Website: thermofisher.com
Customer Service (US): 1 800 955 6288 ext. 1
Technical Support (US): 1 800 955 6288 ext. 441
thermofisher.com/contactus

Performance guaranteed

CD273 (B7-DC) Polyclonal Antibody

Catalog Number PA5-20344

Product data sheet

Details	
Size	100 µg
Host/Isotope	Rabbit / IgG
Class	Polyclonal
Type	Antibody
Immunogen	A 16 amino acid peptide from near the center of human PDL-2.
Conjugate	Unconjugated
Form	Liquid
Concentration	1 mg/mL
Purification	Antigen affinity chromatography
Storage buffer	PBS
Contains	0.02% sodium azide
Storage Conditions	Maintain refrigerated at 2-8°C for up to 3 months. For long term storage store at -20°C

Species Reactivity	
Species reactivity	Human, Mouse
Published species	Rat, Human

Tested Applications	Dilution *
Immunohistochemistry (Paraffin) (IHC (P))	2.5-10 µg/mL
Western Blot (WB)	0.5-1 µg/mL
Immunocytochemistry (ICC/IF)	20 µg/mL

Published Applications	
Western Blot (WB)	See 1 publications below

* Suggested working dilutions are given as a guide only. It is recommended that the user titrate the product for use in their own experiment using appropriate negative and positive controls.

Product specific information

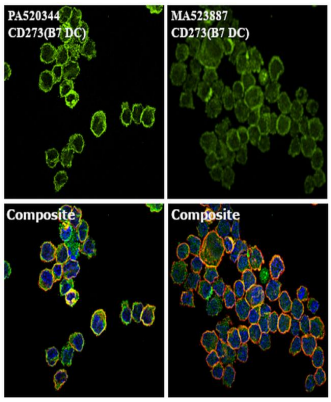
A suggested positive control is Raji cell lysate. PA5-20344 can be used with blocking peptide PEP-0464.

Background/Target Information

Programmed death-ligand 2 (PD-L2), or B7-DC, is a member of the B7 ligand family within the immunoglobulin superfamily that, along with programmed death-ligand 1 (PD-L1), acts as a ligand for programmed cell death protein 1 (PD-1). Though expressed primarily in dendritic cells, PD-L2 expression can be induced on a wide variety of immune and non-immune cells depending on the microenvironment. PD-L2 expression is particularly upregulated in the presence of Th2 cytokine, IL-4, as well as Th1 cytokines, TNF-alpha and IFN-gamma to a lesser degree. While generally expressed at lower levels compared to PD-L1, PD-L2 demonstrates a 2 to 6 times higher relative affinity to PD-1 than PD-L1. PD-1 and its ligands are referred to as inhibitory immune checkpoint molecules in that they provide useful negative feedback during physiological homeostasis. Ligation of PD-L2 or PD-L1 inhibits activation, proliferation, and cytokine secretion (e.g. IFN-gamma, IL-10) in T cells, ultimately dampening immune response. Conversely, studies have shown that PD-L2 can also stimulate T cell proliferation and cytokine production, even in PD-1-deficient T cells, suggesting additional receptors. Recent studies have concluded that PD-L2 also binds to a second receptor, repulsive guidance molecule b (RGMb), which was originally identified as a receptor for bone morphogenetic proteins (BMPs). RGMb is expressed in the central nervous system, as well as in macrophages, however, its role in immunity is only beginning to emerge. Interaction between PD-L2 and RGMb regulates the development of respiratory tolerance in the lung through BMP and/or neogenin signaling pathways. The naturally occurring human PD-L2 monomer consists of a 201-amino-acid extracellular domain, a 21-amino-acid transmembrane domain, and a 32-amino-acid cytoplasmic domain.

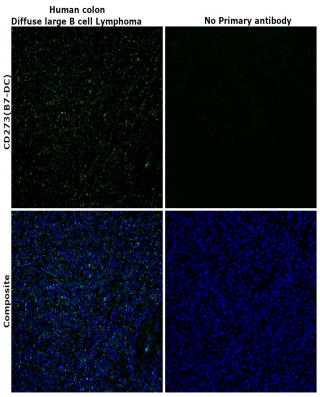
For Research Use Only. Not for use in diagnostic procedures. Not for resale without express authorization.

Product Images For CD273 (B7-DC) Polyclonal Antibody



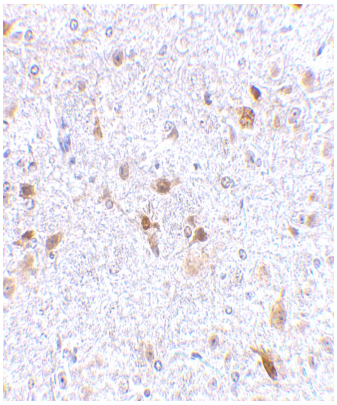
CD273 (B7-DC) Antibody (PA5-20344)

Antibody specificity was demonstrated by showing that antibodies raised against the same target protein perform similarly. Immunofluorescence of CD273 (B7DC) using CD273 (B7DC) Polyclonal Antibody (Product # PA5-20344) along with another CD273 (B7DC) Monoclonal Antibody (176611) (Product # MA5-23887) shows similar expression of CD273 (B7DC). {IAV}



