abcam

ab205921

Anti-PD-L1/CD274 antibody [28-8]

Anti-PD-L1/CD274 antibody [28-8] (ab205921) is a rabbit monoclonal antibody detecting PD-L1/CD274 in **Western Blot, Flow Cytometry, IHC-P, IHC-Fr, ICC/IF, mIHC**. Suitable for **Human**.

- KO validated for confirmed specificity
- Multiplex IHC validated on the Leica BOND® MAX using Opal reagents
- Biophysical QC for unrivalled batch-batch consistency
- Over 470 publications

Recombinant | RabMAb | Advanced Validation | 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	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	28-8
Purification technique	Affinity purification Protein A
Concentration	0.994 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

mIHC

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Transfected cell line, Mouse
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	2 µg/mL
Notes	Please refer to protocol booklet. For antigen buffer for FFPE tissue, it is recommended to use Universal HIER antigen retrieval reagent (ab208572). Perform heat-mediated antigen retrieval before commencing with IHC staining protocol.

Species	Transfected cell line
Dilution info	2 µg/mL
Notes	Please refer to protocol booklet. For antigen buffer for FFPE tissue, it is recommended to use Universal HIER antigen retrieval reagent (ab208572). Perform heat-mediated antigen retrieval before commencing with IHC staining protocol.

Not recommended

Species	Mouse
Dilution info	-
Notes	Please refer to protocol booklet. For antigen buffer for FFPE tissue, it is recommended to use Universal HIER antigen retrieval reagent (ab208572). Perform heat-mediated antigen retrieval before commencing with IHC staining protocol.

ICC/IF

Tested

Species	Transfected cell line
Dilution info	2 µg/mL
Notes	-

Species	Human
Dilution info	2 µg/mL
Notes	-

Not recommended

Species	Mouse
Dilution info	-
Notes	-

Flow Cyt

Tested

Species	Human
Dilution info	-
Notes	Please refer to protocol in Support&downloads section ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Not recommended

Species	Transfected cell line, Mouse
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	-
Notes	Please refer to protocol in Support&downloads section ab228415 works better than ab205921 in western blot testing.

Not recommended

Species	Transfected cell line, Mouse
Dilution info	-
Notes	-

IHC-Fr

Tested

Species	Human
Dilution info	2 µg/mL
Notes	-

Not recommended

Species	Transfected cell line, Mouse
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 [28-8] (ab205921) was developed by Abcam using patented rabbit monoclonal antibody technology and is validated for use in Flow Cyt, ICC/IF, IHC-Fr, IHC-P, WB, mIHC in human samples.

Anti-PD-L1 antibody [28-8] (ab205921) has been cited over 476 times in peer reviewed journals and is trusted by the scientific community.

Abcam's high quality manufacturing and validation processes ensure Anti-PD-L1 antibody [28-8] (ab205921) has high sensitivity and specificity alongside high lot-to-lot consistency and reproducibility.

The specificity of Anti-PD-L1 antibody [28-8] (ab205921) has been confirmed by testing in knockout samples.

Anti-PD-L1 antibody [28-8] (ab205921) has 13 independent reviews from customers.

Anti-PD-L1 antibody [28-8] (ab205921) specifically detects PD-L1 (UniProt ID: Q9NZQ7; Molecular weight: 31kDa) and is sold in a convenient trial size to enable initial testing (20 µL) and larger sizes for subsequent scaling up experiments (50 µL).

Conjugation-ready, carrier free format available for antibody clone 28-8 - ab228413.

Antibody clone 28-8 is also available pre-conjugated to a variety of labels for your convenience - HRP, PE, Alexa Fluor® 555, Alexa Fluor® 568, Alexa Fluor® 594, FITC (ab209961, ab209962, ab213358, ab213359, ab213360, ab224027).

PD-L1 (Programmed Death-Ligand 1) is a protein that plays a significant role in oncology, particularly in the immune response to cancer. It is expressed on the surface of cancer cells and interacts with the PD-1 receptor on T-cells, effectively inhibiting the immune system's ability to attack the tumor. This interaction is a key target for immunotherapy drugs, such as checkpoint inhibitors, which block the PD-1/PD-L1 pathway, allowing the immune system to recognize and destroy cancer cells.

FURTHER INFORMATION ON POSITIVE CONTROLS (Chinese version)

Tissue:
- Tonsil - with hyperreactive changes. Screening of hyper-reactive tonsils is recommended to find tonsil with the highest expression of PD-L1 in crypt epithelium, macrophages homing the germinal centers and interfollicular mononuclear leukocytes.
- Tumor tissues - prescreened for positive tumor and inflammatory infiltrates. PD-L1 expression varies by tumor type so screening is recommended to find positive and negative tumor controls.
- The following publication is useful for finding suitable tumor types for PD-L1 expression: http://www.immunotherapyofcancer.org/content/1/S1/P53
- Note: Look for specimens with high numbers of inflammatory macrophages and mononuclear leukocytes.

Cell Lines:
- Positive controls: B-CPAP (high), ES-2 (medium), HCC70 (low).

Primary antibody negative control: Recombinant Rabbit IgG isotype control antibody, ab172730.

Recombinant protein positive control: Recombinant human PD-L1 protein, ab167713.

Immunohistochemistry usage:
For IHC usage on FFPE tissues, we recommend using PD-L1 IHC panel ab236676, which contains PD-L1 antibody clone 28-8 (ab205921), HIER antigen retrieval reagent (ab208572) and IHC detection kit HRP/DAB (ab209101).

Western blot usage:
For clone 28-8, it is recommended to use Odyssey system. This system has the advantages of a wider dynamic range and less background than chemiluminescence.

This PD-L1 antibody [28-8] has been used as detector antibody in Human PD-L1 SimpleStep ELISA® kit: ab214565 and ab277712.

To support you in your biomarker discovery and validation studies, we offer a comprehensive suite of solutions including high quality, validated recombinant antibodies and assay kits.

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 plays an important role in the immune system. It is a protein expressed on antigen-presenting cells and some tumor cells helping them interact with T-cells. The molecular weight of PD-L1 is approximately 33 kDa. PD-L1 expression can occur in various tissues and is often upregulated in tumor microenvironments. This increased expression helps tumors evade the immune response.
Biological function summary	PD-L1 modulates the immune response by interacting with its receptor PD-1 on T-cells and other immune cells leading to immune suppression. It is part of the PD-1/PD-L1 complex an important regulator of T-cell activity and immune tolerance. The binding of PD-L1 to PD-1 inhibits T-cell proliferation and cytokine production reducing the ability of the immune system to attack cancer cells.
Pathways	PD-L1 is involved in the immune checkpoint pathway and the cancer immunity cycle. Its interaction with PD-1 influences important signaling cascades within these pathways affecting the immune system's capacity to target tumor cells effectively. Another protein important in this context is CTLA-4 another immune checkpoint regulator which along with PD-1 modulates immune system activity against cancer.
Associated diseases and disorders	PD-L1 has strong connections to cancer and autoimmune diseases. Many cancers including melanoma and non-small cell lung cancer exploit PD-L1 to protect themselves from the immune system. Increased PD-L1 expression can also impact autoimmune disorders where the immune system reacts against healthy body tissues. This interaction further involves PD-1 and CTLA-4 illustrating their shared roles in these disease contexts.

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

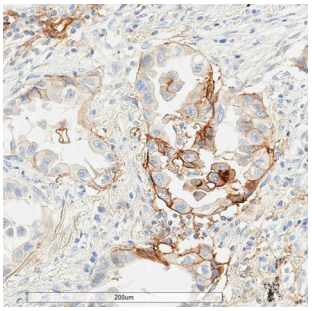
We do not recommend this combination. It is not covered by our product promise.

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.

