Human CD28 Antibody
Monoclonal Mouse IgG1 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 IgG1 Clone # 37407
Purification: Protein A or G purified from ascites
Immunogen: S. frugiperda insect ovarian cell line ST21-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.

**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.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Recommended Concentration</th>
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<tbody>
<tr>
<td>Western Blot</td>
<td>1 μg/mL</td>
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<tr>
<td>Agonist Activity</td>
<td>0.2-0.6 μg/mL</td>
</tr>
<tr>
<td>Immunocytochemistry</td>
<td>8-25 μg/mL</td>
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</table>

**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 ED50 for this effect is typically 0.2-0.6 μg/mL.

**Immunocytochemistry**
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.
Stability & Storage:
- *Small pack size (-SP) is supplied either lyophilized or as a 0.2 μm filtered solution in PBS.*
- 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.

*Small pack size (-SP) is supplied either lyophilized or as a 0.2 μm filtered solution in PBS.
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-xL. CD28 ligation has also been shown to regulate Th1/Th2 differentiation.

References: