abcam

Product datasheet

Anti-PD-L1 antibody [28-8] - Low endotoxin, Azide free ab209889

敲除验证 重组 RabMAb

31 图像

概述

产品名称	Anti-PD-L1抗体[28-8] - Low endotoxin, Azide free
描述	兔单克隆抗体[28-8] to PD-L1 - Low endotoxin, Azide free
宿主	Rabbit
经测试应用	**适用于:** ICC/IF, IHC-P, Flow Cyt, WB, IHC-Fr
种属反应性	**与反应:** Human
免疫原	Fusion protein. This information is proprietary to Abcam and/or its suppliers.
阳性对照	Tissue: Human tonsil, head and neck squamous cell carcinoma and placenta tissues; L2987 cell line. Cell Lines: Positives: B-CPAP (high), ES-2 (medium), HCC70 (low), CHO-PDL1, U-87 MG For additional information - please refer to this publication:Programmed death-ligand 1 (PD-L1) expression in various tumor types - http://www.immunotherapyofcancer.org/content/1/S1/P53 IHC-Fr: Frozen human tonsil tissue sections
常规说明	ab209889 is the Low endotoxin, azide-free version of **ab205921**. Anti-PD-L1 antibody [28-8] has been used as detector antibody in **Human PD-L1 SimpleStep ELISA® kit (ab214565)**. **Additional information on positive controls:** Tissue: Tonsil- with hyperreactive changes Note: Tonsil Specimens- is recommended to screen several hyper-reactive tonsils to find those with 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 Note: Tumor Specimens- PD-L1 expression varies by tumor type so screening is recommended to find positive and negative tumor controls. Refer to web link publication below to find some suggested tumor types. Many tumor specimens have some inflammatory macrophages and mononuclear leukocytes. Best to look for specimens with high numbers of these cells Cell Lines: Positives: B-CPAP- high, ES-2- medium, HCC70 - low **For primary negative control, isotype control, RabMAb negative control antibody (ab172730)** is recommended.

For negative control sample, cell line COLO205 is recommended.

For PD-L1 protein, see **ab167713**

Recommended protocols:

For recommended Immunohistochemistry (IHC) protocol, please refer to the protocol book in the protocol section and/or **here (downloadable copy)**

For IHC usage on FFPE tissues, the following antigen solution is recommended with clone 28-8 - Universal HIER antigen retrieval reagent (**ab208572**) **KO Validated**

For recommended Flow Cytometry (Flow Cyt) protocol, please refer to the protocol book **here (downloadable copy)**

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.

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 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**.

Our **Low endotoxin, azide-free formats** have low endotoxin level (≤ 1 EU/ml, determined by the LAL assay) and are free from azide, to achieve consistent experimental results in functional assays.

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C. Do Not Freeze.
存储溶液	pH: 7.2 Constituent: PBS
无载体	**是**
纯度	Protein A purified
克隆	单克隆

克隆编号	28-8
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab209889于以下的经测试应用**

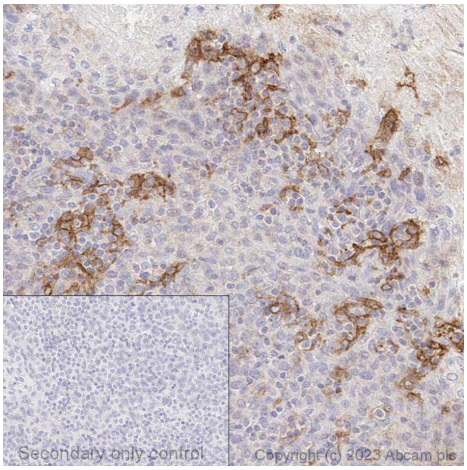
"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
ICC/IF		Use a concentration of 2 µg/ml.
IHC-P		Use a concentration of 2 µg/ml. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
Flow Cyt		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB		Use at an assay dependent concentration. Detects a band of approximately 33-43 kDa (predicted molecular weight: 33 kDa). Please check the parent abID, **ab205921**, for more information on dilutions.
IHC-Fr		Use a concentration of 1 µg/ml.

靶标

功能	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.
组织特异性	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.
序列相似性	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.
细胞定位	Cell membrane and Endomembrane system.

