

Anti-PD-L1 CD274 Antibody

Catalog Number: A00109-1

About CD274

PD-L1 plays a critical role in induction and maintenance of immune tolerance to self. As a ligand for the inhibitory receptor PDCD1/CD279, PD-L1 modulates the activation threshold of T-cells and limits T-cell effector response (1). The PDCD1/CD279-mediated inhibitory pathway is exploited by tumors to attenuate anti-tumor immunity and facilitate tumor survival (2,3). Through a yet unknown activating receptor, it may costimulate T-cell subsets that predominantly produce interleukin-10 (IL10) (4).

Overview

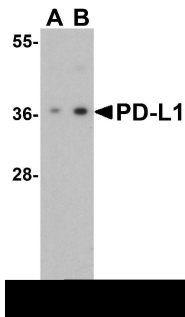
Product Name	Anti-PD-L1 CD274 Antibody
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-PD-L1 CD274 Antibody (Catalog # A00109-1). Tested in ELISA, WB, IHC-P, IF, Flow Cytometry applications. This antibody reacts with Human, Mouse, Rat.
Application	ELISA, Flow Cytometry, IF, IHC-P, WB
Clonality	Polyclonal
Formulation	PD-L1 Antibody is supplied in PBS containing 0.02% sodium azide.
Storage Instructions	PD-L1 antibody can be stored at 4°C for three months and -20°C, stable for up to one year. Avoid repeated freeze-thaw cycles. Antibodies should not be exposed to prolonged high temperatures.
Host	Rabbit
Uniprot ID	Q9NZQ7

Technical Details

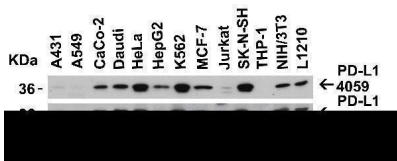
Immunogen	Anti-PD-L1 antibody was raised against a peptide corresponding to 17 amino acids near the center of human PD-L1 isoform 1. The immunogen is located within amino acids 60 - 110 of PD-L1.
Predicted Reactive Species	Mouse, Rat
Cross Reactivity	PD-L1 antibody has no cross-reactivity to PD-L2.
Isotype	IgG
Form	Liquid
Concentration	1 mg/mL
Purification	PD-L1 Antibody is affinity chromatography purified via peptide column.
Suggested Dilutions	WB: 1-2 ug/mL; IHC-P: 2.5-5 ug/mL; IF: 20 ug/mL; Flow Cyt: 0.5 ug/mL.

Antibody validated: Western Blot in human and mouse samples; Immunohistochemistry in human and rat samples; Immunofluorescence in human and rat samples and Flow Cytometry in mouse samples. All other applications and species not yet tested. Optimal dilutions for each application should be determined by the researcher.

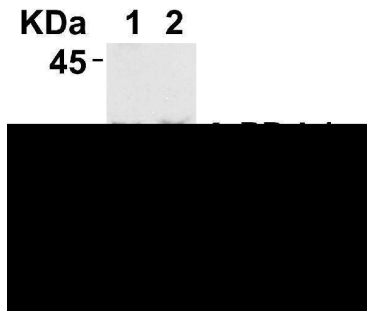
Anti-PD-L1 CD274 Antibody (A00109-1) Images



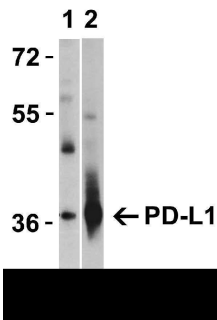
Western Blot Validation of PD-L1 in HeLa Cells Loading: 15 ug of lysates per lane. Antibodies: A00109-1 (A: 1 ug/mL, B: 2 ug/mL), 1 h incubation at RT in 5% NFDm/TBST. Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution.



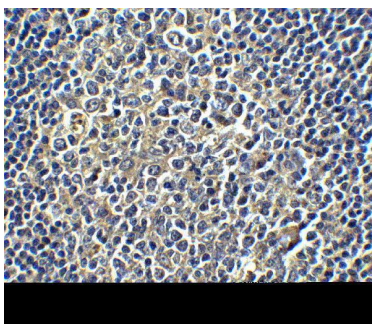
Independent Antibody Validation (IAV) via Protein Expression Profile in Human and Mouse cell lines Loading: 15 ug of lysates per lane. Antibodies: A00109-1 (2 ug/mL), RF16035 (2 ug/mL), and beta-actin (1 ug/mL), 1 h incubation at RT in 5% NFDm/TBST. Secondary: Goat anti-rabbit and or anti-mouse IgG HRP conjugate at 1:10000 and 1:5000 dilution, respectively.



