abcam

Product datasheet

Anti-PD-L1 antibody [73-10] - BSA and Azide free ab226766

Recombinant RabMAb

1 Abreviews 26 Images

Overview

Product name	Anti-PD-L1 antibody [73-10] - BSA and Azide free
Description	Rabbit monoclonal [73-10] to PD-L1 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** IHC-Fr, Flow Cyt (Intra), WB, ICC/IF, IHC-P, IP
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Chinese hamster ovary cell lysate stably expressing PD-L1; MDA-MB-231, H1975, NCI-H1299, A549, PC3, DU145, A375, U-87 MG, BXPC-3, NIH:OVCAR-3, SK-OV-3, and HeLa whole cell lysates; Human thymus and placenta tissue lysate. IHC-P: Human placenta, lung carcinoma and tonsil tissues. ICC/IF: CHO-PD-L1 cells. Flow Cyt (intra): CHO-PD-L1 cells. IP: NCI-H1975 whole cell lysate. IHC-Fr: Frozen human tonsil tissue sections
General notes	ab226766 is the carrier-free version of **ab228415**. **Clone 73-10 is also known as clone MKP1A07310.** **Clone 73-10 has been tested within Blueprint Phase 2 project.** **See here for more details.** **ab226766 (PD-L1 clone 73-10) is a catalogue antibody for Research Use Only. Not for use in diagnostic procedures.** Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. 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. 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.

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 production

For more information **see here**.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.20 Constituent: 100% PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	73-10
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab226766 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

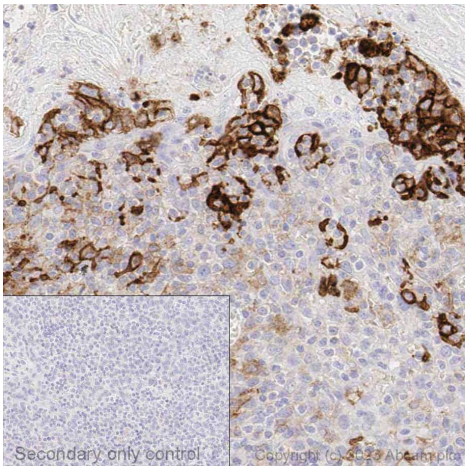
Application	Abreviews	Notes
IHC-Fr		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 33 kDa.
ICC/IF		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
IP		Use at an assay dependent concentration.

Target

Function Involved in the costimulatory signal, essential for T-cell proliferation and production of IL10 and

	IFNG, in an IL2-dependent and a PDCD1-independent manner. Interaction with PDCD1 inhibits T-cell proliferation and cytokine production.
Tissue specificity	Highly expressed in the heart, skeletal muscle, placenta and lung. Weakly expressed in the thymus, spleen, kidney and liver. Expressed on activated T- and B-cells, dendritic cells, keratinocytes and monocytes.
Sequence similarities	Belongs to the immunoglobulin superfamily. BTN/MOG family. Contains 1 Ig-like C2-type (immunoglobulin-like) domain. Contains 1 Ig-like V-type (immunoglobulin-like) domain.
Cellular localization	Cell membrane and Endomembrane system.

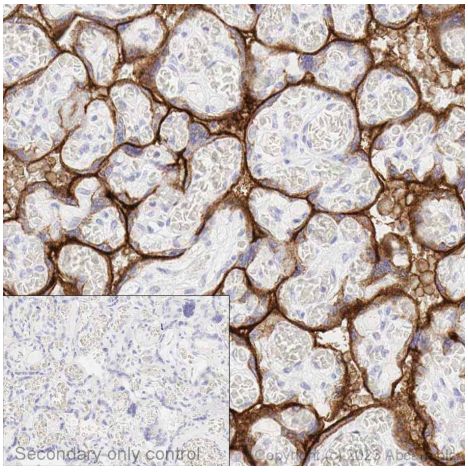
Images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [73-10] - BSA and Azide free (ab226766)

