

DESCRIPTION

Species Reactivity	Mouse
Specificity	Detects recombinant mouse PD-L1 protein in Direct ELISA.
Source	Recombinant Monoclonal Rabbit IgG Clone # 2096A
Purification	Protein A or G purified from cell culture supernatant
Immunogen	Mouse myeloma cell line, NS0 derived mouse PD-L1/B7-H1 Phe19-His239 Accession # Q9EP73
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose.

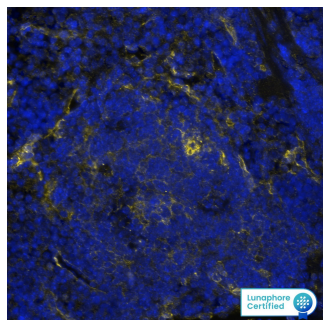
APPLICATIONS

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
Multiplex Immunofluorescence	5 µg/mL	Immersion fixed paraffin-embedded sections of Mouse Spleen
Immunohistochemistry	0.05-10 µg/mL	Perfusion fixed frozen sections of mouse thymus and immersion fixed paraffin-embedded sections of mouse thymus

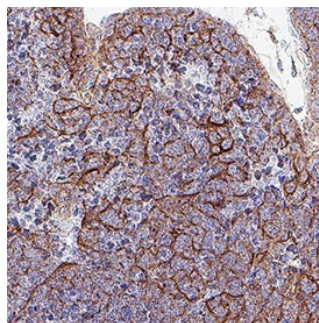
DATA

Multiplex Immunofluorescence



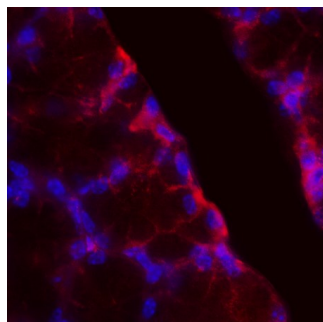
Detection of PD-L1/B7-H1 in Mouse Spleen via Multiplex Immunofluorescence staining on COMET™. PD-L1/B7-H1 was detected in immersion fixed paraffin-embedded sections of mouse spleen using Rabbit Anti-Mouse PD-L1/B7-H1 Recombinant Monoclonal Antibody (Catalog # MAB11630) at 5µg/mL at 37 ° Celsius for 4 minutes. Before incubation with the primary antibody, tissue underwent an all-in-one dewaxing and antigen retrieval preprocessing using PreTreatment Module (PT Module) and Dewax and HIER Buffer H (pH 9). Tissue was stained using the Alexa Fluor™ Plus 647 Goat anti-Rabbit IgG Secondary Antibody at 1:200 at 37 ° Celsius for 2 minutes. (Yellow; Lunaphore Catalog # [DR647RB](#)) and counterstained with DAPI (blue; Lunaphore Catalog # [DR100](#)). Specific staining was localized to the membrane. Protocol available in [COMET™ Panel Builder](#).

Immunohistochemistry



Detection of PD-L1/B7-H1 in Mouse Thymus. PD-L1/B7-H1 was detected in immersion fixed paraffin-embedded sections of mouse thymus using Rabbit Anti-Mouse PD-L1/B7-H1 Monoclonal Antibody (Catalog # MAB11630) at 0.075 µg/ml for 1 hour at room temperature followed by incubation with the Anti-Rabbit IgG VisUCyte™ HRP Polymer Antibody (Catalog # [VC003](#)) or the HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # [HAF008](#)). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using VisUCyte Antigen Retrieval Reagent-Basic (Catalog # [VCTS021](#)). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to the membrane. View our protocol for [IHC Staining with VisUCyte HRP Polymer Detection Reagents](#).

Immunofluorescence



Detection of 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 # MAB11630) at 1 µg/ml overnight at 4 °C. Tissue was stained using the NorthernLights™ 557-conjugated Anti-Rabbit IgG Secondary Antibody (red; Catalog # [NL004](#)) and counterstained with hematoxylin (blue). Specific staining was localized to the membrane. View our protocol for [Fluorescent IHC Staining of Frozen Tissue Sections](#).

PREPARATION AND STORAGE

Reconstitution	Reconstitute lyophilized material at 0.2 mg/ml in sterile PBS. For liquid material, refer to CoA for concentration.
Shipping	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.
Stability & Storage	<p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <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

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 been reported (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.