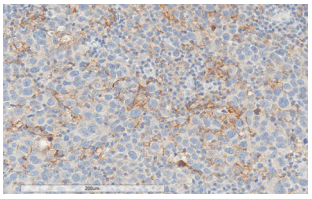
26 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [28-8] (ab205921)

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

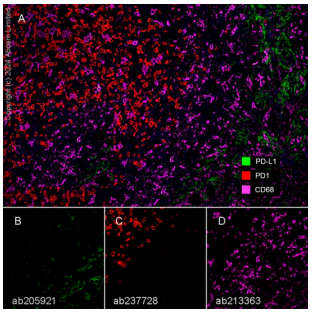
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human non-small cell lung cancer tissue labelling PD-L1 with ab205921. Tumor cells and immuno cells localized within the stroma show PD-LA plasma membrane staining.
For antigen retrival buffer, Universal HIER antigen retrieval reagent (ab208572) was used.
For IHC detection kit, Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) is recommended.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [28-8] (ab205921)

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

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human melanoma tissue labelling PD-L1 with ab205921. Tumor cells show weak and partial postive PD-L1 expresseion in the plasma membrane. PD-L1 positive tumor associated immunoe cells are also stained.
For antigen retrival buffer Universal HIER antigen retrieval reagent (ab208572) was used.
For IHC detection kit Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) is recommended.



Multiplex immunohistochemistry - Anti-PD-L1/CD274 antibody [28-8] (ab205921)

PD-L1/CD274 Multiplex immunohistochemistry staining of Human tonsil tissue using rabbit Anti-PD-L1/CD274 antibody

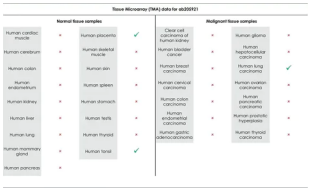
Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded Human tonsil tissue staining PD-L1 with ab205921 at a 1:500 (2.19 ug/ml) dilution; PD1 with ab237728 at 1:2000 (0.525 ug/ml) dilution and CD68 with ab213363 at 1:500 (1.26 ug/ml) dilution followed by a ready to use secondary antibody Opal Polymer HRP Ms + Rb.

Panel A: merged staining of anti-CD68 (magenta; Opal™690), anti-PD-L1 (green; Opal™520) and anti-PD1 (red; Opal™570) on human tonsil.
Panel B: anti-PD-L1 stained on cells involved in T cell inhibition.
Panel C: anti-PD1 stained on antigen-stimulated T cells.
Panel D: anti-CD68 stained on macrophages.

The section was incubated in three rounds of staining: in the order of ab213363 and ab205921 for 30 mins, then ab237728 for 10 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.

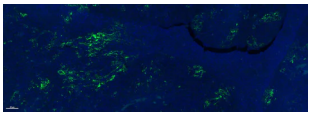
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [28-8] (ab205921)

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

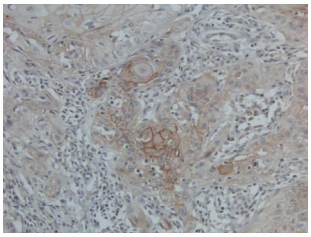
Tissue Microarrays stained for Anti-PD-L1 antibody [28-8] using ab205921 in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negative (cross mark) staining per sample type tested. The sections were pre-treated using Heat mediated antigen retrieval using ab208572 (Universal HIER antigen retrieval reagent). The sections were incubated with ab205921 at +4°C overnight. For IHC detection kit, Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) is recommended.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [28-8] (ab205921)

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

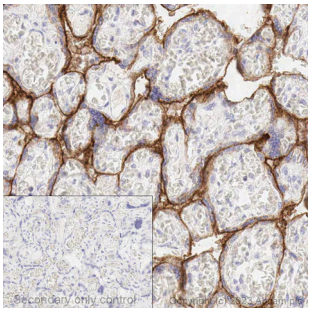
Anti-PD-L1 antibody [28-8] (ab205921)
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human tonsil tissue labelling PD-L1 with ab205921 at a dilution of 1:400. Heat mediated antigen retrieval was performed using AR9 antigen retrieval solution, and microwave treatment for 15 min at 20% power. Anti-Rabbit/Mouse HRP polymer (PerkinElmer Opal Polymer HRP Ms Plus Rb) 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 Phenochart 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 [28-8] (ab205921)

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

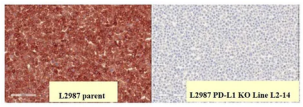
Immunohistochemical analysis of formalin-fixed, paraffin-embedded Human head and neck squamous cell carcinoma tissue labeling PD-L1 with ab205921 at 2 µg/ml. Counterstained with Hematoxylin.
For antigen retrival buffer, Universal HIER antigen retrieval reagent (ab208572) was used.
For IHC detection kit, Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) is recommended.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [28-8] (ab205921)

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 ab205921 at a dilution of 2µg/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). ab205921 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.