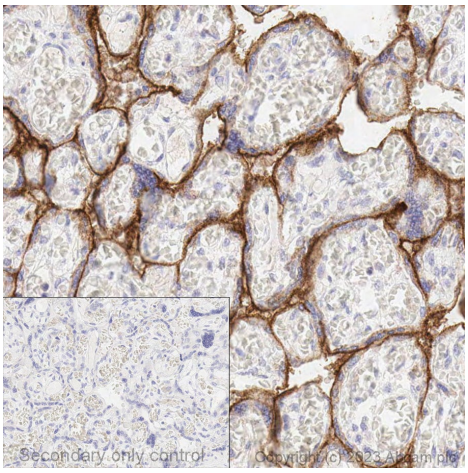
图片



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [28-8] - Low endotoxin, Azide free (ab209889)

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.

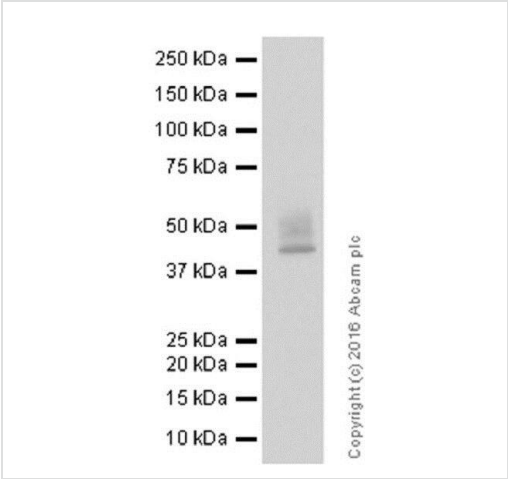
This data was developed using **ab205921**, the same antibody clone in a different buffer formulation.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [28-8] - Low endotoxin, Azide free (ab209889)

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.

This data was developed using **ab205921**, the same antibody clone in a different buffer formulation.

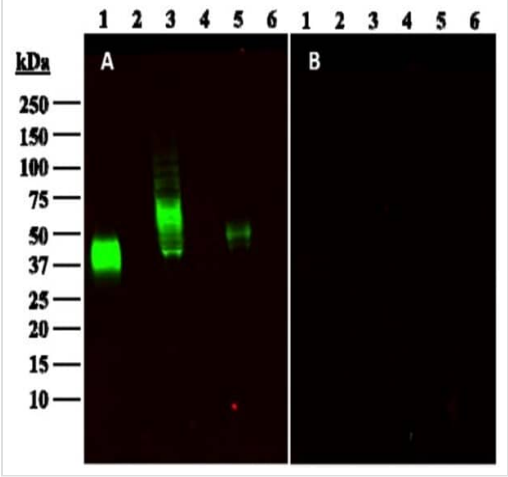


Western blot - Anti-PD-L1 antibody [28-8] - Low endotoxin, Azide free (ab209889)

This data was developed using **ab205921**, the same antibody clone in a different buffer formulation:

Primary ab Dilution 1:100 dilution, Secondary ab **Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody**, 1:20,000 dilution, Blocking and diluting buffer and concentration 5% NFDM/TBST, Lane 1: NCI-H1975 (Human non-small cell lung cancer epithelia), Observed MW 40-60 kDa.

For recommended Western Blot (WB) protocol for endogenous PD-L1 expression, please refer to the protocol book in the protocol section and/or **here (downloadable copy).**



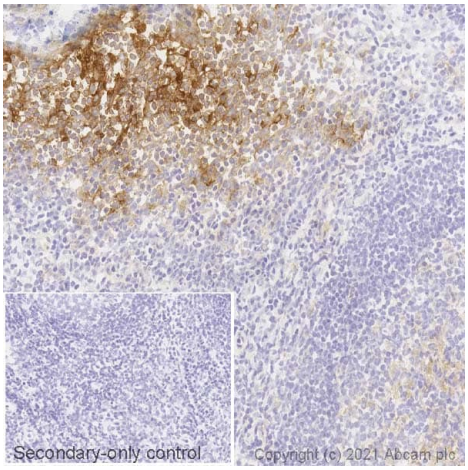
Western blot - Anti-PD-L1 antibody [28-8] - Low endotoxin, Azide free (ab209889)