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

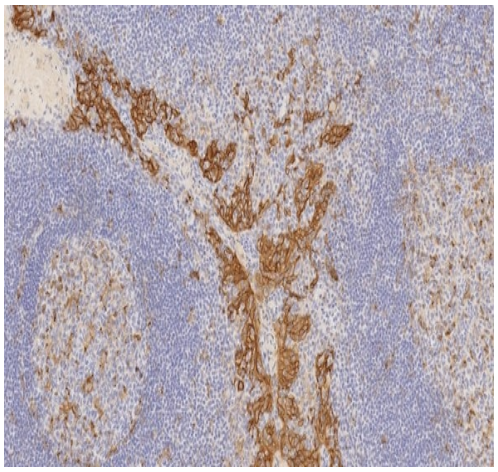
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab228415**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [73-10] - BSA and Azide free (ab226766)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab228415**).



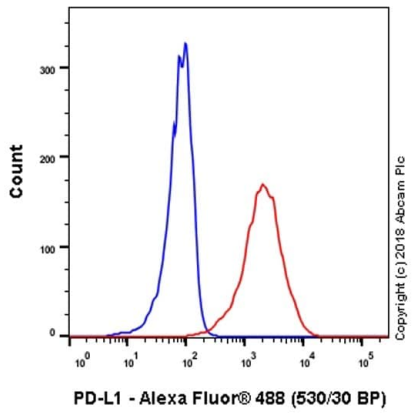
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [73-10] - BSA and Azide free (ab226766)

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

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

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

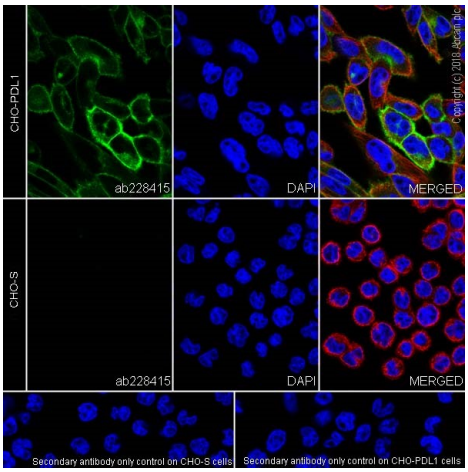
This image was generated using **ab228415**, the same antibody but with BSA and Azide



Flow Cytometry (Intracellular) - Anti-PD-L1 antibody [73-10] - BSA and Azide free (ab226766)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab228415**).



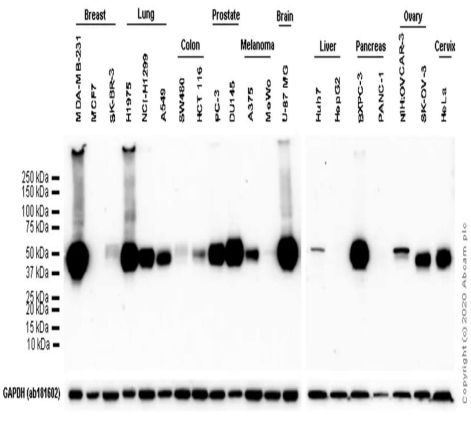
Immunocytochemistry/ Immunofluorescence - Anti-PD-L1 antibody [73-10] - BSA and Azide free (ab226766)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized CHO-PD-L1 (PD-L1 stably expressed Chinese hamster ovary epithelial cell) cells labeling PD-L1 with **ab228415** at 1/200 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1,000 dilution (green). Confocal image showing membranous staining on CHO-PD-L1 cells.

The nuclear counter stain is DAPI (blue). Tubulin is detected with anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) (red) at 1/200 dilution.

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab228415**).