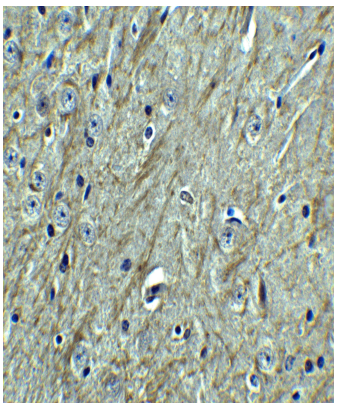
CD273 (B7-DC) Antibody (PA5-20344) in IHC (P)

Immunohistochemical analysis of CD273 (B7-DC) was performed using formalin-fixed paraffin-embedded human colon (diffuse large B cell lymphoma) tissue sections. To expose the target protein, heat-induced epitope retrieval was performed on de-paraffinized sections using eBioscience™ IHC Antigen Retrieval Solution - Low pH (10X) (Product # 00-4955-58) diluted to 1X solution in water in a decloaking chamber at 110 degree Celsius for 15 minutes. Following antigen retrieval, the sections were blocked with 2% normal goat serum in 1X PBS for 45 minutes at room temperature and then probed with or without CD273 (B7-DC) Polyclonal Antibody (Product # PA5-20344) at 10 µg/mL concentration in 0.1% normal goat serum overnight at 4 degree Celsius in a humidified chamber. Detection was performed using Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 488 (Product # A32731) at a dilution of 1:2000 in 0.1% normal goat serum for 45 minutes at room temperature. ReadyProbes™ Tissue Autofluorescence Quenching Kit (Product # R37630) was used to quench autofluorescence from the tissues. Nuclei were stained with DAPI (Product # D1306) and the sections were mounted using ProLong™ Glass Antifade Mountant (Product # P36984). The images were captured on EVOS™ M7000 Imaging System (Product # AMF7000) at 20X magnification.



CD273 (B7-DC) Antibody (PA5-20344) in IHC (P)

Immunohistochemical analysis of paraffin-embedded mouse brain tissue using CD273 (B7-DC) Polyclonal Antibody (Product # PA5-20344) at 2.5 µg/mL. Tissue was fixed with formaldehyde and blocked with 0.1 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 4C. A goat anti-rabbit IgG H&L (HRP) at 1/250 was used as secondary. Counter stained with Hematoxylin.

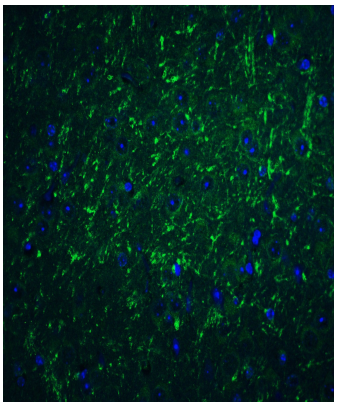


CD273 (B7-DC) Antibody (PA5-20344) in IHC (P)

Immunohistochemical analysis of paraffin-embedded mouse brain tissue using CD273 (B7-DC) Polyclonal Antibody (Product # PA5-20344) at 2.5 µg/mL. Tissue was fixed with formaldehyde and blocked with 0.1 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 4C. A goat anti-rabbit IgG H&L (HRP) at 1/250 was used as secondary. Counter stained with Hematoxylin.

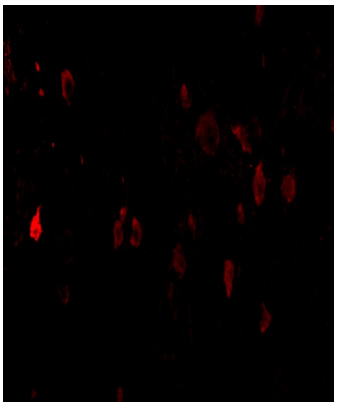
Thermo Fisher Scientific
3747 N. Meridian Road
Rockford, IL 61105 USA

thermofisher.com/contactus



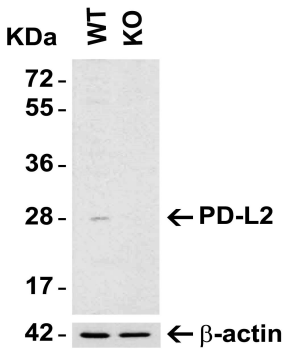
CD273 (B7-DC) Antibody (PA5-20344) in IHC (P)

Immunofluorescent analysis of 4% paraformaldehyde-fixed mouse brain tissue labeling PD-L2 with CD273 (B7-DC) Polyclonal Antibody (Product # PA5-20344) at 20 µg/mL, followed by goat anti-rabbit IgG secondary antibody at 1:500 dilution (green) and DAPI staining (blue).



