abcam

ab279292

Anti-PD-L1/CD274 antibody [CAL10] - Mouse IgG1 (Chimeric)

Anti-PD-L1/CD274 antibody [CAL10] - Mouse IgG1 (Chimeric) (ab279292) is a mouse monoclonal antibody detecting PD-L1/CD274 in **Western Blot, Flow Cytometry (Intra), IHC-P, ICC/IF**. Suitable for **Human**.

- KO validated for confirmed specificity
- Biophysical QC for unrivalled batch-batch consistency

Recombinant | KO Validated | 20ul selling size

Key facts

Isotype	IgG1
Host species	Mouse
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	CAL10
Purification technique	Affinity purification Protein A
Concentration	1.053 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	-
Notes	Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Not recommended

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

ICC/IF

Tested

Species	Transfected cell line - Human
Dilution info	1/100
Notes	-

Expected

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

Not recommended

Species	Transfected cell lysate - Human
Dilution info	-
Notes	-

WB

Tested

Species	Transfected cell lysate - Human

Dilution info	1/1000
Notes	-

Species	Human
Dilution info	1/1000
Notes	-

Not recommended

Species	Transfected cell line - Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Transfected cell line - Human
Dilution info	1/50
Notes	-

Expected

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

Not recommended

Species	Transfected cell lysate - Human
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	Blue Ice
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

What is this antibody validated in?
Anti-PD-L1 antibody [CAL10] - Mouse IgG1 (Chimeric) (ab279292) is a mouse recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Immunohistochemistry (IHC-P), Immunocytochemistry/immunofluorescence (ICC/IF) in Human samples.

Specificity confirmed
The specificity of Anti-PD-L1 antibody [CAL10] - Mouse IgG1 (Chimeric) (ab279292) has been confirmed by Western blot testing in CD274 Knockout A549 cell line, ab267054.

Other related products
We have a range of other formats of antibody clone [CAL10] also available for your convenience: ab237726, Carrier free - ab251611, Alexa Fluor® 647 - ab267565, ab279292, ab279293, ab279294, Carrier free - ab279304, Carrier free -

Trial sizes available!
Test your antibody or perform pre-screening before committing to a larger quantity. Sold in 20µl. Discover our selection of trial-size 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.

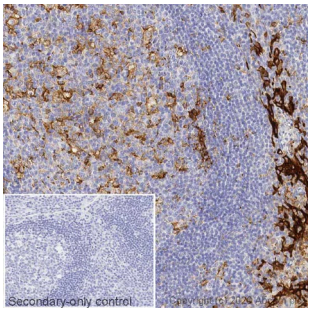
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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.

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6 product images



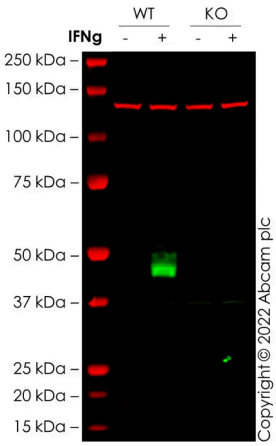
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [CAL10] - Mouse IgG1 (Chimeric) (ab279292)

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

IHC image of PD-L1 staining in a section of formalin-fixed paraffin-embedded normal human tonsil* performed on a Leica BOND^{TM} system using the standard protocol F.
The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab279292, 1ug/ml, for 15 mins at room temperature. A rabbit anti-mouse IgG1, ab125913, was added for 8 mins at room temperature and detected using an HRP conjugated goat anti-rabbit 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

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Western blot - Anti-PD-L1/CD274 antibody [CAL10] - Mouse IgG1 (Chimeric) (ab279292)

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

False colour image of Western blot: Anti-PD-L1 antibody [CAL10] – Mouse IgG1 (Chimeric); Rabbit anti-Vinculin antibody (ab219649) loading control staining at 1/1000 dilution, shown in red. In Western blot, ab279292 was shown to bind specifically to PD-L1. A band was observed at 45 kDa in wild-type A549 cell lysates with no signal observed at this size in Cd274 knockout cell line. 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-Mouse IgG H&L 800CW and Goat anti-Rabbit IgG H&L 680RD at 1/20000 dilution.

All lanes:
Western blot - Anti-PD-L1/CD274 antibody [CAL10] - Mouse IgG1 (Chimeric) (ab279292) at 1/1000 dilution

Lanes 1 - 2:
Western blot - Human wild-type A549 cell line (ab255450)

Lane 1:
Wild-type A549 Control IFN-gamma (0 ng/mL, 48 h) at 20 µg

Lane 2:
Wild-type A549 Treated IFN-gamma (100 ng/mL, 48 h) at 20 µg

Lanes 3 - 4:
Western blot - Human CD274 (PD-L1) knockout A549 cell line (ab267055)

Lane 3:
CD274 knockout A549 Control IFN-gamma (0 ng/mL, 48 h) at 20 µg

Lane 4:
CD274 knockout A549 Treated IFN-gamma (100 ng/mL, 48 h) at 20 µg

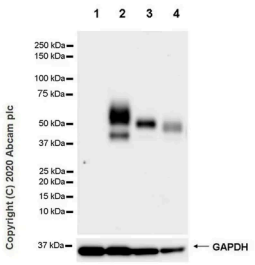
Secondary

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

Performed under reducing conditions.