Western blot - Anti-PD-L1 antibody [73-10] - BSA and Azide free (ab226766)

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

Lane 1 : MDA-MB-231 (Human breast adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate
Lane 3 : SK-BR-3 (Human breast adenocarcinoma epithelial cell) whole cell lysate
Lane 4 : H1975 (Human non-small cell lung cancer epithelial cell) whole cell lysate
Lane 5 : NCI-H1299 (Human lung carcinoma epithelial cell) whole cell lysate
Lane 6 : A549 (Human lung carcinoma epithelial cell) whole cell lysate
Lane 7 : SW480 (Human colorectal adenocarcinoma epithelial cell) whole cell lysate
Lane 8 : HCT 116 (Human colorectal carcinoma epithelial cell) whole cell lysate
Lane 9 : PC-3 (Human prostate adenocarcinoma epithelial cell) whole cell lysate
Lane 10 : DU 145 (Human prostate carcinoma epithelial cell) whole

cell lysate
Lane 11 : A375 (Human malignant melanoma epithelial cell) whole cell lysate
Lane 12 : MeWo (Human malignant melanoma fibroblast) whole cell lysate
Lane 13 : U-87 MG (Human glioblastoma-astrocytoma epithelial cell) whole cell lysate
Lane 14 : Huh7 (Human hepatocellular carcinoma epithelial cell) whole cell lysate
Lane 15 : HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysate
Lane 16 : BXPC-3 (Human pancreas adenocarcinoma epithelial cell) whole cell lysate
Lane 17 : PANC-1 (Human pancreatic epithelioid carcinoma epithelial cell) whole cell lysate
Lane 18 : NIH:OVCAR-3 (Human ovary adenocarcinoma epithelial cell) whole cell lysate
Lane 19 : SK-OV-3 (Human ovarian cancer epithelial cell) whole cell lysate
Lane 20 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate

Lysates/proteins at 20 µg per lane.

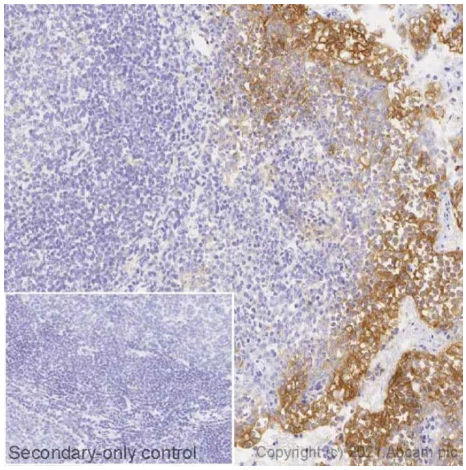
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 33 kDa
Observed band size: 40-60 kDa

Exposure time: 120 seconds

This data was produced using **ab228415**, the same antibody clone in a different buffer formulation.

Blocking/Diluting buffer and concentration: 5% NFDM/TBST.
Expression of PD-L1 varied widely among the tumor cell lines.



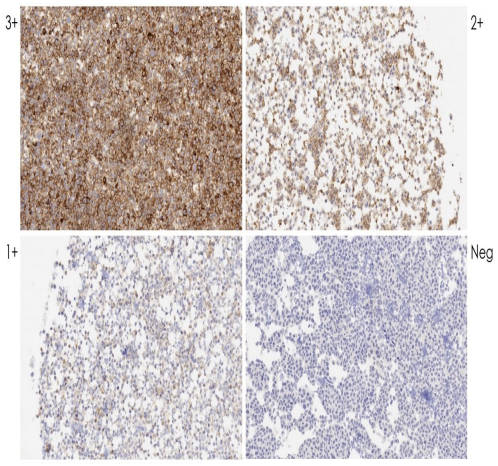
Immunohistochemistry (Frozen sections) - Anti-PD-L1 antibody [73-10] - BSA and Azide free (ab226766)

This data was produced using **ab228415**, the same antibody clone in a different buffer formulation.

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

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

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

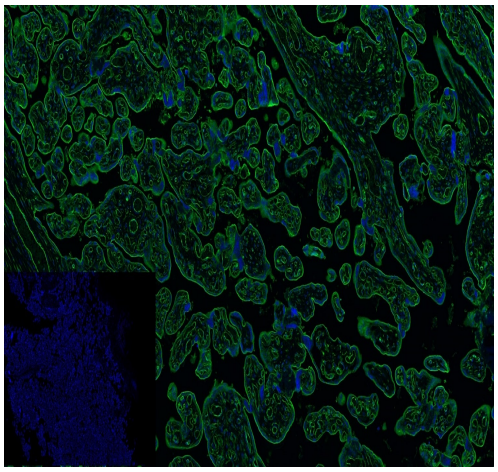


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [73-10] - BSA and Azide free (ab226766)

