

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human CD28 in direct ELISAs and Western blots. In direct ELISAs, approximately 20% cross-reactivity with recombinant mouse CD28 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	<i>S. frugiperda</i> insect ovarian cell line Sf 21-derived recombinant human CD28
Endotoxin Level	<0.10 EU per 1 µg of the antibody by the LAL method.
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or 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
Western Blot	0.1 µg/mL	Recombinant Human CD28 Fc Chimera (Catalog # 342-CD)
Agonist Activity	0.3-0.6 µg/mL	See Below
Flow Cytometry	2.5 µg/10 ⁶ cells	Human whole blood CD3 ⁺ T cells
Immunocytochemistry	5-15 µg/mL	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

DATA

<p>Agonist Activity</p> <p>Human CD28 Antibody Enhances IL-2 Secretion in Jurkat Cells. 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 ED₅₀ for this effect is typically 0.3-0.6 µg/mL.</p>	<p>Immunocytochemistry</p> <p>CD28 in Human PBMCs. 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 counterstained with DAPI (blue). View our protocol for <i>Fluorescent ICC Staining of Non-adherent Cells</i>.</p>
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PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	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
Stability & Storage	<p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <ul style="list-style-type: none"> ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

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_L. 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.