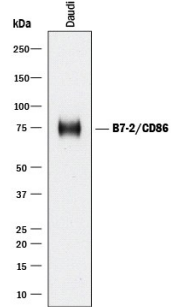
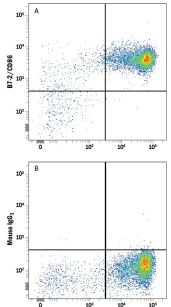
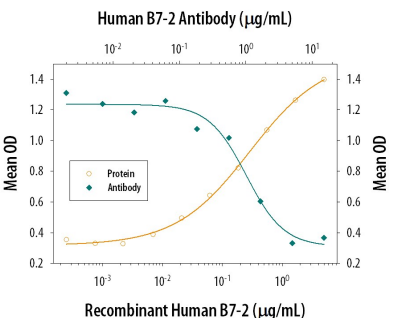
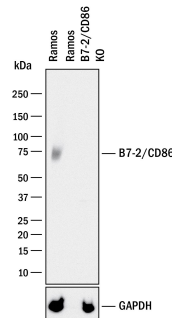


DESCRIPTION	
<b>Species Reactivity</b>	Human
<b>Specificity</b>	Detects human B7-2/CD86 in direct ELISAs and Western blots. In direct ELISAs, no cross-reactivity with recombinant human (rh) B7-1, recombinant mouse B7-2, recombinant rat B7-2, rhB7-H1, rhB7-H2, rhB7-H3, rhB7-H3b, rhB7-H4, or rhB7-L2 is observed.
<b>Source</b>	Monoclonal Mouse IgG <sub>1</sub> Clone # 37301
<b>Purification</b>	Protein A or G purified from ascites
<b>Immunogen</b>	<i>S. frugiperda</i> insect ovarian cell line Sf 21-derived recombinant human B7-2/CD86 Ala23-His244 Accession # P42081
<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	
<b>Please Note:</b> Optimal dilutions should be determined by each laboratory for each application. <i>General Protocols</i> are available in the <i>Technical Information</i> section on our website.	
	<b>Recommended Concentration      Sample</b>
<b>Western Blot</b>	5 µg/mL      See Below
<b>Flow Cytometry</b>	0.25 µg/10 <sup>6</sup> cells      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.
<b>Knockout Validated</b>	B7-2/CD86 is specifically detected in Ramos human Burkitt's lymphoma parental cell line but is not detectable in B7-2/CD86 knockout Ramos cell line
<b>Neutralization</b>	Measured by its ability to neutralize B7-2/CD86-induced IL-2 secretion in the Jurkat human acute T cell leukemia cell line. Freeman, G. J. <i>et al.</i> (1993) <i>Science</i> <b>262</b> :909. The Neutralization Dose (ND <sub>50</sub> ) is typically 0.5-2.5 µg/mL in the presence of 2 µg/mL Recombinant Human B7-2/CD86 Fc Chimera.

DATA	
<p><b>Western Blot</b></p>  <p><b>Detection of Human B7-2/CD86 by Western Blot.</b> Western blot shows lysates of Daudi human Burkitt's lymphoma cell line. PVDF membrane was probed with 5 µg/mL of Mouse Anti-Human B7-2/CD86 Monoclonal Antibody (Catalog # MAB141) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for B7-2/CD86 at approximately 75 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.</p>	<p><b>Flow Cytometry</b></p>  <p><b>Detection of B7-2/CD86 in Human Blood Monocytes by Flow Cytometry.</b> Human peripheral blood monocytes were stained with Mouse Anti-Human CD14 PE-conjugated Monoclonal Antibody (Catalog # FAB3832P) and either (A) Mouse Anti-Human B7-2/CD86 Monoclonal Antibody (Catalog # MAB141) or (B) Mouse IgG<sub>1</sub> Isotype Control (Catalog # MAB002) followed by Allophycocyanin-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # F0101B).</p>
<p><b>Neutralization</b></p>  <p><b>Cell IL-2 Secretion Induced by B7-2/CD86 and Neutralization by Human B7-2/CD86 Antibody.</b> Recombinant Human B7-2/CD86 Fc Chimera induces IL-2 secretion in the Jurkat human acute T cell leukemia cell line in a dose-dependent manner (orange line), as measured by the Human IL-2 Quantikine kit (Catalog # D2050). Under these conditions, IL-2 secretion elicited by B7-2/CD86 is neutralized (green line) by increasing concentrations of Mouse Anti-Human B7-2/CD86 Monoclonal Antibody (Catalog # MAB141). The ND<sub>50</sub> is typically 0.5-2.5 µg/mL.</p>	<p><b>Knockout Validated</b></p>  <p><b>Western Blot Shows Human B7-2/CD86 Specificity by Using Knockout Cell Line.</b> Western blot shows lysates of Ramos human Burkitt's lymphoma parental cell line and B7-2/CD86 knockout Ramos cell line (KO). PVDF membrane was probed with 5 µg/mL of Mouse Anti-Human B7-2/CD86 Monoclonal Antibody (Catalog # MAB141) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for B7-2/CD86 at approximately 74 kDa (as indicated) in the parental Ramos cell line, but is not detectable in knockout Ramos cell line. GAPDH (Catalog # AF5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.</p>

## PREPARATION AND STORAGE

<b>Reconstitution</b>	Reconstitute at 0.5 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

B7-1 and B7-2, together with their receptors CD28 and CTLA-4, constitute one of the dominant costimulatory pathways that regulate T- and B-cell responses. 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 and is involved in the down-regulation of the immune response. B7-1 is expressed on activated B cells, activated T cells, and macrophages. B7-2 is constitutively expressed on interdigitating dendritic cells, Langerhans cells, peripheral blood dendritic cells, memory B cells, and germinal center B cells. Additionally, B7-2 is expressed at low levels on monocytes and can be up-regulated through interferon  $\gamma$ . B7-1 and B7-2 are both members of the immunoglobulin superfamily. Human B7-2 is a 329 amino acid (aa) protein containing a putative 23 aa signal peptide, a 224 aa extracellular domain, a 21 aa transmembrane domain, and a 61 aa cytoplasmic domain. Human B7-2 and B7-1 share 26% amino acid identity. Human and mouse B7-2 share 50% amino acid identity. However, it has been observed that both human and mouse B7-1 and B7-2 can bind to either human or mouse CD28 and CTLA-4, suggesting that there are conserved amino acids which form the B7-1/B7-2/CD28/CTLA-4 critical binding sites.

## References:

1. Azuma, M. *et al.* (1993) *Nature* **366**:76.
2. Freeman, G.J. *et al.* (1993) *Science* **262**:909.
3. Freeman, G. *et al.* (1991) *J. Exp. Med.* **174**:625.
4. Selvakumar, A. *et al.* (1993) *Immunogenetics* **38**:292.
5. Chen, C. *et al.* (1994) *J. Immunol.* **152**:4929.
6. Freeman, G.J. *et al.* (1993) *J. Exp. Med.* **178**:2185.