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

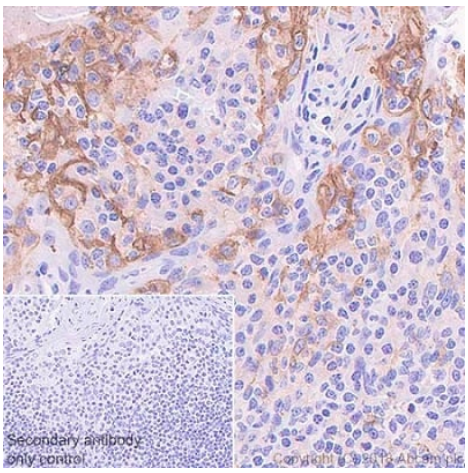
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

This image was generated using **ab228415**, the same antibody but with BSA and Azide



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [73-10] - BSA and Azide free (ab226766)

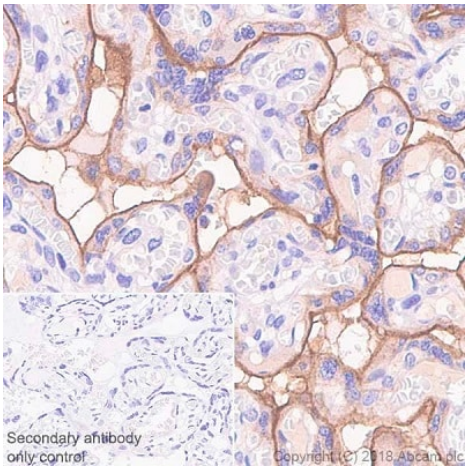
Anti-PD-L1 antibody [73-10] (**ab228415**)
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human tonsil tissue labelling PD-L1 with **ab228415** at a dilution of 1:2500. Heat mediated antigen retrieval was performed using AR9 antigen retrieval solution, and microwave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



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [73-10] - BSA and Azide free (ab226766)

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

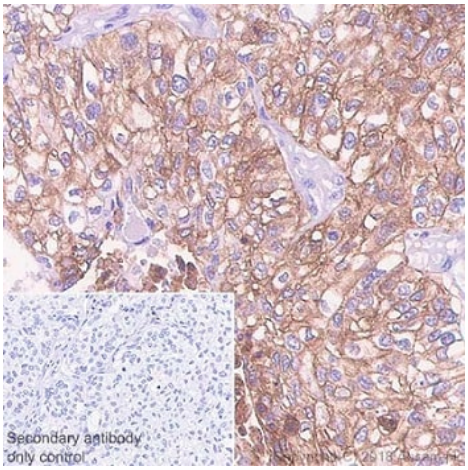
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab228415**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [73-10] - BSA and Azide free (ab226766)

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

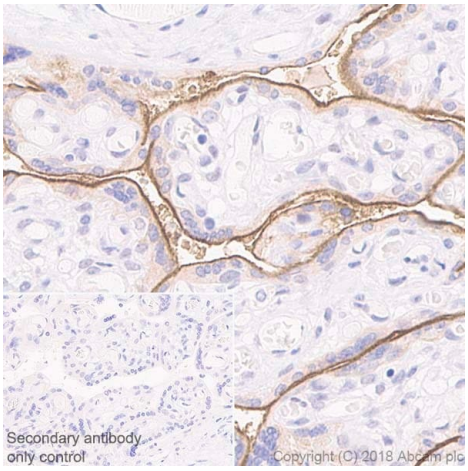
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab228415**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [73-10] - BSA and Azide free (ab226766)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab228415**).



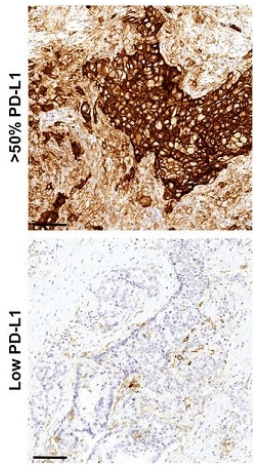
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [73-10] - BSA and Azide free (ab226766)

Immunohistochemical analysis of paraffin-embedded human placenta tissue labeling PD-L1 with **ab228415** at 1/5000 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) ready to use. Membranous and cytoplasmic staining in human placenta (PMID: 12538684) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab228415**).

