abcam

ab228415

Anti-PD-L1/CD274 antibody [73-10]

Anti-PD-L1/CD274 antibody [73-10] (ab228415) is a rabbit monoclonal antibody detecting PD-L1/CD274 in **Western Blot, Flow Cytometry (Intra), IP, IHC-P, IHC-Fr, ICC/IF**. Suitable for **Human**.

- KO validated for confirmed specificity
- Biophysical QC for unrivalled batch-batch consistency
- Over 20 publications

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	73-10
Purification technique	Affinity purification Protein A
Concentration	0.12 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	1/500
Notes	Recommended incubation time: 10 mins at room temperature Perform heat-mediated antigen retrieval before commencing with IHC staining protocol.

Not recommended

Species	Transfected cell lysate, Transfected cell line
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	1/30
Notes	-

Not recommended

Species	Transfected cell lysate, Transfected cell line
Dilution info	-
Notes	-

WB

Tested

Species	Transfected cell lysate
Dilution info	1/1000
Notes	-

Species	Human
Dilution info	1/1000

Notes	-

Not recommended

Species	Transfected cell line
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Transfected cell line
Dilution info	1/200
Notes	-

Expected

Species	Human
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Transfected cell lysate
Dilution info	-
Notes	-

IHC-Fr

Tested

Species	Human
Dilution info	0.05 µg/mL
Notes	-

Not recommended

Species	Transfected cell lysate, Transfected cell line
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Transfected cell line
Dilution info	1/100
Notes	-

Expected

Species	Human
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Transfected cell lysate
Dilution info	-
Notes	-

Target data

See full target information PD-L1/CD274

Function Plays a critical role in induction and maintenance of immune tolerance to self (PubMed:11015443, PubMed:28813410, PubMed:28813417, PubMed:31399419). As a ligand for the inhibitory receptor PDCD1/PD-1, modulates the activation threshold of T-cells and limits T-cell effector response (PubMed:11015443, PubMed:28813410, PubMed:28813417, PubMed:36727298). Through a yet unknown activating receptor, may costimulate T-cell subsets that predominantly produce interleukin-10 (IL10) (PubMed:10581077). Can also act as a transcription coactivator: in response to hypoxia, translocates into the nucleus via its interaction with phosphorylated STAT3 and promotes transcription of GSDMC, leading to pyroptosis (PubMed:32929201). The PDCD1-mediated inhibitory pathway is exploited by tumors to attenuate anti-tumor immunity and escape destruction by

the immune system, thereby facilitating tumor survival (PubMed:28813410, PubMed:28813417). The interaction with PDCD1/PD-1 inhibits cytotoxic T lymphocytes (CTLs) effector function (By similarity). The blockage of the PDCD1-mediated pathway results in the reversal of the exhausted T-cell phenotype and the normalization of the anti-tumor response, providing a rationale for cancer immunotherapy (By similarity).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Anti-PD-L1 antibody [73-10] (ab228415) was developed by Abcam using patented rabbit monoclonal antibody technology and is validated for use in Flow Cyt (Intra), ICC/IF, IHC-Fr, IHC-P, IP and WB.

Abcam's high quality manufacturing and validation processes ensure Anti-PD-L1 antibody [73-10] (ab228415) has high sensitivity and specificity alongside high lot-to-lot consistency and reproducibility.

The specificity of Anti-PD-L1 antibody [73-10] (ab228415) has been confirmed by Western Blot testing in PD-L1 knockout A549 cells (ab267054).

Anti-PD-L1 antibody [73-10] (ab228415) specifically detects PD-L1 (UniProt ID: Q9NZQ7; Molecular weight: 32kDa) and is sold in 50 µL selling sizes.

Conjugation-ready, carrier free format available for antibody clone 73-10 - ab226766.

Antibody clone 73-10 is also available pre-conjugated to a variety of labels for your convenience - Alexa Fluor® 488, Alexa Fluor® 647, Alexa Fluor® 555, Alexa Fluor® 594 (ab237402, ab237403, ab274896, ab275355).