This data was developed using **ab205921**, the same antibody clone in a different buffer formulation:

(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 protocol section and/or **here (downloadable copy).**



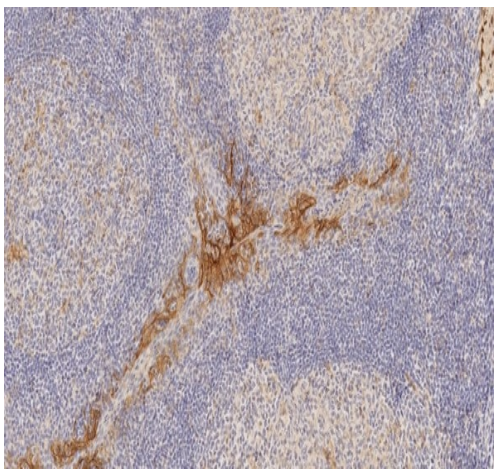
Immunohistochemistry (Frozen sections) - Anti-PD-L1 antibody [28-8] - Low endotoxin, Azide free (ab209889)

This data was developed using **ab205921**, 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 **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.

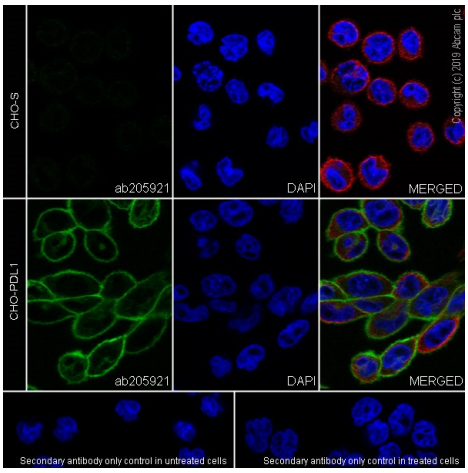
*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [28-8] - Low endotoxin, Azide free (ab209889)

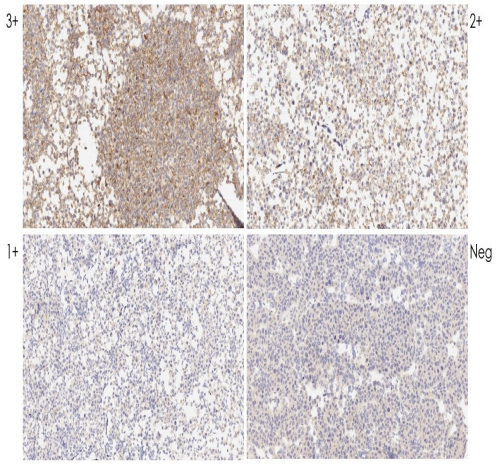
IHC image of **ab205921** staining PD-L1 in human tonsil formalin fixed paraffin embedded tissue sections*, performed on a Leica BOND RX (Polymer Refine kit). The section was pre-treated using heat mediated antigen retrieval with EDTA buffer (pH9, epitope retrieval solution 2) for 30 mins at 98°C. The 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.

This image was generated using **ab205921**, the same antibody but with BSA and Azide



Immunocytochemistry/ Immunofluorescence - Anti-PD-L1 antibody [28-8] - Low endotoxin, Azide free (ab209889)

This data was developed using the same antibody clone in a different buffer formulation (**ab205921**).
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 control: PBS instead of the primary antibody.

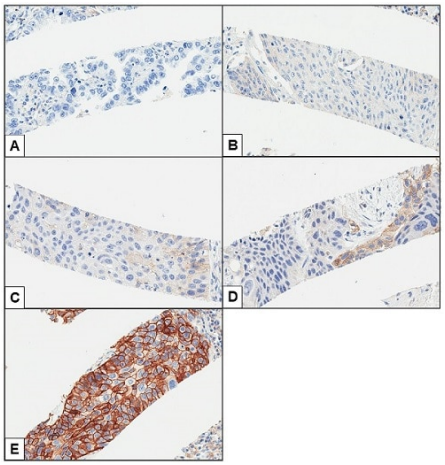


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [28-8] - Low endotoxin, Azide free (ab209889)

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.