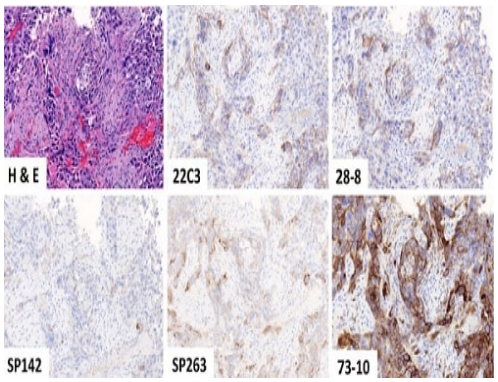


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [73-10] - BSA and Azide free (ab226766)

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

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) Staining PD-L1 in human non-small cell lung cancer tissue with >50% PD-L1-positive tumor cells were compared with tissue with lower PD-L1 expression using **ab228415** at 0.25µg/ml incubated for 30 minutes at room temperature. Antigen Retrieval was done with Target Retrieval Solution, high pH. Detection was done with EnVision FLEX/HRP. Hematoxylin EnVision FLEX was used as a counter stain.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab228415**).



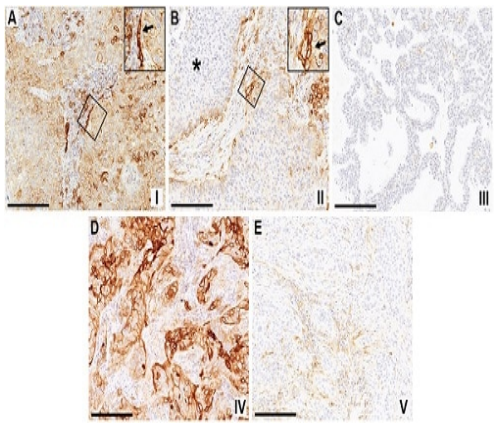
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [73-10] - BSA and Azide free (ab226766)

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

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) of lung cancer tissue samples. Comparing the staining PD-L1 with different monoclonal antibodies. **ab228415** (73-10) showed higher sensitivity to PD-L1 compared to the other clones. For further details on this image please see PubMed ID: 29800747.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab228415**).

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



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [73-10] - BSA and Azide free (ab226766)

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

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) Staining PD-L1 in human non-small cell lung cancer tissue using **ab228415** at 0.25µg/ml incubated for 30 minutes at room temperature. Antigen Retrieval was done with Target Retrieval Solution, high pH. Detection was done with EnVision FLEX/HRP. Hematoxylin EnVision FLEX was used as a counter stain.

A: Diffuse expression of PD-L1 (IHC) on tumor cell membranes of a squamous cell carcinoma, including central regions of trabeculae. Prominent labeling of cells in the TME compartment at the tumor-nest-TME interface suggesting presence of an immunological synapse (inset arrow).

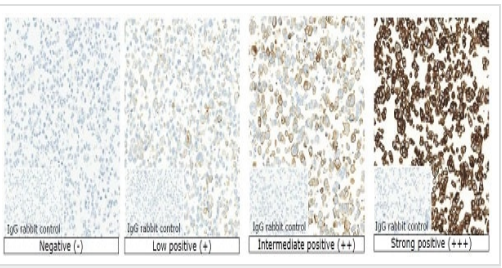
B: Patchy expression of PD-L1 in a squamous cell carcinoma at the tumor-nest-TME interface (inset arrow). Minimal to no PD-L1 expression in the trabeculae (asterisk) if compared with (**A**)

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

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

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

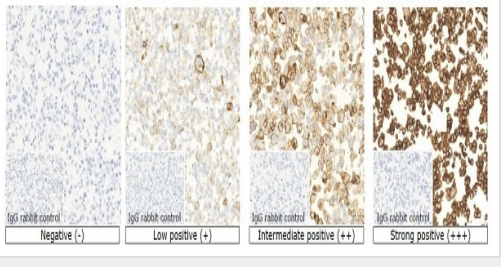
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab228415**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [73-10] - BSA and Azide free (ab226766)

