Mouse PD-L1/B7-H1 Antibody

Recombinant Monoclonal Rabbit IgG Clone # 2096C
Catalog Number: MAB90781

DESCRIPTION

Species Reactivity	Mouse
Specificity	Detects mouse PD-L1/B7-H1 in direct ELISAs and Western blots.
Source	Recombinant Monoclonal Rabbit IgG Clone # 2096C
Purification	Protein A or G purified from cell culture supernatant
Immunogen	Mouse myeloma cell line NS0-derived recombinant mouse PD-L1/B7-H1 Met1-His239 Accession # Q9EP73
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.

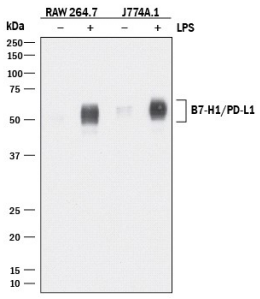
APPLICATIONS

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	2 µg/mL	See Below
Flow Cytometry	0.25 µg/10^6 cells	See Below
Immunohistochemistry	5-25 µg/mL	See Below

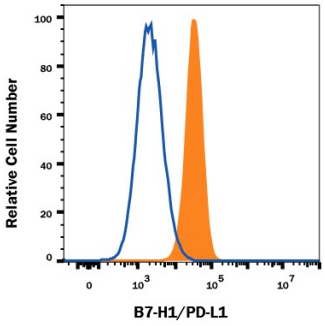
DATA

Western Blot



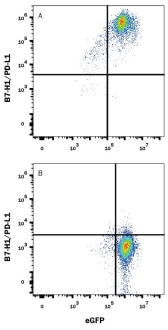
Detection of Mouse PD-L1/B7-H1 by Western Blot. Western blot shows lysates of RAW 264.7 mouse monocyte/macrophage cell line and J774A.1 mouse reticulum cell sarcoma macrophage cell line untreated (-) or treated (+) with 10 µg/mL LPS for 4 hours. PVDF membrane was probed with 2 µg/mL of Rabbit Anti-Mouse PD-L1/B7-H1 Monoclonal Antibody (Catalog # MAB90781) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). A specific band was detected for PD-L1/B7-H1 at approximately 50-55 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Flow Cytometry



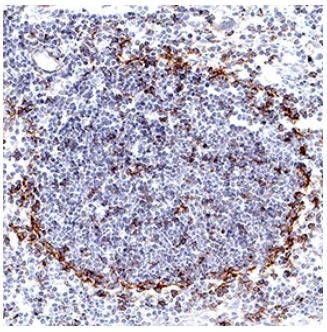
Detection of PD-L1/B7-H1 in RAW 264.7 Mouse Cell Line by Flow Cytometry. RAW 264.7 mouse monocyte/macrophage cell line either treated with LPS overnight (filled histogram) or untreated (open histogram) was stained with Rabbit Anti-Mouse PD-L1/B7-H1 Monoclonal Antibody (Catalog # MAB90781), followed by Allophycocyanin-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # F0111). View our protocol for Staining Membrane-associated Proteins.

Flow Cytometry



Detection of PD-L1/B7-H1 in HEK293 Human Cell Line Transfected with Mouse PD-L1/B7-H1 and eGFP by Flow Cytometry. HEK293 human embryonic kidney cell line transfected with either (A) mouse PD-L1/B7-H1 or (B) irrelevant transfectants and eGFP was stained with Rabbit Anti-Mouse PD-L1/B7-H1 Monoclonal Antibody (Catalog # MAB90781) followed by Allophycocyanin-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # F0111). Quadrant markers were set based on control antibody staining (Catalog # MAB1050). View our protocol for Staining Membrane-associated Proteins.

Immunohistochemistry



PD-L1/B7-H1 in Mouse Thymus. PD-L1/B7-H1 was detected in perfusion fixed frozen sections of mouse thymus using Rabbit Anti-Mouse PD-L1/B7-H1 Monoclonal Antibody (Catalog # MAB90781) at 5 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Rabbit IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC003). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to thymocytes. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

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

Mouse B7 homolog 1(B7-H1), also called programmed death ligand 1 (PD-L1) and programmed cell death 1 ligand 1 (PDCD1L1), is a member of the B7 family of proteins that provide signals for regulating T-cell activation and tolerance (1-4). Other family members include B7-1, B7-2, B7-H2, B7-H3 and PD-L2. B7 proteins are immunoglobulin (Ig) superfamily members with extracellular Ig-V-like and Ig-C-like domains and a short cytoplasmic region. Among the family members, they share from 20-40% amino acid (aa) sequence identity. The cloned mouse B7-H1/PD-L1 cDNA encodes a 290 aa type I membrane precursor protein with a putative 18 aa signal peptide, a 220 aa extracellular region containing one V-like and one C-like Ig domain, a 22 aa transmembrane region, and a 30 aa cytoplasmic domain. Mouse and human B7-H1/PD-L1 share approximately 70% aa sequence identity. B7-H1/PD-L1 is one of two ligands for programmed death-1 (PD-1), a member of the CD28 family of immunoreceptors. The other identified ligand is PD-L2. Mouse B7-H1/PD-L1 and PD-L2 share approximately 34% aa sequence identity and have similar functions. B7-H1/PD-L1 is constitutively expressed in various lymphoid and non-lymphoid organs including placenta, heart, pancreas, lung, liver, and endothelium (1-4). The expression of B7-H1/PD-L1 is detected on B cells, T cells, monocytes, dendritic cells and thymic epithelial cells. IFN-γ treatment induces B7-H1/PD-L1 expression in monocytes, dendritic cells, and endothelial cells. B7-H1/PD-L1 expression is also upregulated in a variety of tumor cell lines. On previously activated T cells, B7-H1/PD-L1 interaction with PD-1 inhibits TCR-mediated proliferation and cytokine production, suggesting an inhibitory role in regulating immune responses. In contrast, a costimulatory function for the PD-1 ligands on resting T cells has also beenreported (1-4).

References:

1. Tamura, H. *et al.* (2001) Blood **97**:1809.
2. Freeman, G. *et al.* (2000) J. Exp. Med. **192**:1027.
3. Sharpe, A.H. and G. J. Freeman (2002) Nat. Rev. Immunol. **2**:116.
4. Coyle, A. and J. Gutierrez-Ramos (2001) Nat. Immunol. **2**:203.

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