Validation with PD-L1 siRNA Knockdown in HeLa Cells HeLa cells were transfected with control siRNAs (lane 1) or PD-L1 siRNAs (lane 2) Loading: 10 ug of HeLa whole cell lysates per lane. Antibodies: A00109-1 (2 ug/mL) and GAPDH (3783, 0.02 ug/mL), 1 h incubation at RT in 5% NFDm/TBST. Secondary: Goat anti-mouse IgG HRP conjugate at 1:5000 dilution.



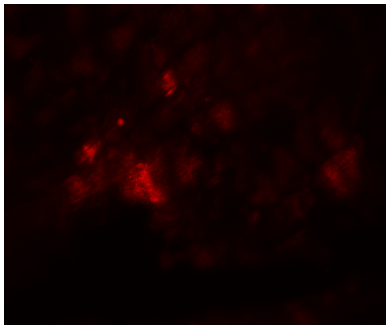
Validation with PD-L1 overexpression in 293 cells Loading: Lysates/proteins at 15 ug per lane. Lane 1: non-transfected 293 cells Lane 2: PD-L1 overexpressed 293 cells Antibodies: A00109-1 (1 ug/mL). 1 h incubation at RT in 5% NFDm/TBST. Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution.



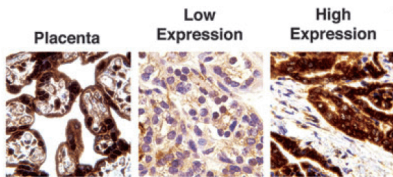
Immunohistochemistry Validation of PD-L1 in Human Tonsil Cells Immunohistochemical analysis of paraffin-embedded human tonsil tissue using anti-PD-L1 antibody (A00109-1) at 5 ug/ml. Tissue was fixed with formaldehyde and blocked with 10% serum for 1 h at RT; antigen retrieval was by heat mediation with a citrate buffer (pH6). Samples were incubated with primary antibody overnight at 4°C. A goat anti-rabbit IgG H&L (HRP) at 1/250 was used as secondary. Counter stained with Hematoxylin.



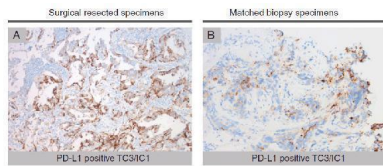
Immunofluorescence Validation of PD-L1 in Human Heart
Immunofluorescent analysis of 4% paraformaldehyde-fixed human heart tissue labeling PD-L1 with A00109-1 at 20 ug/ml, followed by goat anti-rabbit IgG secondary antibody at 1/500 dilution (red). Image showing both membrane and cytoplasmic staining on human heart tissue.



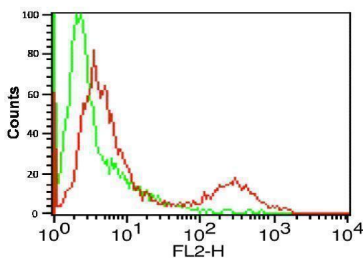
Immunofluorescence Validation of PD-L1 in Rat Heart
Immunofluorescence analysis of 4% paraformaldehyde-fixed rat heart tissue labeling PD-L1 with A00109-1 at 20 ug/ml, followed by goat anti-rabbit IgG secondary antibody at 1/250 dilution (red).



Immunohistochemistry Validation of PD-L1 in Human thyroid cancer (Angell et al., 2014) Immunohistochemical analysis of PD-L1 expression in human thyroid cancer with anti-PD-L1 antibodies (A00109-1). Placenta was used as a positive control.

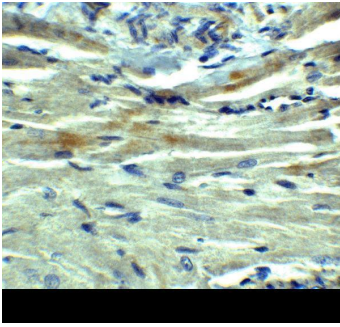


Immunohistochemistry Validation of PD-L1 in Human Lung Cancer (Ilie et al., 2015) Surgical specimens (left panel) and matched biopsy specimens (right panel). PD-L1-positive (A,B) and PD-L1-negative (C,D) tumors.