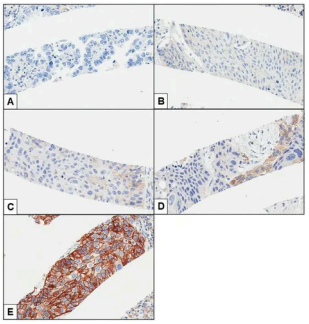
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [28-8] (ab205921)

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

ab205921 specificity testing by Immunohistochemistry (KO testing): Loss of detection on KO Cells. Strong IHC detection with anti-PD-L1 (ab205921, clone 28-8) is seen in human lung adenocarcinoma tumor cell line L2987. PDL1 gene was edited in L2987 cells using TALEN constructs targeting exon4 of human PD-L1, transcript variant 1 (NM_014143.3) and complete knock out (K.O) confirmed by deep sequencing in clone L2-14. IHC detection is completely eliminated in the L2987 L2-14 K.O. cell line.

For recommended Immunohistochemistry (IHC) protocol, please refer to the protocol book in the product protocol section.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Nakamura et al PLoS One. 2017; 12(10): e0186192. Published online 2017 Oct 19. doi: 10.1371/journal.pone.0186192

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [28-8] (ab205921)

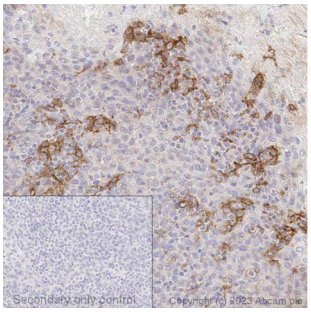
PD-L1/CD274 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of human lung cancer tissue using rabbit Anti-PD-L1/CD274 antibody

Formalin-fixed paraffin-embedded human lung cancer tissue stained for PD-L1 using ab205921. Representative images of PD-L1 expression.
(A) <1.0% (B) 1.0–4.9% (C) 5.0–9.9% (D) 10.0–49.9% and (E) ≥50.0% PD-L1-positive cells (magnification 200×).

From image 3 of Nakamura et al.

Nakamura et al PLoS One. 2017; 12(10): e0186192. Published online 2017 Oct 19. doi: 10.1371/journal.pone.0186192' PMID: 29049375

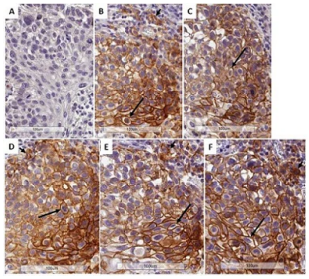
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Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [28-8] (ab205921)

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 ab205921 at a dilution of 2µg/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). ab205921 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 [28-8] (ab205921)

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

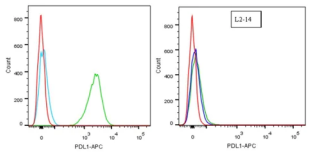
Immunohistochemical analysis of Human Lung NSCLC with ab205921 at 2 µg/ml.
High power view
A) Rabbit IgG 5 µg/mL. No staining
B) Anti PD-L1 2 µg/mL (ab205921 batches 1)
C) Anti PD-L1 2 µg/mL (ab205921 batches 3)
D) Anti PD-L1 2 µg/mL (ab205921 batches 4)
E) Anti PD-L1 2 µg/mL (ab205921 batches 5)
F) Anti PD-L1 2 µg/mL (ab205921 batches 6)

All batches/lots (13456) showed consistent results.

Note linear and complete or partial (arrows) PD-L1 staining of tumor cells. Tumor associated immune cells localized over the tumor margin exhibit positive plasma membrane staining (small arrows).

For recommended Immunohistochemistry (IHC) protocol please refer to the protocol book in the Product Protocol section.

