Human CD28 Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF-342-PB

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human CD28 in direct ELISAs and Western blots.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	*S. frugiperda* insect ovarian cell line *Sf* 21-derived recombinant human CD28 Asn19-Pro152 Accession # P10747
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.

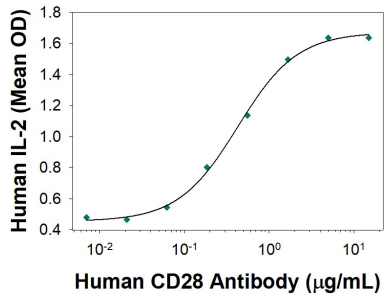
APPLICATIONS

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
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
Immunohistochemistry	3-15 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.	

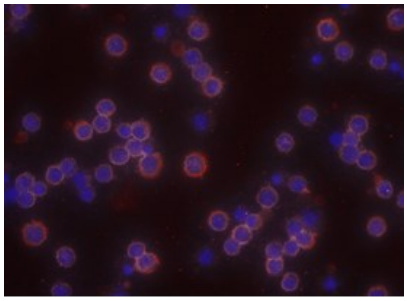
DATA

Agonist Activity



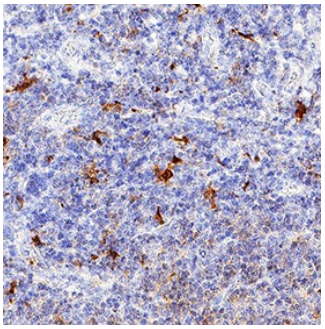
Human CD28 Antibody Enhances IL-2 Secretion in Jurkat Cells. Human CD28 Antigen Affinity-purified Polyclonal Antibody enhances IL-2 secretion in Jurkat human acute T cell leukemia cell line stimulated with 10 ng/mL phorbol myristate acetate (PMA) and 0.5 µM calcium ionophore, in a dose-dependent manner, as measured using the Quantikine Human IL-2 ELISA Kit (Catalog # Catalog # D2050). The ED_{50} for this effect is typically 0.3-0.6 µg/mL.

Immunocytochemistry



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 # Catalog # NL001) and counter-stained with DAPI (blue). View our protocol for Fluorescent ICC Staining of Non-adherent Cells.

Immunohistochemistry



CD28 in Human Tonsil. CD28 was detected in immersion fixed paraffin-embedded sections of human tonsil using Goat Anti-Human CD28 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-342-PB) at 3 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Goat IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC004). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to cell surfaces in lymphocytes. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

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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	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 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.

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