Predicted band size: 33 kDa

Observed band size: 45 kDa



Western blot - Anti-PD-L1/CD274 antibody [CAL10] - Mouse IgG1 (Chimeric) (ab279292)

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

Blocking/Dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-PD-L1/CD274 antibody [CAL10] - Mouse IgG1 (Chimeric) (ab279292) at 1/1000 dilution

Lane 1:

CHO-S (Chinese hamster ovary epithelial cell) whole cell lysate at 20 µg

Lane 2:
CHO-PD-L1 (PD-L1 stably expressed Chinese hamster ovary epithelial cell) whole cell lysate at 20 µg

Lane 3:
Human placenta tissue lysate at 20 µg

Lane 4:
NCI-H1299 (human lung carcinoma epithelial cell), whole cell lysate at 20 µg

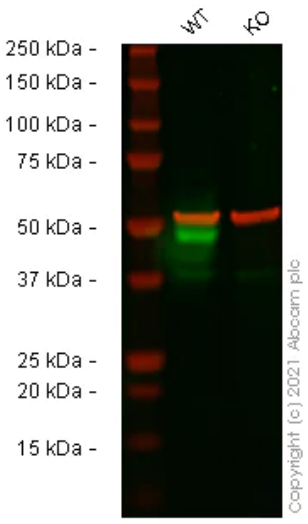
Secondary

All lanes:
Peroxidase-Conjugated Goat anti-Mouse IgG (H+L) at 1/5000 dilution

Predicted band size: 33 kDa

Observed band size: 40-60 kDa

Exposure time: 15s



Western blot - Anti-PD-L1/CD274 antibody [CAL10] - Mouse IgG1 (Chimeric) (ab279292)

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

False colour image of Western blot: Anti-PD-L1 antibody [CAL10] - Mouse IgG1 staining at 1/1000 dilution, shown in green; Rabbit anti-alpha Tubulin antibody [EP1332Y] (ab52866) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab279292 was shown to bind specifically to PD-L1. A band was observed at 48 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-Mouse IgG H&L (IRDye® 800CW) preabsorbed (ab216772) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (ab216777) at 1/20000 dilution.
Lanes 1 - 2:
Western blot - Anti-PD-L1/CD274 antibody [CAL10] - Mouse IgG1 (Chimeric) (ab279292) at 1/1000 dilution

Lanes 1 - 2:
Western blot - Anti-PD-L1/CD274 antibody [CAL10] (ab237726) at 1/1000 dilution

Lane 1:
Wild-type A549 Treated IFN-gamma (100 ng/ml) for 48 hours cell lysate at 20 µg

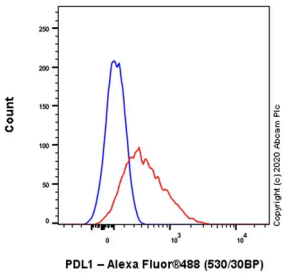
Lane 2:
CD274 knockout A549 Treated IFN-gamma (100 ng/ml) for 48 hours cell lysate at 20 µg

Lane 2:
Western blot - Human CD274 (PD-L1) knockout A549 cell line (ab267054)

Performed under reducing conditions.

Predicted band size: 33 kDa

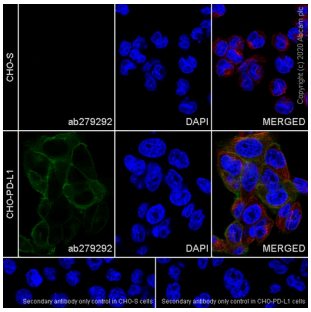
Observed band size: 48 kDa



Flow Cytometry (Intracellular) - Anti-PD-L1/CD274 antibody [CAL10] - Mouse IgG1 (Chimeric) (ab279292)

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

Flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized CHO-s (Chinese hamster ovary epithelial cell, Blue) / CHO-PDL1 (PD-L1 stably expessed Chinese hamster ovary epithelial cell, Red) labelling PD-L1 with ab279292 at 1/50 dilution (0.1µg).
Goat Anti-Mouse IgG (Alexa Fluor® 488, ab150113) at 1/2000 dilution was used as the secondary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-PD-L1/CD274 antibody [CAL10] - Mouse IgG1 (Chimeric) (ab279292)

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

Immunocytochemical analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100-fixed permeabilized CHO-PD-L1 cells labeling PD-L1 with ab279292 at 1/100 dilution, followed by ab150113 Goat Anti-MouseIgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green). ab179513 Anti-beta Tubulin rabbit monoclonal antibody was used to counterstain tubulin at 1/200 dilution, followed by ab150080 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) at a 1/500 dilution (Red). The nuclear counterstain was DAPI (Blue). Confocal image showing membranous and cytoplasmic staining in CHO-PD-L1 cells.
Negative control 1: ab279292 at a 1/100 dilution followed by ab150080 at a 1/200 dilution.

Negative control 2: ab179513 at a 1/200 dilution followed by ab150157 at a 1/1000 dilution.

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