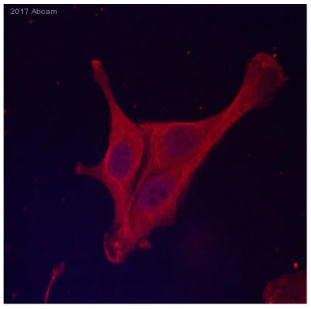
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Flow Cytometry - Anti-PD-L1/CD274 antibody [28-8] (ab205921)

PD-L1/CD274 Flow Cytometry staining of PDL-1 KO cells using rabbit Anti-PD-L1/CD274 antibody

ab205921 specificity testing by Flow Cytometry (KO testing): Loss of detection on KO cells.Strong detection with anti-PD-L1 (ab205921 clone 28-8) TALEN constructs targeting exon4 of human PD-L1 transcript variant 1 (NM_014143.3) and complete knock out (K.O) confirmed by deep sequencing in clone L2-14. Cell surface staining is almost completely eliminated in the L2987 L2-14 KO cell line. For recommended Flow Cytometry (Flow Cyt) protocol please refer to the protocol book in the protocol section. Alexa Fluor® 488 (ab209959) and Alexa Fluor® 647 (ab209960) conjugated versions are available for this clone.

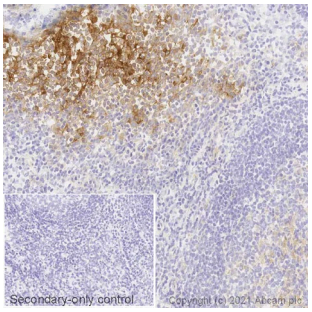


This image is courtesy of a customer review submitted by Dr. Dimitra Kalamida.

Immunocytochemistry/ Immunofluorescence - Anti-PD-L1/CD274 antibody [28-8] (ab205921)

PD-L1/CD274 Immunocytochemistry/ Immunofluorescence staining of U-87 MG (human glioblastoma-astrocytoma epithelial cell line) cells using rabbit Anti-PD-L1/CD274 antibody

Paraformaldehyde-fixed Triton X-100 permeabilized U-87 MG (human glioblastoma-astrocytoma epithelial cell line) cells stained for PD-L1 (red) using ab205921 at 1/200 dilution in ICC/IF followed by CF568 Donkey anti-rabbit IgG(H+L) secondary antibody at 1/500 dilution.
Alexa Fluor® 488 (ab209959) and Alexa Fluor® 647 (ab209960) conjugated versions are available for this clone.



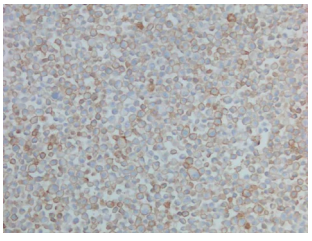
Immunohistochemistry (Frozen sections) - Anti-PD-L1/CD274 antibody [28-8] (ab205921)

PD-L1/CD274 Immunohistochemistry (Frozen sections) staining of human tonsil tissue 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 ab205921 1ugml 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.

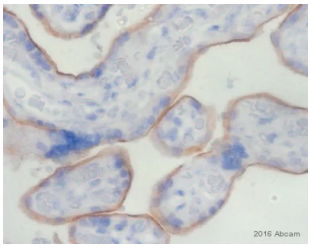
*Tissue obtained from the Human Research Tissue Bank supported by the NIHR Cambridge Biomedical Research Centre



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [28-8] (ab205921)

PD-L1/CD274 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of L2987 cells using rabbit Anti-PD-L1/CD274 antibody

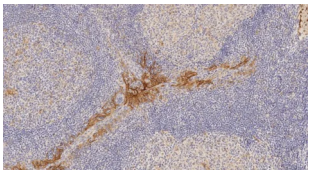
Immunohistochemical analysis of formalin-fixed paraffin-embedded L2987 (Human lung adenocarcinoma cell line with endogenous PD-L1 expression) cells labeling PD-L1 with ab205921 at 2 µg/ml. Counterstained with Hematoxylin.
For antigen retrival buffer Universal HIER antigen retrieval reagent (ab208572) was used.
For IHC detection kit Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) is recommended.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [28-8] (ab205921)

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

Paraformaldehyde-fixed paraffin-embedded human placenta tissue stained for PD-L1 using ab205921 at 1/100 dilution in immunohistochemical analysis.