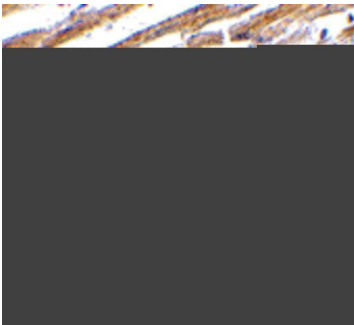


Flow Cytometry Validation of PD-L1 Overlay histogram showing A-20 cells stained with A00109-1 (red line, 1ug/1x10⁶ cells). 1 h incubation at 4 °C in 2% FBS/PBS. Followed by secondary antibody 488 goat anti-rabbit IgG (H+L) at 1/500 dilution for 1 h 4 °C. Isotype control antibody (Green line) was mouse IgG1 (1ug/1x10⁶ cells) used under the same conditions. Acquisition of >10,000 events was performed.

Immunohistochemistry Validation of PD-L1 in Rat Heart
Immunohistochemical analysis of paraffin-embedded rat heart tissue using anti-PD-L1 antibody (A00109-1) at 5 ug/ml. Tissue was fixed with formaldehyde and blocked with



10% serum for 1 h at RT; antigen retrieval was by heat mediation with a citrate buffer (pH6). Samples were incubated with primary antibody overnight at 4°C. A goat anti-rabbit IgG H&L (HRP) at 1/250 was used as secondary. Counter stained with Hematoxylin.

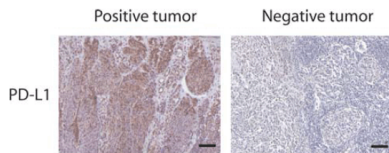


Immunohistochemistry Validation of PD-L1 in Human Heart
Immunohistochemical analysis of paraffin-embedded human heart tissue using anti-PD-L1 antibody (A00109-1) at 2.5 µg/ml. Tissue was fixed with formaldehyde and blocked with 10% serum for 1 h at RT; antigen retrieval was by heat mediation with a citrate buffer (pH6). Samples were incubated with primary antibody overnight at 4°C. A goat anti-rabbit IgG H&L (HRP) at 1/250 was used as secondary. Counter stained with Hematoxylin.

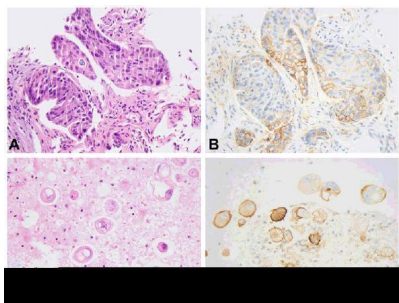
I ^g	BioLegend (12.5 µg/ml)			ProSci (50 µg/ml)			eBioscience (25 µg/ml)	
	RBC	WBC	HeLa	RBC	WBC	HeLa	RBC	HeLa
2 µg/ml	-	-	++	NA	-	++	-	-
4 µg/ml	-	-	+	-	+	-	-	-



Immunofluorescence Validation of PD-L1 in tumors in Human Cells (Dhar et al., 2018) (A) Several antibody brands were first tested with RBCs, WBCs, and HeLa cells: BioLegend, ProSci, and eBioscience. ProSci (A00109-1) was chosen as it provided the highest staining intensity. (B, C) Using the optimal conditions of anti-PDL1 (ProSci Inc) at a concentration of 50ug/mL, following by goat anti-rabbit Alexa Fluor 647, PDL-1 staining was tested on several lung cancer cell lines: A549 (adenocarcinoma), H1703 (adenocarcinoma), H3255 (squamous) and WBCs as a control. (D) Once validated, patient samples were stained for PD-L1, CK, CD45, DAPI.



Immunohistochemistry Validation of PD-L1 in Human Tumors (Gadiot et al., 2011) Immunohistochemical analysis of patient tumors labeling PD-L1 with anti-PD-L1 antibodies (A00109-1). Several anti-PD-L1 antibodies were tested for staining, “Only 1 antibody gave no background staining and was competitively blocked by the addition of PD-L1Fc protein (ProSci, #A00109-1)”.



Immunohistochemistry Validation of PD-L1 in Human Lung adenocarcinoma (Heymann et al., 2017) HE staining (left panel) and PD-L1 expression (right panel) in the tumor and pleural fluid for a patient with lung adenocarcinoma. PD-L1 expression detected by anti-PD-L1 antibodies (A00109-1) demonstrated membranous staining in approximately 80% of tumor cells (C) and in approximately 75% of tumor cells (D), respectively.

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Anti-PD-L1 CD274 Antibody

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