Programmed death-ligand 1 (PD-L1) is a critical protein in immuno-oncology, playing a significant role in tumor immune evasion. It is expressed on the surface of tumor cells and binds to the PD-1 receptor on T cells, inhibiting their activity and allowing tumors to escape immune detection.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For

details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	PD-L1 also known as Programmed Death-Ligand 1 or CD274 is a protein involved in immune modulation. Mechanically PD-L1 interacts with its receptors particularly PD-1 to regulate cellular immune responses. This transmembrane protein has a calculated molecular weight of approximately 33 kDa. PD-L1 is expressed on various cell types including tumor cells and immune cells such as dendritic cells macrophages and B cells. Its expression is often upregulated in response to inflammatory cytokines.
Biological function summary	PD-L1 plays a central role in immune evasion mechanisms utilized by tumors. It is not part of a larger protein complex but directly interacts with PD-1 and CD80. When PD-L1 binds to PD-1 it sends inhibitory signals leading to decreased T cell activation and proliferation allowing cancer cells to avoid immune destruction. PD-L1 expression provides a mechanism for tumors to suppress immune surveillance facilitating tumor progression.
Pathways	PD-L1 is integral to the immune checkpoint pathway which is an important regulator of immune response. The interaction between PD-L1 and PD-1 provides a mechanism for immune tolerance which is part of the broader adaptive immune system pathway. PD-L1 is related to other immune checkpoint proteins such as CTLA-4 in its function to limit autoreactivity and promote immune homeostasis.
Associated diseases and disorders	PD-L1 is most associated with cancer particularly in tumors such as melanoma and non-small cell lung cancer. PD-L1 expression on tumor cells often correlates with poor prognosis. PD-L1 directly interacts with PD-1 in these cancers a target for immunotherapies such as checkpoint inhibitors which aim to block this interaction and restore immune activity against tumors. PD-L1 involvement extends to autoimmune disorders where altered expression can impact tolerance and lead to immune-related tissue damage.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

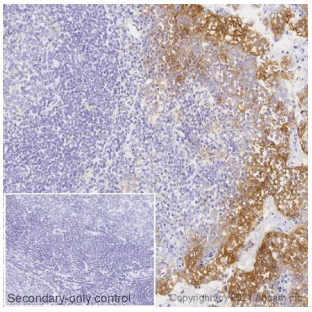
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

---

25 product images



Immunohistochemistry (Frozen sections) - Anti-PD-L1/CD274 antibody [73-10] (ab228415)

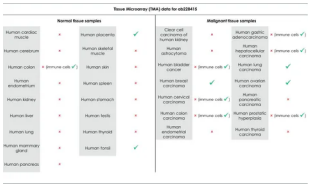
PD-L1/CD274 Immunohistochemistry (Frozen sections) staining using rabbit Anti-PD-L1/CD274 antibody

IHC image of PD-L1 staining in a section of frozen normal human tonsil* performed on a Leica BOND™ system using the standard protocol. The section was fixed in 10% paraformaldehyde (10 min) prior to staining. The section was incubated with ab228415, 0.05ugml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre

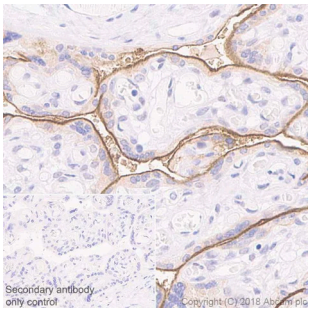
---



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [73-10] (ab228415)

PD-L1/CD274 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-PD-L1/CD274 antibody

Tissue Microarrays stained for "Anti-PD-L1 antibody [73-10]" using "ab228415"in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negative (cross mark) staining per sample type tested. The sections were pre-treated using Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) for 20 minutes. The sections were incubated with ab228415 for 10 mins at room temperature followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

