

#### DESCRIPTION

<b>Species Reactivity</b>	Human
<b>Specificity</b>	Detects human PD-L1/B7-H1 in direct ELISAs. In direct ELISAs, no cross-reactivity with recombinant human (rh) B7-1, -2, -H2, -H3, -H3b, -H4, rhPD-L2, recombinant mouse B7-H1, recombinant rat (rr) B7-1, or rrB7-2 is observed.
<b>Source</b>	Monoclonal Mouse IgG <sub>1</sub> Clone # 130021
<b>Purification</b>	Protein A or G purified from hybridoma culture supernatant
<b>Immunogen</b>	Mouse myeloma cell line NS0-derived recombinant human PD-L1/B7-H1 Phe19-Thr239 Accession # Q9NZQ7
<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 either lyophilized or as a 0.2 µm filtered solution in PBS.

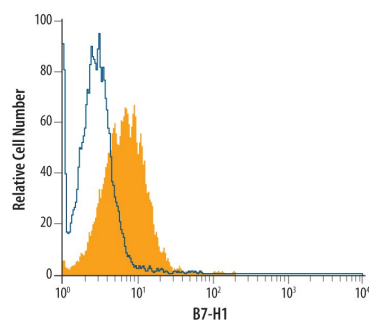
#### APPLICATIONS

**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.

	<b>Recommended Concentration</b>	<b>Sample</b>
<b>Dual RNAscope ISH-IHC Compatible</b>	3-25 µg/mL	Immersion fixed paraffin-embedded sections of human colon cancer
<b>Flow Cytometry</b>	0.25 µg/10 <sup>6</sup> cells	See Below
<b>Immunohistochemistry</b>	8-25 µg/mL	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.	

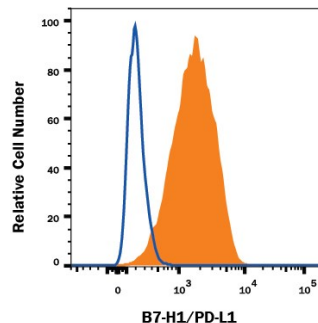
## DATA

### Flow Cytometry



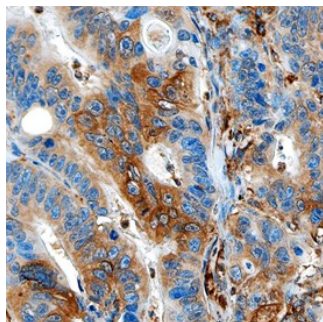
**Detection of PD-L1/B7-H1 in Jurkat Human Cell Line by Flow Cytometry.** Jurkat human acute T cell leukemia cell line was stained with Mouse Anti-Human PD-L1/B7-H1 Monoclonal Antibody (Catalog # MAB1561, filled histogram) or isotype control antibody (Catalog # Catalog # MAB002, open histogram), followed by Phycoerythrin-conjugated Anti-Mouse IgG F(ab')<sub>2</sub> Secondary Antibody (Catalog # Catalog # F0102B). View our protocol for [Staining Membrane-associated Proteins](#).

### Flow Cytometry



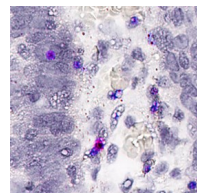
**Detection of PD-L1/B7-H1 in MDA-MB-231 Human Cell Line by Flow Cytometry.** MDA-MB-231 human breast adenocarcinoma cell line was stained with Mouse Anti-Human PD-L1/B7-H1 Monoclonal Antibody (Catalog # MAB1561, filled histogram) or isotype control antibody (Catalog # Catalog # MAB002, open histogram), followed by Phycoerythrin-conjugated Anti-Mouse IgG F(ab')<sub>2</sub> Secondary Antibody (Catalog # Catalog # F0102B). Adherent cells were prepared by either manual scraping or with TrypLE Express treatment with similar results. View our protocol for [Staining Membrane-associated Proteins](#).

### Immunohistochemistry

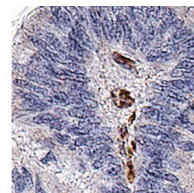


**PD-L1/B7-H1 in Human Colon Cancer.** PD-L1/B7-H1 was detected in formalin fixed paraffin-embedded sections of human colon cancer using Mouse Anti-Human PD-L1/B7-H1 Monoclonal Antibody (Catalog # MAB1561) at 15 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Mouse HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS002) and counterstained with hematoxylin (blue). Specific staining was observed in the cytoplasm. View our protocol for [Chromogenic IHC Staining of Paraffin-embedded Tissue Sections](#).

### In-situ Hybridization



In Situ Hybridization (ISH)



Immunohistochemistry (IHC)

**Detection of PD-L1/B7-H1 in Human Colon Cancer.** Formalin-fixed paraffin-embedded tissue sections of human colon cancer were probed for PDL1 mRNA (ACD RNAScope Probe, catalog # 600861; Fast Red chromogen, ACD catalog # 322360). Adjacent tissue section was processed for immunohistochemistry using mouse anti-human PDL1 monoclonal antibody (R&D Systems catalog # Catalog # MAB1561) at 5µg/mL with overnight incubation at 4 degrees Celsius followed by incubation with anti-mouse IgG VisUCyte HRP Polymer Antibody (Catalog # Catalog # VC001) and DAB chromogen (yellow-brown). Tissue was counterstained with hematoxylin (blue). Specific staining was localized to lymphocytes.

## PREPARATION AND STORAGE

<b>Reconstitution</b>	Reconstitute at 0.5 mg/mL in sterile PBS. For liquid material, refer to CoA for concentration.
<b>Shipping</b>	Lyophilized product is shipped at ambient temperature. Liquid small pack size (-SP) is shipped with polar packs. Upon receipt, store immediately at the temperature recommended below.
<b>Stability &amp; Storage</b>	<p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</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

Human 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 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, there is 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 291 amino acid (aa) type I membrane precursor protein with a putative 18 aa signal peptide, a 220 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- $\gamma$  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 Immunol. **22**:265.
2. Freeman, G.J. *et al.* (2000) J. Exp. Med. **192**:1027.
3. Latchman, Y. *et al.* (2001) Nat. Immunol. **2**:261.