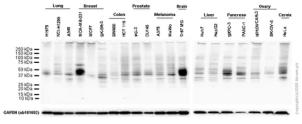


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [28-8] (ab205921)

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

IHC image of ab205921 staining PD-L1 in human tonsil formalin fixed paraffin embedded tissue sections* performed on a Leica BOND RX (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 ab205921 5µg/ml working concentration for 60 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



Western blot - Anti-PD-L1/CD274 antibody [28-8] (ab205921)

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 [28-8] (ab205921) at 1/100 dilution

Lane 1:
H1975 (Human non-small cell lung cancer epithelial cell) whole cell lysate at 20 µg

Lane 2:
NCI-H1299 (Human lung carcinoma epithelial cell) whole cell lysate at 20 µg

Lane 3:
A549 (Human lung carcinoma epithelial cell) whole cell lysate at 20 µg

Lane 4:
MDA-MB-231 (Human breast adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 5:
MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 6:
SK-BR-3 (Human breast adenocarcinoma 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

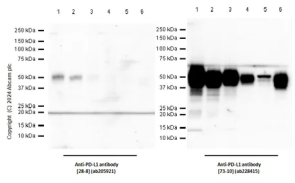
Secondary

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: 3min



Western blot - Anti-PD-L1/CD274 antibody [28-8] (ab205921)

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

ab228415 works better than ab205921 in western blot testing. Blocking/Diluting buffer and concentration: 5% NFDM/TBST
All lanes:
Western blot - Anti-PD-L1/CD274 antibody [28-8] (ab205921) at 1/1000 dilution

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

Lane 2:
U-87 MG (human glioblastoma astrocytoma epithelial cell) whole cell lysate at 20 µg

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

Lane 4:
A375 (human malignant melanoma epithelial cell) whole cell lysate at 20 µg

Lane 5:
HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate at 20 µg

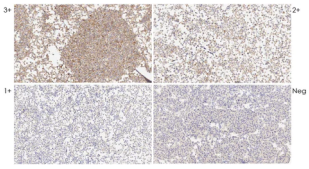
Lane 6:
A549 (human lung carcinoma epithelial cell) whole cell lysate at 20 µg

Secondary

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

Observed band size: 40-60 kDa

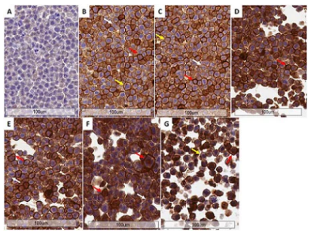
Exposure time: 100s



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [28-8] (ab205921)

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

IHC image of ab205921 staining PD-L1 in PD-L1 Dynamic Range Analyte Control formalin fixed paraffin embedded cell lines (HistoCyte Laboratories), performed on a Leica BOND RX (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 ab205921, 5µg/ml working concentration, for 60 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 [28-8] (ab205921)

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

Immunohistochemical analysis of CHO PD-L1 cells with ab205921 at 2 µg/ml.
High power view
A) Rabbit IgG, 5 µg/mL. No staining
B) Anti PD-L1, 2 µg/mL (ab205921 batches 1)
C) Anti PD-L1, 2 µg/mL (ab205921 batches 3)
D) Anti PD-L1, 2 µg/mL (ab205921 batches 4)

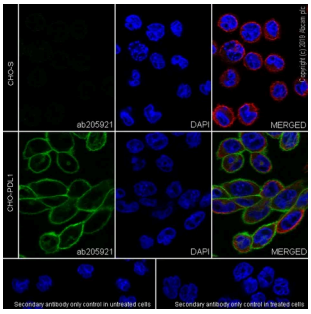
E) Anti PD-L1, 2 µg/mL (ab205921 batches 5)
F) Anti PD-L1, 2 µg/mL (ab205921 batches 6)
G) Anti PD-L1, 2 µg/mL (ab205921 batches 7)

All batches/lots (1,3,4,5,6,7) showed consistent results.

Note strong, moderate, and weak (red, yellow, and white arrows respectively) plasma membrane staining of CHO PD-L1 transfected cells

For recommended Immunohistochemistry (IHC) protocol, please refer to the protocol book in the Product Protocol section.

