abcam

ab279306

Anti-PD-L1/CD274 antibody [CAL10] - Rat IgG2a (Chimeric) - BSA and Azide free

Recombinant | KO Validated

Key facts

Isotype	IgG2a
Host species	Rat
Storage buffer	Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	CAL10
Purification technique	Affinity purification Protein A

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

WB

Tested

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

Not recommended

Species	Transfected cell line - Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Transfected cell line - Human
Dilution info	-
Notes	-

Expected

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

Not recommended

Species	Transfected cell lysate - Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Transfected cell line - Human
Dilution info	-
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 conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab279306 is the carrier free version of ab279294.
This rat monoclonal chimeric antibody has been engineered from a RabMAb parent antibody (ab237726). By necessity, some rabbit sequence is retained as part of the variable domain. When multiplexing with other rabbit-derived antibodies, using cross absorbed Fc-reactive secondary antibodies are recommended.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

Compatibility
This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

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

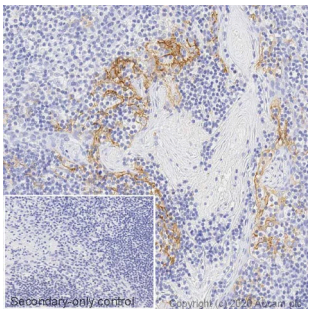
We do not recommend this combination. It is not covered by our product promise.

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.

6 product images



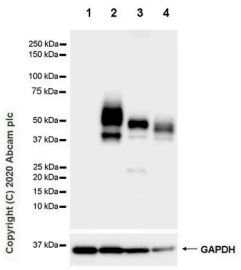
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [CAL10] - Rat IgG2a (Chimeric) - BSA and Azide free (ab279306)

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

This data was produced using ab279294, the same clone in a different formulation.
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 ab279294, 1ug/ml, for 15 mins at room temperature. A rabbit anti-rat IgG2a, ab102248, 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



Western blot - Anti-PD-L1/CD274 antibody [CAL10] - Rat IgG2a (Chimeric) - BSA and Azide free (ab279306)

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

This data was produced using ab279294, the same clone in a different formulation.
Blocking/Dilution buffer: 5% NFDM/TBST.

All lanes:
Western blot - Anti-PD-L1/CD274 antibody [CAL10] - Rat IgG2a (Chimeric) (ab279294) 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

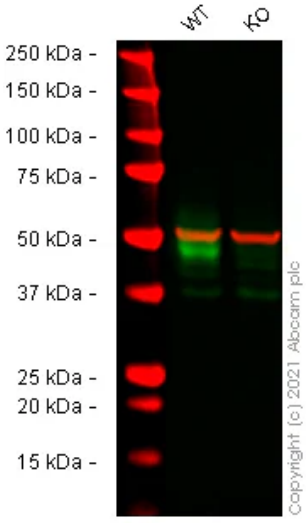
Secondary

All lanes:
Western blot - Goat Anti-Rat IgG H&L (HRP) (ab205720) at 1/5000 dilution

Predicted band size: 33 kDa

Observed band size: 40-60 kDa

Exposure time: 8s



Western blot - Anti-PD-L1/CD274 antibody [CAL10] - Rat IgG2a (Chimeric) - BSA and Azide free (ab279306)

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

False colour image of Western blot: Anti-PD-L1 antibody [CAL10] – Rat IgG2a (Chimeric) 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, ab279294 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-Rat IgG H&L (IRDye® 800CW) preabsorbed (ab253031) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (ab216777) at 1/20000 dilution.

All lanes:
Western blot - Anti-PD-L1/CD274 antibody [CAL10] - Rat IgG2a (Chimeric) (ab279294) at 1/1000 dilution

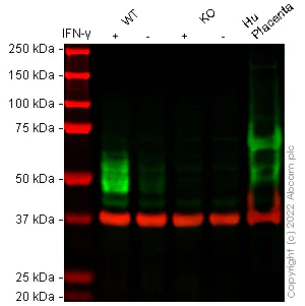
Lane 1:
Wild-type A549 Treated IFN-gamma (100 ng/ml) for 48 hours cell lysate at 20 µg

Lane 2:
Western blot - Human CD274 (PD-L1) knockout A549 cell lysate (ab256831) at 20 µg

Performed under reducing conditions.

Predicted band size: 33 kDa

Observed band size: 48 kDa



Western blot - Anti-PD-L1/CD274 antibody [CAL10] - Rat IgG2a (Chimeric) - BSA and Azide free (ab279306)

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

False colour image of Western blot: Anti-PD-L1 antibody [CAL10] – Mouse IgG2a (Chimeric) staining at 1/1000 dilution, shown in green; Rabbit Anti-GAPDH antibody [EPR16891] (ab181602) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab279293 was shown to bind specifically to PD-L1. A band was observed at 45-65 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 fluorescent western blot (TBS-based) blocking solution 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 IgG2a (Chimeric) (ab279293) 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

Lane 5:
Human Placenta cell lysate at 20 µg

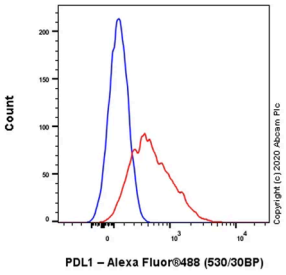
Secondary

Lanes 1 - 5:
Goat anti-Mouse IgG H&L 800CW at 1/20000 dilution

Lanes 1 - 5:
Goat anti-Rabbit IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Observed band size: 45-65 kDa

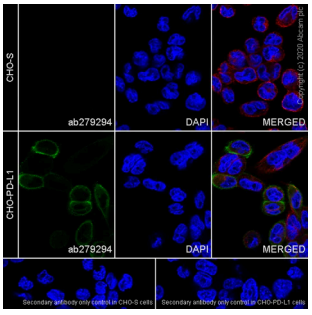


Flow Cytometry (Intracellular) - Anti-PD-L1/CD274 antibody [CAL10] - Rat IgG2a (Chimeric) - BSA and Azide free (ab279306)

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

This data was produced using ab279294, the same clone in a different formulation.
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 ab279294 at 1/50 dilution (0.1µg).

Goat F(ab)2 Anti-Rat IgG Fc (Alexa Fluor® 488, ab150161) at 1/2000 dilution was used as the secondary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-PD-L1/CD274 antibody [CAL10] - Rat IgG2a (Chimeric) - BSA and Azide free (ab279306)

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

This data was produced using ab279294, the same clone in a different formulation.
Immunocytochemical analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100-fixed permeabilized CHO-PD-L1 cells labeling PD-L1 with ab279294 at 1/100 dilution, followed by ab150157 Goat Anti-Rat IgG H&L (Alexa Fluor® 488) 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: ab279294 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.

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