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

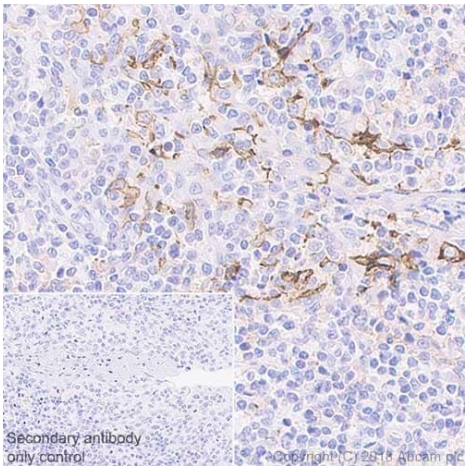
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab228415**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [73-10] - BSA and Azide free (ab226766)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab228415**).



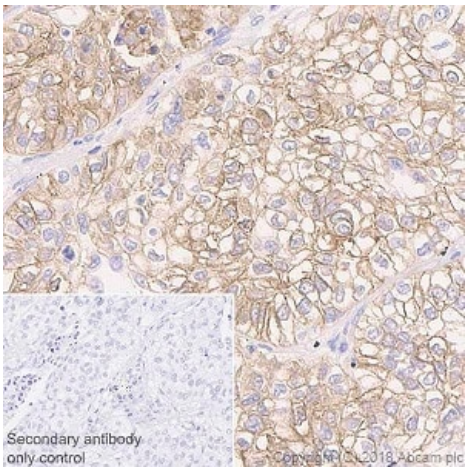
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [73-10] - BSA and Azide free (ab226766)

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

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab228415**).



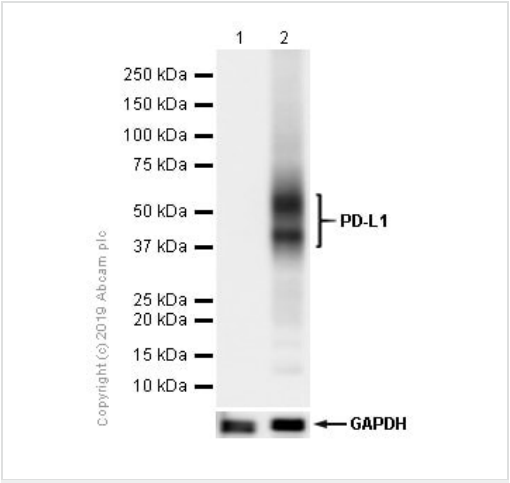
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [73-10] - BSA and Azide free (ab226766)

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

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab228415**).



Western blot - Anti-PD-L1 antibody [73-10] - BSA and Azide free (ab226766)

All lanes : Anti-PD-L1 antibody [73-10] - BSA and Azide free (ab226766) at 1/50000 dilution

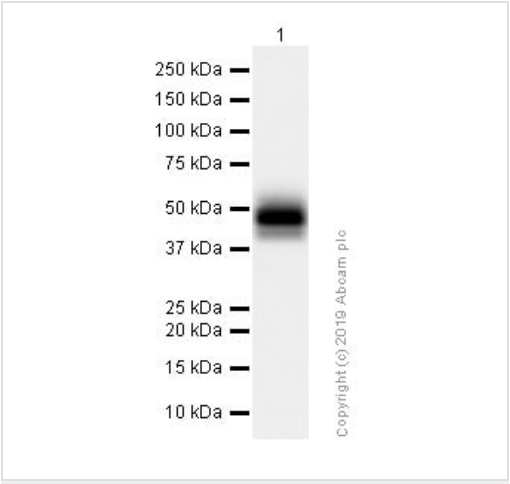
Lane 1 : CHO-S (Chinese hamster ovary epithelial cell) whole cell lysates
Lane 2 : CHO-PD-L1 (PD-L1 stably expressed Chinese hamster ovary epithelial cell) whole cell lysates

Lysates/proteins at 15 µg per lane.

