

DESCRIPTION

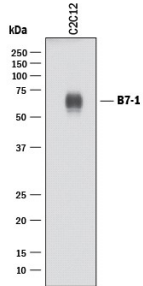
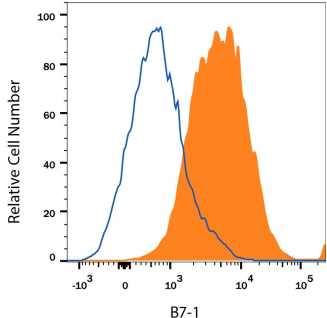
Species Reactivity	Mouse
Specificity	Detects mouse B7-1/CD80 in ELISAs and Western blots.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant mouse B7-1/CD80 Asp37-Lys245 Accession # Q00609
Endotoxin Level	<0.10 EU per 1 µg of the 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

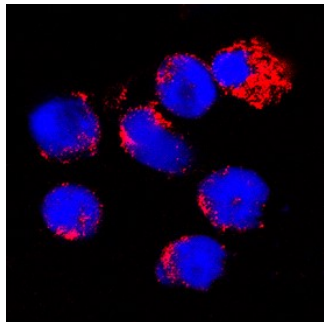
Please Note: Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.

	Recommended Concentration	Sample
Western Blot	1 µg/mL	See Below
Flow Cytometry	0.25 µg/10 ⁶ cells	See Below
Immunocytochemistry	5-15 µg/mL	See Below
Mouse B7-1/CD80 Sandwich Immunoassay		Reagent
ELISA Capture	0.2-0.8 µg/mL	Mouse B7-1/CD80 Antibody (Catalog # AF740)
ELISA Detection	0.1-0.4 µg/mL	Mouse B7-1/CD80 Biotinylated Antibody (Catalog # BAF740)
Standard		Recombinant Mouse B7-1/CD80 Fc Chimera (Catalog # 740-B1)
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	
Neutralization	Measured by its ability to neutralize B7-1/CD80-induced IL-2 secretion in the Jurkat human acute T cell leukemia cell line. The Neutralization Dose (ND ₅₀) is typically 0.15-0.6 µg/mL in the presence of 0.1 µg/mL Recombinant Mouse B7-1/CD80 Fc Chimera and 10 µg/mL PHA.	

DATA

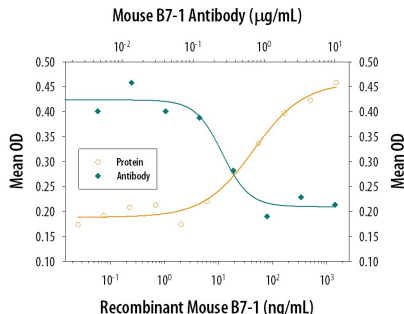
<p>Western Blot</p>  <p>Detection of Mouse B7-1/CD80 by Western Blot. Western blot shows lysates of C2C12 mouse myoblast cell line. PVDF membrane was probed with 1 µg/mL of Goat Anti-Mouse B7-1/CD80 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF740) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for B7-1/CD80 at approximately 60 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.</p>	<p>Flow Cytometry</p>  <p>Detection of B7-1/CD80 in Mouse Splenocytes by Flow Cytometry. Mouse splenocytes either treated with 200 ng/mL LPS (filled histogram) or unstimulated (open histogram) were stained with Goat Anti-Mouse B7-1/CD80 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF740), followed by Phycoerythrin-conjugated Anti-Goat IgG Secondary Antibody (Catalog # F0107). View our protocol for Staining Membrane-associated Proteins.</p>
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Immunocytochemistry



B7-1/CD80 in Mouse Splenocytes.
B7-1/CD80 was detected in immersion fixed mouse splenocytes using Goat Anti-Mouse B7-1/CD80 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF740) at 15 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # NL001) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for [Fluorescent ICC Staining of Non-adherent Cells](#).

Neutralization



IL-2 secretion Induced by B7-1/CD80 and Neutralization by Mouse B7-1/CD80 Antibody.
Recombinant Mouse B7-1/CD80 Fc Chimera (Catalog # 740-B1) co-stimulates IL-2 secretion in the Jurkat human acute T cell leukemia cell line in the presence of PHA in a dose-dependent manner (orange line), as measured by the Human IL-2 Quantikine ELISA Kit (Catalog # D2050). IL-2 secretion elicited by Recombinant Mouse B7-1/CD80 Fc Chimera (0.1 µg/mL) and PHA (10 µg/mL) is neutralized (green line) by increasing concentrations of Goat Anti-Mouse B7-1/CD80 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF740). The ND₅₀ is typically 0.15-0.6 µg/mL.

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. <ul style="list-style-type: none"> ● 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. Mouse B7-1 is a 306 amino acid (aa) protein containing a putative 37 aa signal peptide, a 190 aa extracellular domain, a 22 aa transmembrane domain, and a 38 aa cytoplasmic domain. Mouse B7-1 and B7-2 share 28% amino acid identity. Mouse and human B7-1 share 44% 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.