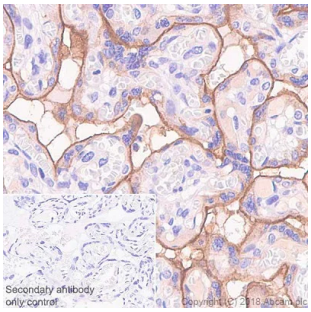


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [73-10] (ab228415)

PD-L1/CD274 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-PD-L1/CD274 antibody

Immunohistochemical analysis of paraffin-embedded human placenta tissue labeling PD-L1 with ab228415 at 1/5000 dilution. The tissue was incubated with ab228415 at 4☒ overnight. followed by Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) ready to use. Membranous and cytoplasmic staining in human placenta (PMID: 12538684) is observed. Counter stained with hematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

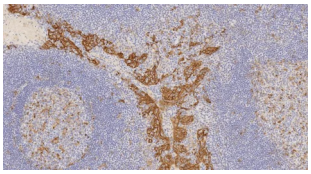
Antigen retrieval: Universal HIER antigen retrieval reagent (10X) (ab208572).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [73-10] (ab228415)

PD-L1/CD274 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-PD-L1/CD274 antibody

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) of human placenta staining PD-L1 with ab228415 at 1/500 dilution. Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) for 10 mins. The section was incubated with ab228415 for 10 mins at room temperature. The secondary antibody used was ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Counter stained Hematoxylin. Performed on a Leica Biosystems BOND® RX instrument.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [73-10] (ab228415)

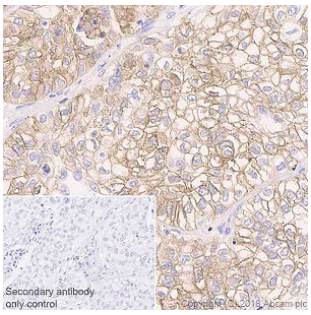
PD-L1/CD274 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-PD-L1/CD274 antibody

IHC image of ab228415 staining PD-L1 in human tonsil formalin fixed paraffin embedded tissue sections*, performed on a Leica BOND RX (standard Protocol F, Polymer Refine kit). The section was pre-treated using heat mediated antigen retrieval with EDTA buffer (pH9, epitope retrieval solution 2) for 30 mins at 98°C. The section was then incubated with ab228415, 0.06µg/ml working concentration, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system for 8 minutes at room temperature. DAB was used as the chromogen for 10 minutes at room temperature. The section was then counterstained with hematoxylin, blued, dehydrated, cleared and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and

antibody incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre

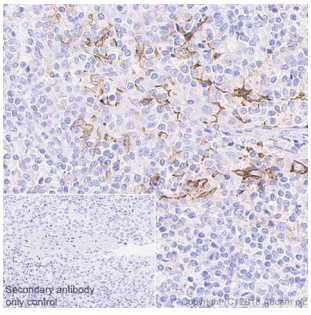


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [73-10] (ab228415)

PD-L1/CD274 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-PD-L1/CD274 antibody

Immunohistochemical analysis of paraffin-embedded human lung carcinoma tissue labeling PD-L1 with ab228415 at 1/5000 dilution. The tissue was incubated with ab228415 at 4☒ overnight, followed by Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) ready to use. Membranous and weakly cytoplasmic staining in human lung carcinoma (PMID: 23460533) is observed. Counter stained with hematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Antigen retrieval: Universal HIER antigen retrieval reagent (10X) (ab208572).