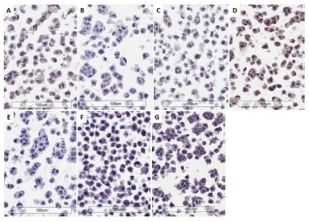
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-PD-L1/CD274 antibody [28-8] (ab205921)

PD-L1/CD274 Immunocytochemistry/ Immunofluorescence staining of CHO-PDL1 (PD-L1 stably expessed Chinese hamster ovary epithelial cell) cells using rabbit Anti-PD-L1/CD274 antibody

Immunocytochemistry analysis of CHO-PDL1 (PD-L1 stably expessed Chinese hamster ovary epithelial cell) labeling PD-L1 with purified ab205921 at 1/400 dilution. Cells were fixed with 4% Paraformaldehyde and permeabilised with 0.1% tritonX-100. Goat anti rabbit IgG (Alexa Fluor® 488 ab150077) at 1/1000 (2 µg/ml) was used as the secondary antibody. ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.32 µg/ml) was used as counterstain. Nuclei were stained blue with DAPI.
Negative controls: Cells not transfected with PD-L1 and both the transfected and mock tranfected cells without the primary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [28-8] (ab205921)

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

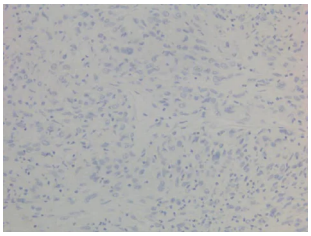
Immunohistochemical analysis of CHO Parental cells with ab205921 at 2 µg/ml.
High power view
A) Rabbit IgG 5 µg/mL. No staining
B) Anti PD-L1 2 µg/mL (ab205921 batches 1)
C) Anti PD-L1 2 µg/mL (ab205921 batches 3)
D) Anti PD-L1 2 µg/mL (ab205921 batches 4)
E) Anti PD-L1 2 µg/mL (ab205921 batches 5)
F) Anti PD-L1 2 µg/mL (ab205921 batches 6)
G) Anti PD-L1 2 µg/mL (ab205921 batches 7)

All batches/lots (134567) showed consistent results.

Note absence of PD-L1 expression in CHO parental cells.

For recommended Immunohistochemistry (IHC) protocol please refer to the protocol book in the Product Protocol section.

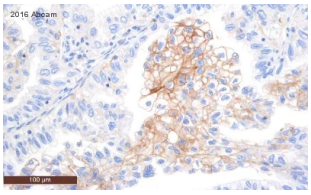
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [28-8] (ab205921)

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

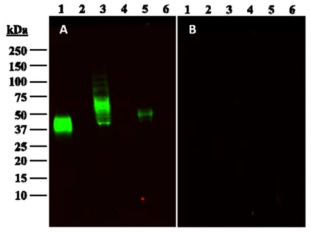
Immunohistochemical analysis of formalin-fixed, paraffin-embedded PD-L1 negative Non-small cell lung carcinoma (NSCLC) tissue with ab205921 at 2 µg/ml. Counterstained with Hematoxylin. For antigen retrival buffer, Universal HIER antigen retrieval reagent (ab208572) was used.For IHC detection kit, Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) is recommended.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1/CD274 antibody [28-8] (ab205921)

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 human non-squamous non-small cell lung cancer (NSQ-NSCLC) using ab205921 at a dilution of 1/400, incubated for an hour at room temperature. Heat mediated antigen retrieval was carried out in low pH buffer and the sample was blocked with peroxidase blocking buffer for 3 minutes.
This image was courteously provided by Dr. Kai Schmitt from the Institute of Pathology, Saarbrücken-Rastpfuhl.



Western blot - Anti-PD-L1/CD274 antibody [28-8] (ab205921)

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

(A and B) Western blots of recombinant PD-L1 protein (Lane 1), cell lysates of CHO-PD-L1 (Lane 3), CHO (Lane 4), ES-2 (Lane 5) and Colo205 (Lane 6) cell lines. In B, anti-PD-L1 (ab205921, clone 28-8) was pre-incubated with purified recombinant PDL1 protein overnight at 4°C.
Blank/no sample (Lane2). Lane 2 is blank on purpose.

For recommended Western Blot (WB) protocol, please refer to the protocol book in the Support&downlods section

All lanes:
Western blot - Anti-PD-L1/CD274 antibody [28-8] (ab205921)

Predicted band size: 33 kDa

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