abcam

ab282458

Anti-PD-L1/CD274 antibody [RM1012]

Recombinant | RabMAb | KO Validated | 20ul selling size

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	Multiclonal
Immunogens	The exact immunogen used to generate this antibody is proprietary information.
Clone number	RM1012
Purification technique	Affinity purification Protein A
Concentration	0.439 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ICC/IF

Tested

Species	Human
Dilution info	1/1000
Notes	-

Not recommended

Species	Mouse, Rat, Recombinant fragment
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	1/30
Notes	-

Not recommended

Species	Mouse, Rat, Recombinant fragment
Dilution info	-
Notes	-

Flow Cyt

Tested

Species	Human
Dilution info	1/50
Notes	-

Not recommended

Species	Mouse, Rat, Recombinant fragment
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	1/10000
Notes	-

Not recommended

Species	Mouse, Rat, Recombinant fragment
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	1/500
Notes	-

Not recommended

Species	Mouse, Rat, Recombinant fragment
Dilution info	-
Notes	-

I-ELISA

Tested

Species	Recombinant fragment
Dilution info	-
Notes	-

Expected

Species	Human
Dilution info	Use at an assay dependent concentration.

Notes	-

Not recommended

Species	Mouse, Rat
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 are recombinant multiclonals?
Recombinant multiclonals are a mixture of recombinant antibodies co-expressed from a library of heavy and light chains. They offer several advantages including:

- The sensitivity of polyclonal antibodies by recognising multiple epitopes
- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

View our range of recombinant multiclonal antibodies.

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.

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

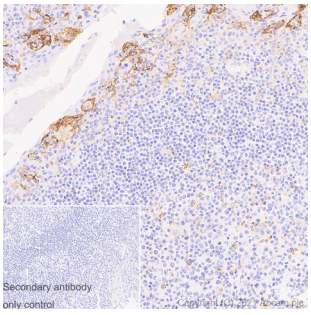
We do not recommend this combination. It is not covered by our product promise.

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.

10 product images

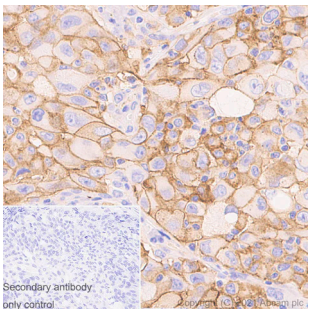


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [RM1012] (ab282458)

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 labelling PD-L1 with ab282458 at 1/500 (0.876 ug/ml) dilution followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Positive staining on human tonsil. The section was incubated with ab282458 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.
Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins

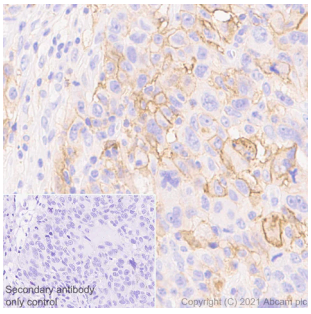


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [RM1012] (ab282458)

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 cancer tissue labelling PD-L1 with ab282458 at 1/500 (0.876 ug/ml) dilution followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Positive staining on human lung cancer. The section was incubated with ab282458 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.
Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins

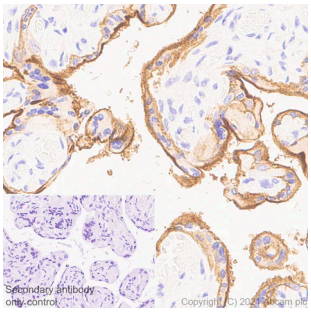


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [RM1012] (ab282458)

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

Immunohistochemical analysis of paraffin-embedded Human breast cancer tissue labelling PD-L1 with ab282458 at 1/500 (0.876 ug/ml) dilution followed by a ready to use LeicaDS9800 (Bond☒ Polymer Refine Detection) . Positive staining on human breast cancer. The section was incubated with ab282458 for 30 mins at room temperature.The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.
Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond☒ Polymer Refine Detection) .

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins

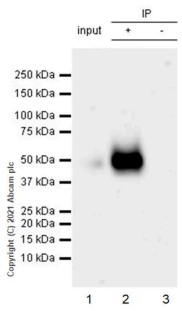


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [RM1012] (ab282458)

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 labelling PD-L1 with ab282458 at 1/500 (0.876 ug/ml) dilution followed by a ready to use LeicaDS9800 (Bond☒ Polymer Refine Detection) . Positive staining on human placenta. The section was incubated with ab282458 for 30 mins at room temperature.The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.
Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond☒ Polymer Refine Detection) .