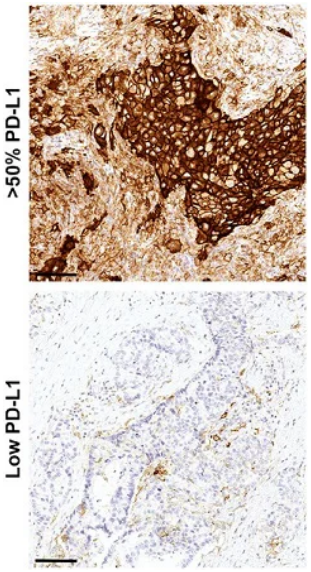


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [73-10] (ab228415)

PD-L1/CD274 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-PD-L1/CD274 antibody

Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling PD-L1 with ab228415 at 1/5000 dilution. The tissue was incubated with ab228415 at 4° overnight, followed by Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) ready to use. Cytoplasmic and membranous staining in human tonsil is observed. Counter stained with hematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Antigen retrieval: Universal HIER antigen retrieval reagent (10X) (ab208572).

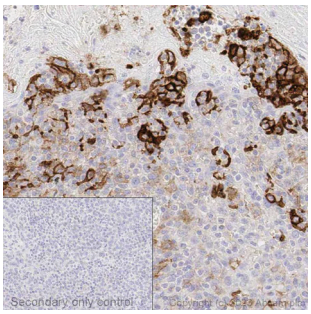


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [73-10] (ab228415)

PD-L1/CD274 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-PD-L1/CD274 antibody

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) Staining PD-L1 in human non-small cell lung cancer tissue with >50% PD-L1-positive tumor cells were compared with tissue with lower PD-L1 expression using 73-10 at 0.25µg/ml incubated for 30 minutes at room temperature. Antigen Retrieval was done with Target Retrieval Solution high pH. Detection was done with EnVision FLEX/HRP. Hematoxylin EnVision FLEX was used as a counter stain.

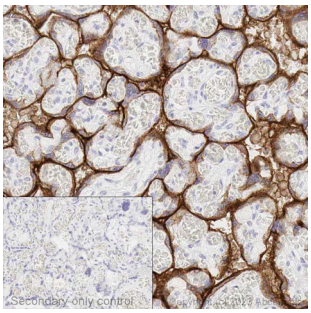
Image from Silva MA et al., PLoS One. 2018;13(6):e0196464. Fig 3(B).; 10.1371/journal.pone.0196464.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [73-10] (ab228415)

PD-L1/CD274 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of human tonsil using rabbit Anti-PD-L1/CD274 antibody

Immunohistochemical analysis of formalin-fixed paraffin-embedded human tonsil labelling PD-L1 with ab228415 at a concentration of 0.1µg/ml. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with an OptiView DAB IHC Detection Kit. Heat mediated antigen retrieval was conducted for 32min with ULTRA cell conditioning solution (CC1 pH8.5). ab228415 anti PD-L1 antibody was incubated at 37°C for 16min. Sections were counterstained is with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [73-10] (ab228415)

PD-L1/CD274 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of human placenta using rabbit Anti-PD-L1/CD274 antibody

Immunohistochemical analysis of formalin-fixed paraffin-embedded human placenta labelling PD-L1 with ab228415 at a concentration of 0.1µg/ml. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with an OptiView DAB IHC Detection Kit. Heat mediated antigen retrieval was conducted for 32min with ULTRA cell conditioning solution (CC1 pH8.5). ab228415 anti PD-L1 antibody was incubated at 37°C for 16min. Sections were counterstained with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.

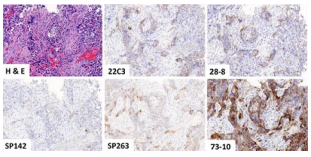
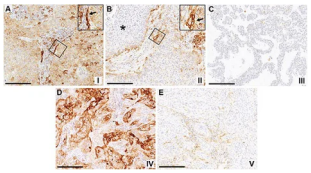


Image from Tsao MS et al., J Thorac Oncol. 2018;13(9):1302-1311. Fig 3.; 10.1016/j.jtho.2018.05.013 with permission from Elsevier.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [73-10] (ab228415)

PD-L1/CD274 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-PD-L1/CD274 antibody

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) of lung cancer tissue samples. Comparing the staining PD-L1 with different monoclonal antibodies. 73-10 showed higher sensitivity to PD-L1 compared to the other clones. For further details on this image please see PubMed ID: 29800747.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [73-10] (ab228415)

Image from Silva MA et al., PLoS One. 2018;13(6):e0196464. Fig 4.; 10.1371/journal.pone.0196464.

