Human PD-L1/B7-H1 Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF156

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human PD-L1/B7-H1 in direct ELISAs and Western blots. In direct ELISAs, less than 15% cross-reactivity with recombinant mouse PD-L1/B7-H1 is observed and less than 1% cross-reactivity with recombinant human PD-L2 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human PD-L1/B7-H1 Phe19-Thr239 Accession # Q9NZQ7
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose.

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
Immunohistochemistry	5-15 µg/mL	See Below
Simple Western	50 µg/mL	See Below
ELISA	This antibody functions as an ELISA detection antibody when paired with Mouse Anti-Human PD-L1/B7-H1 Monoclonal Antibody (Catalog # MAB1561R). *This product is intended for assay development on various assay platforms requiring antibody pairs. We recommend the Human PD-L1/B7-H1 DuoSet ELISA Kit (Catalog # DY156) for convenient development of a sandwich ELISA or the Human/Cynomolgus Monkey PD-L1/B7-H1 Quantikine ELISA Kit (Catalog # DB7H10) for a complete optimized ELISA.*	

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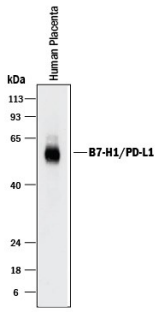
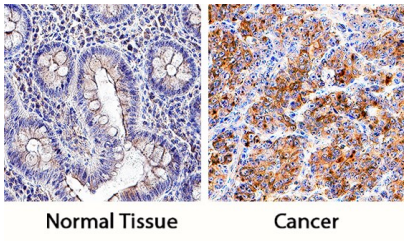
DATA

Western Blot



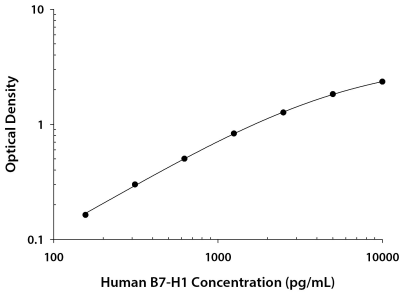
Detection of Human PD-L1/B7-H1 by Western Blot. Western blot shows lysates of human placenta tissue. PVDF membrane was probed with 2 µg/mL of Goat Anti-Human PD-L1/B7-H1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF156) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # Catalog # HAF017). 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.

Immunohistochemistry



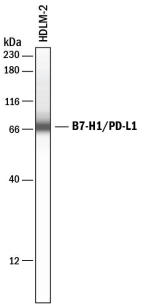
PD-L1/B7-H1 in Human Colon and Colon Cancer Tissue. PD-L1/B7-H1 was detected in immersion fixed paraffin-embedded sections of normal human colon (left panel) and human colon cancer tissue (right panel) using Goat Anti-Human PD-L1/B7-H1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF156) at 5 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # Catalog # CTS008) and counterstained with hematoxylin (blue). Specific staining was localized to cell membranes and cytoplasm. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

ELISA



Human PD-L1/B7-H1 ELISA Standard Curve. Recombinant Human PD-L1/B7-H1 protein was serially diluted 2-fold and captured by Mouse Anti-Human PD-L1/B7-H1 Monoclonal Antibody (Catalog # Catalog # MAB1561R) coated on a Clear Polystyrene Microplate (Catalog # Catalog # DY990). Goat Anti-Human PD-L1/B7-H1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF156) was biotinylated and incubated with the protein captured on the plate. Detection of the standard curve was achieved by incubating Streptavidin-HRP (Catalog # Catalog # DY998) followed by Substrate Solution (Catalog # Catalog # DY999) and stopping the enzymatic reaction with Stop Solution (Catalog # Catalog # DY994).

Simple Western



Detection of Human PD-L1/B7-H1 by Simple Western™. Simple Western lane view shows lysates of HDLM-2 human Hodgkin's lymphoma cell line, loaded at 0.2 mg/mL. A specific band was detected for PD-L1/B7-H1 at approximately 72 kDa (as indicated) using 50 µg/mL of Goat Anti-Human PD-L1/B7-H1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF156) followed by 1:50 dilution of HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # Catalog # HAF109). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

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.
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

Human B7 homolog 1 (B7-H1), also called programmed cell death 1 ligand 1 (PDCD1L1) and programmed death ligand 1 (PDL1), is a member of the growing B7 family of immune proteins that provide signals for both stimulating and inhibiting T cell activation. Other family members include B7-1, B7-2, B7-H2, PDL2 and B7-H3. B7 proteins are members of the immunoglobulin (Ig) superfamily, their extracellular domains contain 2 Ig-like domains and all members have short cytoplasmic domains. Among the family members, they share about 20-25% amino acid identity. Human and mouse B7-H1 share approximately 70% amino acid sequence identity. B7-H1 has been identified as one of two ligands for programmed death-1 (PD-1), a member of the CD28 family of immunoreceptors. The B7-H1 gene encodes a 290 amino acid (aa) type I membrane precursor protein with a putative 18 aa signal peptide, a 221 aa extracellular domain, a 21 aa transmembrane region, and a 31 aa cytoplasmic domain. Human B7-H1 is constitutively expressed in several organs such as heart, skeletal muscle, placenta and lung, and in lower amounts in thymus, spleen, kidney and liver. B7-H1 expression is upregulated in a small fraction of activated T and B cells and a much larger fraction of activated monocytes. B7-H1 expression is also induced in dendritic cells and keratinocytes after IFN-γ stimulation. Interaction of B7-H1 with PD-1 results in inhibition of TCR-mediated proliferation and cytokine production. The B7-H1:PD-1 pathway is involved in the negative regulation of some immune responses and may play an important role in the regulation of peripheral tolerance.

References:

1. Nishimura, H. and T. Honjo (2001) Trends in Immunology **22**:265.
2. Freeman, G.J. *et al.* (2000) J. Exp. Med. **192**:1027.
3. Latchman, Y. *et al.* (2001) Nat. Immunol. **2**:261.

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