activated T cells via the up-regulation of Bcl-x<sub>L</sub>. CD28 ligation has also been shown to regulate Th1/Th2 differentiation. Agonist activity has been reported using MAB4831 (4,5).

**References:**

1. Lenschow, D.J. *et al.* (1996) *Annu. Rev. Immunol.* **14**:233.
2. Hathcock, K.S. and R.J. Hodes (1996) *Advances in Immunol.* **62**:131.
3. Ward, S.G. (1996) *Biochem. J.* **318**:361.
4. Nguyen, P. *et al.* (2003) *Blood* **13**:4320.
5. Orbach, A. *et al.* (2007) *J. Immunol.* **179**:7287.