PD-L1/CD274 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-PD-L1/CD274 antibody

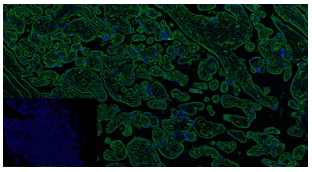
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) Staining PD-L1 in human non-small cell lung cancer tissue using 73-10 at 0.25µg/ml incubated for 30 minutes at room temperature. Antigen Retrieval was done with Target Retrieval Solution, high pH. Detection was done with EnVision FLEX/HRP. Hematoxylin EnVision FLEX was used as a counter stain.
A: Diffuse expression of PD-L1 (IHC) on tumor cell membranes of a squamous cell carcinoma, including central regions of trabeculae. Prominent labeling of cells in the TME compartment at the tumor-nest-TME interface suggesting presence of an immunological synapse (inset arrow).

B: Patchy expression of PD-L1 in a squamous cell carcinoma at the tumor-nest-TME interface (inset arrow). Minimal to no PD-L1 expression in the trabeculae (asterisk) if compared with (**A**)

C: No to minimal PD-L1 expression in both tumor and TME compartments in an adenocarcinoma.

D: Diffuse expression of PD-L1 by tumor-nests in an adenocarcinoma with minimal TME staining.

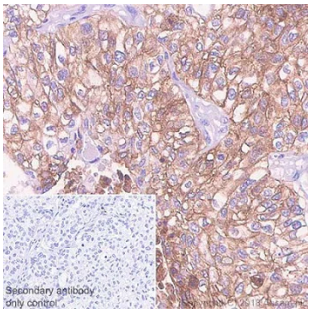
F: TME expression only. No to minimal PD-L1 expression in tumor cells of a squamous cell carcinoma, with widespread staining in the TME compartment.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [73-10] (ab228415)

PD-L1/CD274 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-PD-L1/CD274 antibody

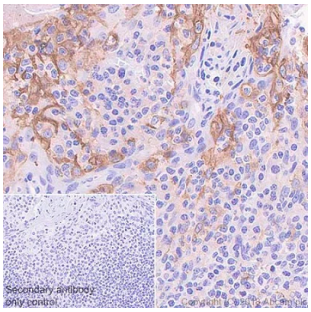
Anti-PD-L1 antibody [73-10] (ab228415)
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human tonsil tissue labelling PD-L1 with ab228415 at a dilution of 1:2500. Heat mediated antigen retrieval was performed using AR9 antigen retrieval solution, and microwave treatment for 15 min at 20% power. Anti-Rabbit/Mouse HRP polymer (Vector Labs) was used as secondary antibody. Opal tyramide amplification was performed using Opal 520 fluorophore.. Counterstained with DAPI stain. Image scanned with Vectra 3.0 and analyzed via software.
This image was courteously provided by Dr. Houssein Abdul Sater, Georgia Cancer Center.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [73-10] (ab228415)

PD-L1/CD274 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-PD-L1/CD274 antibody

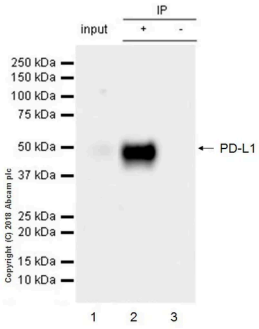
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) of human lung carcinoma staining PD-L1 with ab228415 at 1/500 dilution. Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) for 10 mins. The section was incubated with ab228415 for 10 mins at room temperature. The secondary antibody used was ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Counter stained Hematoxylin. Performed on a Leica Biosystems BOND® RX instrument.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [73-10] (ab228415)

PD-L1/CD274 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-PD-L1/CD274 antibody

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) of human tonsil staining PD-L1 with ab228415 at 1/500 dilution. Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) for 10 mins. The section was incubated with ab228415 for 10 mins at room temperature. The secondary antibody used was ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Counter stained Hematoxylin. Performed on a Leica Biosystems BOND® RX instrument.



