

#### DESCRIPTION

<b>Species Reactivity</b>	Human
<b>Specificity</b>	Detects human CD28 in direct ELISAs and Western blots. In direct ELISAs, approximately 20% cross-reactivity with recombinant mouse CD28 is observed.
<b>Source</b>	Polyclonal Goat IgG
<b>Purification</b>	Antigen Affinity-purified
<b>Immunogen</b>	<i>S. frugiperda</i> insect ovarian cell line Sf 21-derived recombinant human CD28
<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.

	Recommended Concentration	Sample
<b>Western Blot</b>	0.1 µg/mL	Recombinant Human CD28 Fc Chimera (Catalog # 342-CD)
<b>Agonist Activity</b>	0.3-0.6 µg/mL	See Below
<b>Flow Cytometry</b>	2.5 µg/10 <sup>6</sup> cells	Human whole blood CD3 <sup>+</sup> T cells
<b>Immunocytochemistry</b>	5-15 µg/mL	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

<p><b>Agonist Activity</b></p> <p><b>Human CD28 Antibody Enhances IL-2 Secretion in Jurkat Cells.</b> Human CD28 Antigen Affinity-purified Polyclonal Antibody enhances IL-2 secretion in Jurkat human acute T cell leukemia cell line stimulated with 10 ng/mL phorbol myristate acetate (PMA) and 0.5 µM calcium ionophore, in a dose-dependent manner, as measured using the Quantikine Human IL-2 ELISA Kit (Catalog # D2050). The ED<sub>50</sub> for this effect is typically 0.3-0.6 µg/mL.</p>	<p><b>Immunocytochemistry</b></p> <p><b>CD28 in Human PBMCs.</b> CD28 was detected in immersion fixed human peripheral blood mononuclear cells (PBMCs) using 10 µg/mL Goat Anti-Human CD28 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-342-PB) for 3 hours at room temperature. Cells were stained with the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # NL001) and counter-stained with DAPI (blue). View our protocol for <a href="#">Fluorescent ICC Staining of Non-adherent Cells</a>.</p>
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#### PREPARATION AND STORAGE

<b>Reconstitution</b>	Reconstitute at 0.2 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>	<p><b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b></p> <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 up-regulated rapidly following T cell activation and CD28 ligation. Cell surface expression of mouse 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 upregulation of Bcl-x<sub>L</sub>. CD28 ligation has also been shown to regulate Th1/Th2 differentiation.

#### 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.