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Immunoprecipitation - Anti-PD-L1/CD274 antibody [RM1012] (ab282458)

PD-L1 was immunoprecipitated from 0.35 mg NCI-H1975 (Human adenocarcinoma lung epithelial cell) whole cell lysate with ab282458 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab282458 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(ab131366) was used at 1/5000 dilution.
Lane 1: NCI-H1975 (Human adenocarcinoma lung epithelial cell) whole cell lysate 10 ug

Lane 2: ab282458 IP in NCI-H1975 whole cell lysate

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab282458 in NCI-H1975 whole cell lysate

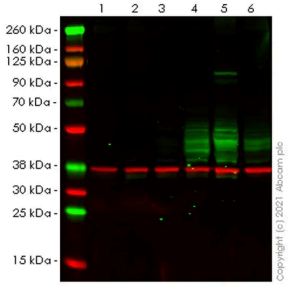
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 32 seconds

All lanes:
Immunoprecipitation - Anti-PD-L1/CD274 antibody [RM1012] (ab282458)

Predicted band size: 33 kDa

Observed band size: 40-60 kDa



Western blot - Anti-PD-L1/CD274 antibody [RM1012] (ab282458)

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

Lysates/proteins at 20 µg per lane.
Performed under reducing conditions.

False colour image of Western blot: Anti-PD-L1 antibody [RM1012] (ab282458) staining at 1/10, 000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red.

In Western blot, ab282458 was shown to bind specifically to PD-L1. Target band was observed at 40-60 kDa in wild-type A549 treated with IFN gamma cell lysates with no signal observed at this size in PD-L1 knockout cell line ab267054 (knockout cell lysate ab256831). To generate this image, wild-type and CD274 (PD-L1) knockout A549 cell lysates were analyzed. 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 (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) at 1/20000 dilution.

All lanes:
Western blot - Anti-PD-L1/CD274 antibody [RM1012] (ab282458) at 1/10000 dilution

Lane 1:
Untreated CD274 (PD-L1) KO A549 (Human lung carcinoma epithelial cell) cell lysate at 20 µg

Lane 2:
CD274 (PD-L1) KO A549 treated with 100 ng/ml human IFN gamma for 48 hours cell lysate at 20 µg

Lane 3:
Untreated wild-type A549 cell lysate at 20 µg

Lane 4:
Wild-type A549 treated with 100ng/ml human IFN gamma for 48 hours cell lysate at 20 µg

Lane 5:
MDA-MB-231 (Human breast adenocarcinoma epithelial cell) cell lysate at 20 µg

Lane 6:
NCI-H1975 (Human adenocarcinoma lung epithelial cell) cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/100000 dilution

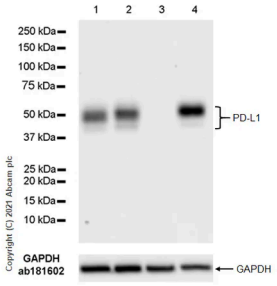
Performed under reducing conditions.

Predicted band size: 33 kDa

Observed band size: 40-60 kDa

Exposure time: 3min

Western blot - Anti-PD-L1/CD274 antibody [RM1012] (ab282458)



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

Blocking and Diluting buffer and concentration: 5% NFDM/TBST.
Low expression control: MCF7 (PMID: 28184013, 31741201).

All lanes:
Western blot - Anti-PD-L1/CD274 antibody [RM1012] (ab282458) at 1/1000 dilution

Lane 1:
NCI-H1975 (Human adenocarcinoma lung epithelial cell) whole cell lysate at 20 µg

Lane 2:
MDA-MB-231 (Human breast adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 3:
MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate at 20 µg

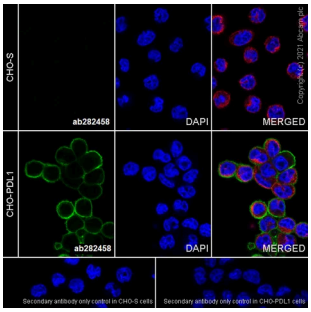
Lane 4:
Human placenta lysate at 20 µg

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

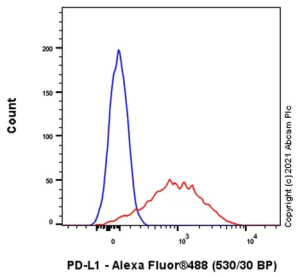


Immunocytochemistry/ Immunofluorescence - Anti-PD-L1/CD274 antibody [RM1012] (ab282458)

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-PDL1 cells labelling PD-L1 with ab282458 at 1/1000 (0.438 ug/ml) dilution, followed by ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green). Confocal image showing membranous staining in CHO-PDL1 cells and no staining in CHO-S cells. ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 2.5ug/ml dilution (Red). The Nuclear counterstain was DAPI (Blue).

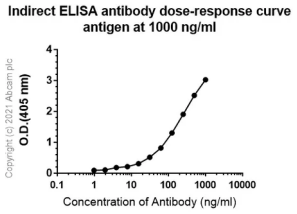
Secondary antibody only control: Secondary antibody is ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 dilution.



Flow Cytometry - Anti-PD-L1/CD274 antibody [RM1012] (ab282458)

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

Flow cytometric analysis of CHO-S (Chinese hamster ovary epithelial cell (Blue)) / CHO-PDL1 (PD-L1 stably expessed Chinese hamster ovary epithelial cell) (Red)) cells labelling PD-L1 with ab282458 at 1/50 dilution (1ug). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as the secondary antibody. Gated on viable cells.



Indirect ELISA - Anti-PD-L1/CD274 antibody [RM1012] (ab282458)

ELISA using ab282458 at varying antibody concentrations and antigen concentration at 1000 ng/ml. An Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) (1/2500) was used as the secondary antibody.

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