Immunoprecipitation - Anti-PD-L1/CD274 antibody [73-10] (ab228415)

PD-L1 was immunoprecipitated from 0.35 mg of NCI-H1975 (human non-small cell lung cancer cell line) whole cell lysate with ab228415 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab228415 at 1/1,000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/5,000 dilution.
Lane 1: NCI-H1975 whole cell lysate 10 µg (Input).

Lane 2: ab228415 IP in NCI-H1975 whole cell lysate (+).

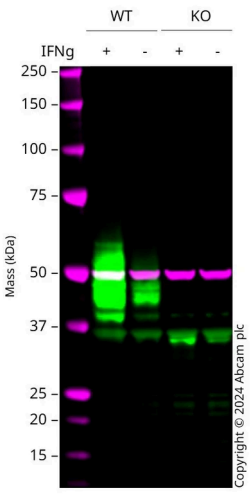
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab228415 in NCI-H1975 whole cell lysate (-).

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: 30 seconds.

All lanes:
Immunoprecipitation - Anti-PD-L1/CD274 antibody [73-10] (ab228415)

Predicted band size: 33 kDa



Western blot - Anti-PD-L1/CD274 antibody [73-10] (ab228415)

PD-L1/CD274 Western blot staining using rabbit Anti-PD-L1/CD274 antibody

Western blot: Anti-CD274 antibody [73-10] (ab228415) staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (ab7291) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab228415 was shown to bind specifically to CD274. A band was observed at 40-60 kDa in treated wild-type A549 cell lysates with no signal observed at this size in CD274 knockout cell line ab267054 (knockout cell lysate ab256831). To generate this image, wild-type and CD274 knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.
All lanes:
Western blot - Anti-PD-L1/CD274 antibody [73-10] (ab228415) at 1/1000 dilution

Lane 1:
Wild-type A549 Treated IFN-gamma (100 ng/mL, 48 h) cell lysate at 20 µg

Lane 2:

Wild-type A549 Vehicle Control IFN-gamma (0 ng/mL, 48 h) cell lysate at 20 µg

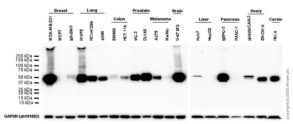
Lane 3:
CD274 knockout A549 Treated IFN-gamma (100 ng/mL, 48 h) cell lysate at 20 µg

Lane 4:
CD274 knockout A549 Vehicle Control IFN-gamma (0 ng/mL, 48 h) cell lysate at 20 µg

Secondary

All lanes:
Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.



Western blot - Anti-PD-L1/CD274 antibody [73-10] (ab228415)

PD-L1/CD274 Western blot staining using rabbit Anti-PD-L1/CD274 antibody

Blocking/Diluting buffer and concentration: 5% NFDM/TBST. Expression of PD-L1 varied widely among the tumor cell lines.
All lanes:
Western blot - Anti-PD-L1/CD274 antibody [73-10] (ab228415) at 1/1000 dilution

Lane 1:
MDA-MB-231 (Human breast adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 2:
MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 3:
SK-BR-3 (Human breast adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 4:
H1975 (Human non-small cell lung cancer epithelial cell) whole cell lysate at 20 µg

Lane 5:
NCI-H1299 (Human lung carcinoma epithelial cell) whole cell lysate at 20 µg

Lane 6:
A549 (Human lung carcinoma epithelial cell) whole cell lysate at 20 µg

Lane 7:
SW480 (Human colorectal adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 8:
HCT 116 (Human colorectal carcinoma epithelial cell) whole cell lysate at 20 µg

Lane 9:
PC-3 (Human prostate adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 10:
DU 145 (Human prostate carcinoma epithelial cell) whole cell lysate at 20 µg