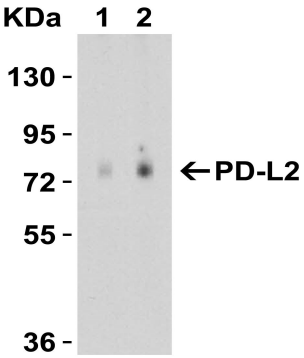
CD273 (B7-DC) Antibody (PA5-20344) in IHC (P)

Immunofluorescent analysis of 4% paraformaldehyde-fixed mouse brain cells labeling PD-L2 with CD273 (B7-DC) Polyclonal Antibody (Product # PA5-20344) at 20 µg/mL, followed by goat anti-rabbit IgG secondary antibody at 1:500 dilution (red).



CD273 (B7-DC) Antibody (PA5-20344) in WB

Western Blot analysis of CD273 in HeLa WT or PD-L2 KO cells. Lysates (15 µg) were loaded onto SDS-PAGE and blots were probed with CD273 (B7-DC) Polyclonal Antibody (Product # PA5-20344) diluted to 4 µg/mL and anti-beta actin diluted to 1 µg/mL. 1 h incubation at RT in 0.05 NFDM/TBST. Secondary: Goat Anti-Rabbit IgG HRP conjugate at 1:10,000 dilution.

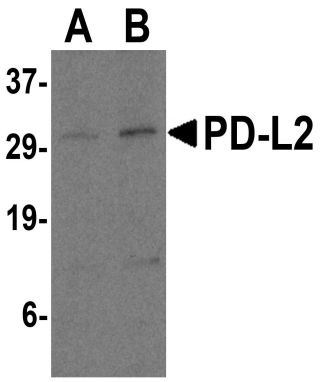


CD273 (B7-DC) Antibody (PA5-20344) in WB

Western Blot analysis of CD273 in HeLa WT or PD-L2 KO cells. Lysates (15 µg) were loaded onto SDS-PAGE and blots were probed with CD273 (B7-DC) Polyclonal Antibody (Product # PA5-20344) diluted to 4 µg/mL and anti-beta actin diluted to 1 µg/mL. 1 h incubation at RT in 0.05 NFDM/TBST. Secondary: Goat Anti-Rabbit IgG HRP conjugate at 1:10,000 dilution.

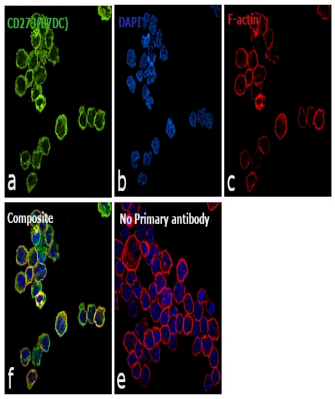
Thermo Fisher Scientific
3747 N. Meridian Road
Rockford, IL 61105 USA

thermofisher.com/contactus



CD273 (B7-DC) Antibody (PA5-20344) in WB

Western Blot Validation in Human Raji Cell Lysate. Loading: 15 µg of lysates per lane. Antibodies: CD273 (B7-DC) Polyclonal Antibody (Product # PA5-20344) (A: 0.5 µg/mL and B: 1 µg/mL), 1h incubation at RT in 0.05 NFDM/TBST. Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10,000 dilution.



CD273 (B7-DC) Antibody (PA5-20344) in ICC/IF

Immunofluorescence analysis of CD273 (B7DC) was performed using 70% confluent log phase Jurkat cells. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 15 minutes, and blocked with 1% BSA for 1 hour at room temperature. The cells were labeled with CD273 (B7DC) Monoclonal Antibody (176611) (Product # PA5-20344) at 20 µg/mL in 0.1% BSA, incubated at 4 degree Celsius overnight and then labeled with Goat anti-Rabbit IgG (Heavy Chain) Superclonal™ Secondary Antibody, Alexa Fluor® 488 conjugate (Product # A27034) at a dilution of 1:2000 for 45 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with ProLong™ Diamond Antifade Mountant with DAPI (Product # P36962). F-actin (Panel c: red) was stained with Rhodamine Phalloidin (Product # R415). Panel d represents the merged image showing membranous localization. Panel e represents control cells with no primary antibody to assess background. The images were captured at 60X magnification.

Thermo Fisher Scientific
3747 N. Meridian Road
Rockford, IL 61105 USA

thermofisher.com/contactus

PubMed References For CD273 (B7-DC) Polyclonal Antibody

1 Western Blot References

Species / Dilution	Summary
	PA5-20344 was used in Western Blot to conclude that PLK1-mediated phosphorylation of vimentin activates TGF- signaling pathway, leading to the metastasis and immune escape through the expression of PD-L1, functioning as a shuttling protein in lung adenocarcinoma.
Human / Not Cited	Cell death and differentiation (2021; 28: 2745) **"PLK1/vimentin signaling facilitates immune escape by recruiting Smad2/3 to PD-L1 promoter in metastatic lung adenocarcinoma."** Author(s):Jang HR,Shin SB,Kim CH,Won JY,Xu R,Kim DE,Yim H PubMed Article URL:http://dx.doi.org/10.1038/s41418-021-00781-4

Thermo Fisher Scientific
3747 N. Meridian Road
Rockford, IL 61105 USA

thermofisher.com/contactus