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

Predicted band size: 33 kDa
Additional bands at: 40-60 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 2 seconds



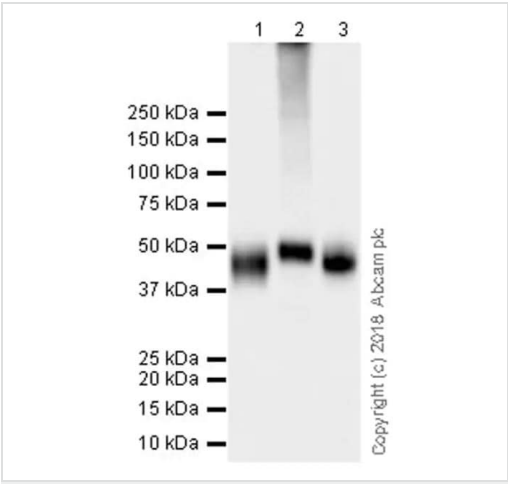
Western blot - Anti-PD-L1 antibody [73-10] - BSA and Azide free (ab226766)

Anti-PD-L1 antibody [73-10] - BSA and Azide free (ab226766) at 1/50000 dilution + H1975 (Human non-small cell lung cancer epithelial cell) whole cell lysates at 15 µg

Secondary
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 33 kDa

Exposure time: 8 seconds



Western blot - Anti-PD-L1 antibody [73-10] - BSA and Azide free (ab226766)

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

Lane 1 : NCI-H1975 (human non-small cell lung cancer cell line), whole cell lysate
Lane 2 : Human placenta
Lane 3 : Human thymus

Lysates/proteins at 20 µg per lane.

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

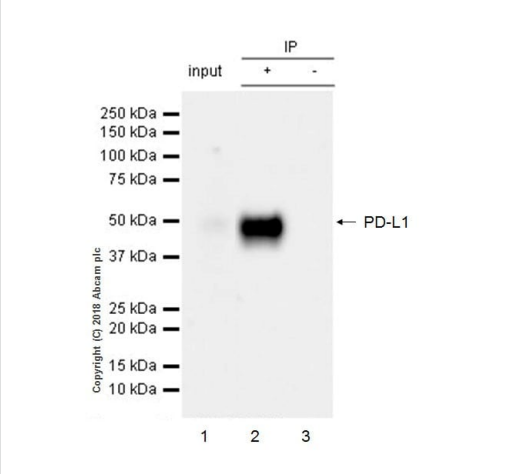
Predicted band size: 33 kDa

Exposure time: 70 seconds

This data was produced using **ab228415**, the same antibody clone in a different buffer formulation.

Blocking/Dilution buffer: 5% NFDM/TBST.

The molecular mass observed is consistent with what has been described in the literature (PMID: 26546452)



Immunoprecipitation - Anti-PD-L1 antibody [73-10] - BSA and Azide free (ab226766)

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

Lane 1: NCI-H1975 whole cell lysate 10 µg (Input).

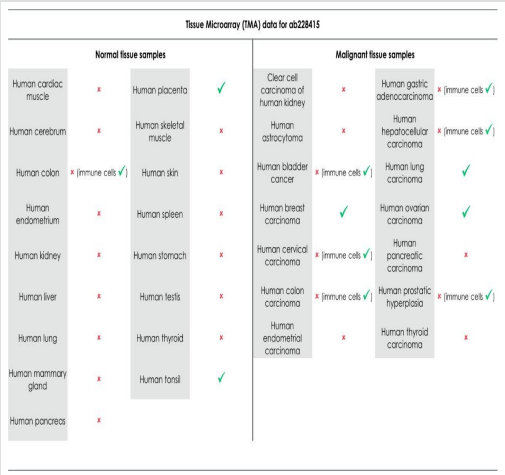
Lane 2: **ab228415** IP in NCI-H1975 whole cell lysate (+).

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

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: 30 seconds.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab228415**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [73-10] - BSA and Azide free (ab226766)

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

Anti-PD-L1 antibody [73-10] - BSA and Azide free (ab226766)

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