Human B7-2/CD86 Antibody

Monoclonal Mouse IgG_1 Clone # 37301

Catalog Number: MAB141

DESCRIPTION

Species Reactivity	Human
Specificity	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.
Source	Monoclonal Mouse IgG_1 Clone # 37301
Purification	Protein A or G purified from ascites
Immunogen	*S. frugiperda* insect ovarian cell line *Sf* 21-derived recombinant human B7-2/CD86 Ala23-His244 Accession # P42081
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.

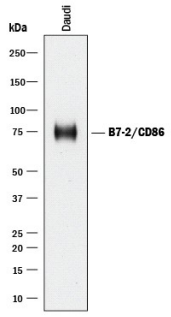
APPLICATIONS

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	5 µg/mL	See Below
Flow Cytometry	0.25 µg/10^6 cells	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	
Knockout Validated	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	
Neutralization	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. *et al*. (1993) Science **262**:909. The Neutralization Dose (ND_{50}) is typically 0.5-2.5 µg/mL in the presence of 2 µg/mL Recombinant Human B7-2/CD86 Fc Chimera.	

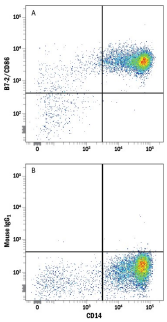
DATA

Western Blot



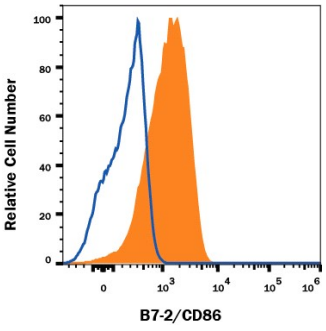
Detection of Human B7-2/CD86 by Western Blot. Western blot shows lysates of Daudi human Burkitt's lymphoma cell line. PVDF membrane was probed with 5 µg/mL of Mouse Anti-Human B7-2/CD86 Monoclonal Antibody (Catalog # MAB141) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). 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.

Flow Cytometry



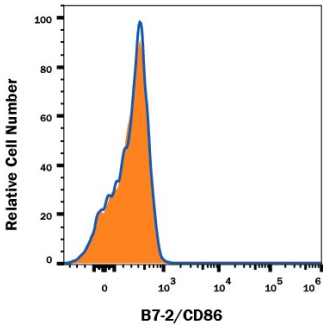
Detection of B7-2/CD86 in Human Blood Monocytes by Flow Cytometry. Human peripheral blood monocytes were stained with Mouse Anti-Human CD14 PE-conjugated Monoclonal Antibody (Catalog # FAB3832P) and either (A) Mouse Anti-Human B7-2/CD86 Monoclonal Antibody (Catalog # MAB141) or (B) Mouse IgG_1 Isotype Control (Catalog # MAB002) followed by Allophycocyanin-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # F0101B).

Flow Cytometry



Detection of B7-2/CD86 in Human Ramos Cell Line by Flow Cytometry. Human Ramos lymphoma cell line was stained with Mouse Anti-Human B7 Formatting the figure legends (adjusted HTML for better line wrapping). Only applies to the website insert. 2/CD86 Monoclonal Antibody (Catalog # MAB141, filled histogram) or isotype control antibody (Catalog # MAB002, open histogram) followed by PE-conjugated Goat Anti-Mouse IgG secondary antibody (Catalog # F0102B). View our protocol for Staining Membrane-associated Proteins.

Knockout Validated



B7-2/CD86 Specificity is Shown by Flow Cytometry in Knockout Cell Line. B7-2/CD86 knockout Ramos human lymphoma cell line was stained with Mouse Anti-Human B7-2/CD86 Monoclonal Antibody (Catalog # MAB141, filled histogram) or isotype control antibody (Catalog # MAB002, open histogram) followed by PE-conjugated Goat anti-Mouse IgG Secondary Antibody (Catalog # F0102B). No staining in the B7-2/CD86 knockout Ramos cell line was observed. View our protocol for Staining Membrane-associated Proteins.

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Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449

Human B7-2/CD86 Antibody

Monoclonal Mouse IgG_1 Clone # 37301

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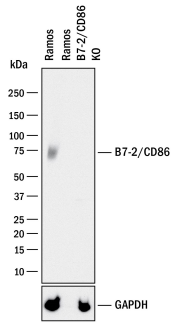
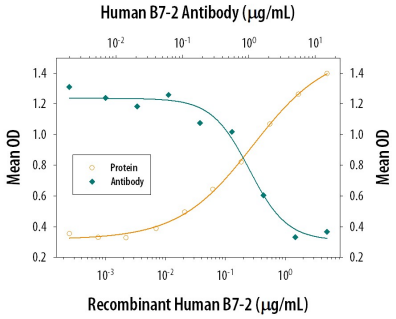
Knockout Validated



Western Blot Shows Human B7-2/CD86 Specificity by Using Knockout Cell Line. 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.

Neutralization



Cell IL-2 Secretion Induced by B7-2/CD86 and Neutralization by Human B7-2/CD86 Antibody. 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_{50} is typically 0.5-2.5 µg/mL.

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.

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 γ. 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.

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Europe | Middle East | Africa TEL +44 (0)1235 529449