Human CD28 Antibody

Monoclonal Mouse IgG_1 Clone # 37407

Catalog Number: MAB342

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human CD28 in Western blots. In Western blots, this antibody does not cross-react with recombinant mouse (rm) CD28, rhCTLA4, or rmCTLA4.
Source	Monoclonal Mouse IgG_1 Clone # 37407
Purification	Protein A or G purified from ascites
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.

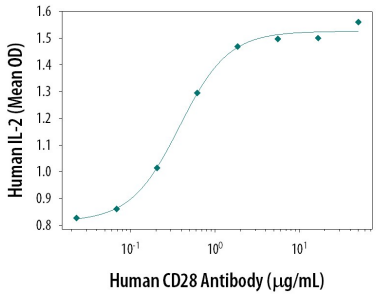
APPLICATIONS

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
Western Blot	1 µg/mL	Recombinant Human CD28 Fc Chimera (Catalog # 342-CD) under non-reducing conditions only
Agonist Activity	0.2-0.6 µg/mL	See Below
Immunocytochemistry	8-25 µg/mL	Immersion fixed U266 human myeloma cell line (Positive) & K562 human chronic myelogenous leukemia cell line (Negative)

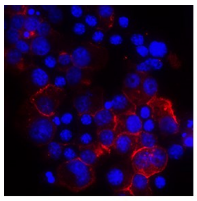
DATA

Agonist Activity

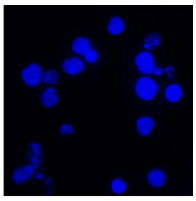


Human CD28 Antibody Enhances IL-2 Secretion in Jurkat Cells. Human CD28 Monoclonal Antibody enhances IL-2 secretion in the Jurkat human acute T cell leukemia cell line stimulated with 10 ng/mL phorbol myristate acetate (PMA) and 0.5 µM calcium ionophore, in a dose-dependent manner, as measured using the Quantikine Human IL-2 ELISA Kit (Catalog # D2050). The ED_{50} for this effect is typically 0.2-0.6 µg/mL.

Immunocytochemistry



U266 (Positive) cells



K562 (Negative) cells

Detection of CD28 in U266 human myeloma cell line (Positive) & K562 human chronic myelogenous leukemia cell line (Negative). CD28 was detected in immersion fixed U266 human myeloma cell line (Positive) & K562 human chronic myelogenous leukemia cell line (Negative) using Mouse Anti-Human CD28 Monoclonal Antibody (Catalog # MAB342) at 8 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to Cytoplasm. View our protocol for Fluorescent ICC Staining of Non-adherent Cells.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 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.

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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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