Lane 11:
A375 (Human malignant melanoma epithelial cell) whole cell lysate at 20 µg

Lane 12:
MeWo (Human malignant melanoma fibroblast) whole cell lysate at 20 µg

Lane 13:
U-87 MG (Human glioblastoma-astrocytoma epithelial cell) whole cell lysate at 20 µg

Lane 14:
Huh7 (Human hepatocellular carcinoma epithelial cell) whole cell lysate at 20 µg

Lane 15:
HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysate at 20 µg

Lane 16:
BXPC-3 (Human pancreas adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 17:
PANC-1 (Human pancreatic epithelioid carcinoma epithelial cell) whole cell lysate at 20 µg

Lane 18:
NIH:OVCAR-3 (Human ovary adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 19:
SK-OV-3 (Human ovarian cancer epithelial cell) whole cell lysate at 20 µg

Lane 20:
HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate at 20 µg

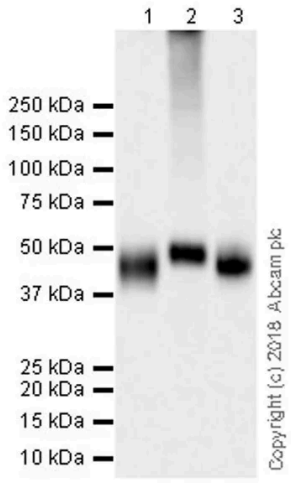
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 33 kDa

Observed band size: 40-60 kDa

Exposure time: 120s



Western blot - Anti-PD-L1/CD274 antibody [73-10] (ab228415)

PD-L1/CD274 Western blot staining using rabbit Anti-PD-L1/CD274 antibody

Blocking/Dilution buffer: 5% NFDM/TBST.
The molecular mass observed is consistent with what has been described in the literature (PMID: 26546452).

All lanes:
Western blot - Anti-PD-L1/CD274 antibody [73-10] (ab228415) at 1/1000 dilution

Lane 1:
NCI-H1975 (human non-small cell lung cancer cell line), whole cell lysate at 20 µg

Lane 2:
Human placenta at 20 µg

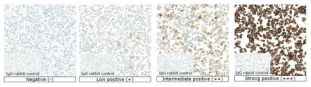
Lane 3:
Human thymus at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

Predicted band size: 33 kDa

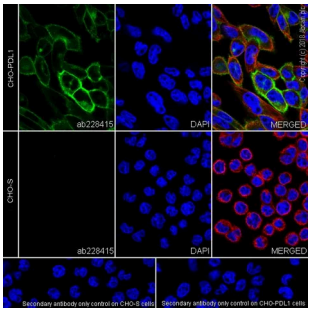
Exposure time: 70s



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [73-10] (ab228415)

PD-L1/CD274 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-PD-L1/CD274 antibody

Immunohistochemical staining of PD-L1 in formalin-fixed, paraffin embedded Formalin-fixed, paraffin-embedded reference standard with negative (-), low positive (+), intermediate positive (++) and strong positive (+++) controlled protein expressing cell lines (,CD274 (PD-L1) Expression IHC Reference Standard', catalog ID HD787, horizon) using clone 73-10 [ab228415] at a dilution of 10µg/ml. Incubate for 30 minutes at 37°C. Heat mediated antigen retrieval in sCC1 (Tris/EDTA buffer, pH 8). Signal detection with BenchMark XT from Roche/Ventana and ultraView Universal DAB Detection Kit (Code 760-500).

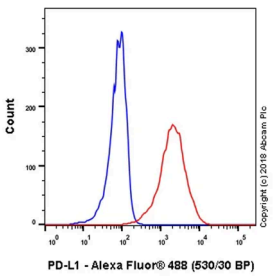


Immunocytochemistry/ Immunofluorescence - Anti-PD-L1/CD274 antibody [73-10] (ab228415)

PD-L1/CD274 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-PD-L1/CD274 antibody

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized CHO-PD-L1 (PD-L1 stably expressed Chinese hamster ovary epithelial cell) cells labeling PD-L1 with ab228415 at 1/200 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1,000 dilution (green). Confocal image showing membranous staining on CHO-PD-L1 cells.
The nuclear counter stain is DAPI (blue). Tubulin is detected with anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (ab195889) (red) at 1/200 dilution.

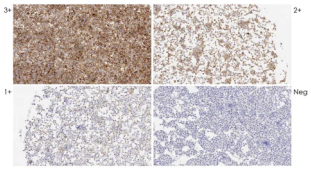
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1,000 dilution.



Flow Cytometry (Intracellular) - Anti-PD-L1/CD274 antibody [73-10] (ab228415)

PD-L1/CD274 Flow Cytometry (Intracellular) staining using rabbit Anti-PD-L1/CD274 antibody

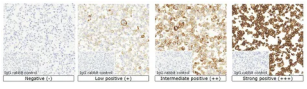
Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized CHO-PD-L1 (PD-L1 stably expressed Chinese hamster ovary epithelial cell, Red) / CHO-S (Chinese hamster ovary epithelial cell, Blue) cell lines labeling PD-L1 with ab228415 at 1/100 dilution (red). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) at 1/2000 dilution was used as the secondary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [73-10] (ab228415)

PD-L1/CD274 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-PD-L1/CD274 antibody

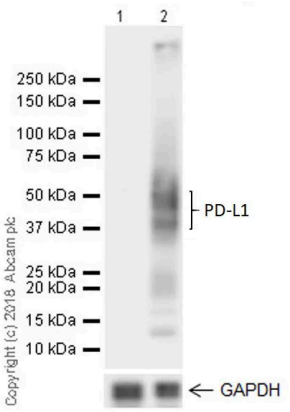
IHC image of ab228415 staining PD-L1 in PD-L1 Dynamic Range Analyte Control formalin fixed paraffin embedded cell lines (HistoCyte Laboratories), performed on a Leica BOND RX (standard Protocol F, Polymer Refine kit). The section was pre-treated using heat mediated antigen retrieval with EDTA buffer (pH9, epitope retrieval solution 2) for 30 mins at 98°C. The section was then incubated with ab228415, 0.06µg/ml working concentration, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system for 8 minutes at room temperature. DAB was used as the chromogen for 10 minutes at room temperature. The section was then counterstained with hematoxylin, blued, dehydrated, cleared and mounted with DPX.
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [73-10] (ab228415)

PD-L1/CD274 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-PD-L1/CD274 antibody

Immunohistochemical staining of PD-L1 in formalin-fixed, paraffin embedded reference standard with negative (-), low positive (+), intermediate positive (++) and strong positive (+++) controlled protein expressing cell lines (,CD274 (PD-L1) Expression IHC Reference Standard, catalog ID HD787, horizon) using clone 73-10 [ab228415] at a dilution of 2µg/ml. Incubate for 30 minutes at room temperature. Heat mediated antigen retrieval in high pH buffer (Tris/EDTA buffer, pH 9, during 20 min at 95°C). Block sample with peroxidase blocking buffer (EnVision Flex Peroxidase-Blocking Reagent) for 5 minutes. Signal detection with Autostainer Link from Dako and EnVision Flex Kit, High pH (Code K8000).



Western blot - Anti-PD-L1/CD274 antibody [73-10] (ab228415)

PD-L1/CD274 Western blot staining using rabbit Anti-PD-L1/CD274 antibody

Blocking/Dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-PD-L1/CD274 antibody [73-10] (ab228415) at 1/1000 dilution

Lane 1:
CHO-S (Chinese hamster ovary epithelial cell) at 10 µg

Lane 2:
CHO-PD-L1 (PD-L1 stably expressed Chinese hamster ovary epithelial cell) at 10 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 33 kDa

Observed band size: 40-60 kDa

Exposure time: 15s

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.