This image was generated using **ab205921**, the same antibody but with BSA and Azide



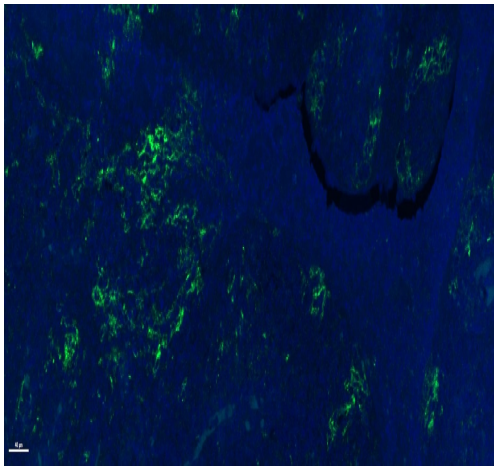
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [28-8] - Low endotoxin, Azide free (ab209889)

Image from Nakamura S HL et al., PLoS One. 2017;12(10):e0186192 Fig 3.; doi: 10.1371/journal.pone.0186192.

Representative images of PD-L1 expression in human lung adenocarcinoma and squamous cell carcinoma specimens.

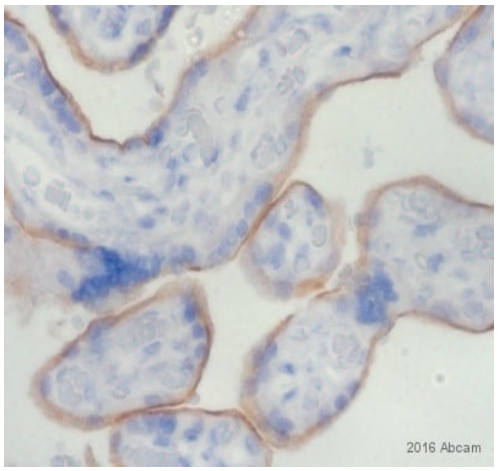
(**A**) <1.0%, (**B**) 1.0–4.9%, (**C**) 5.0–9.9%, (**D**) 10.0–49.9%, and (**E**) ≥50.0% PD-L1-positive cells.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab205921**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [28-8] - Low endotoxin, Azide free (ab209889)

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.

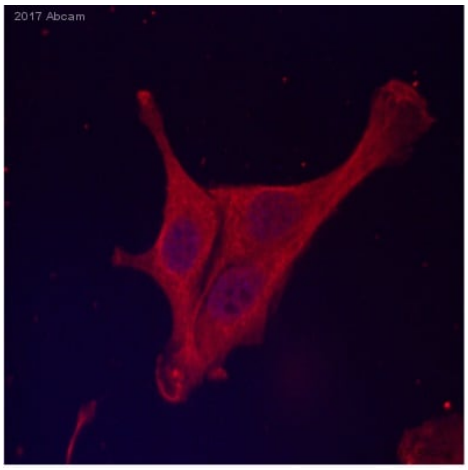


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [28-8] - Low endotoxin, Azide free (ab209889)

This image is courtesy of an Abreview submitted by Mr. Rudolf Jung.

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab205921**).

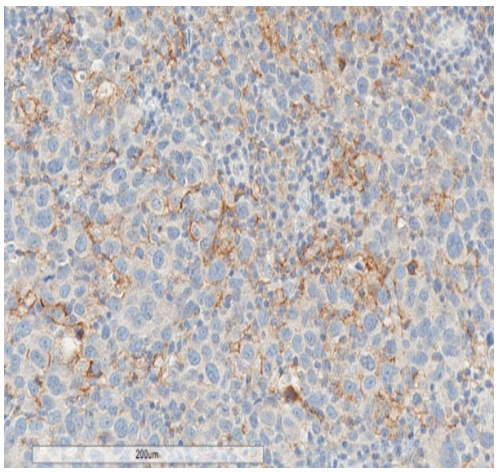


Immunocytochemistry/ Immunofluorescence - Anti-PD-L1 antibody [28-8] - Low endotoxin, Azide free (ab209889)

This image is courtesy of an Abreview submitted by Dr. Dimitra Kalamida.

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab205921**).

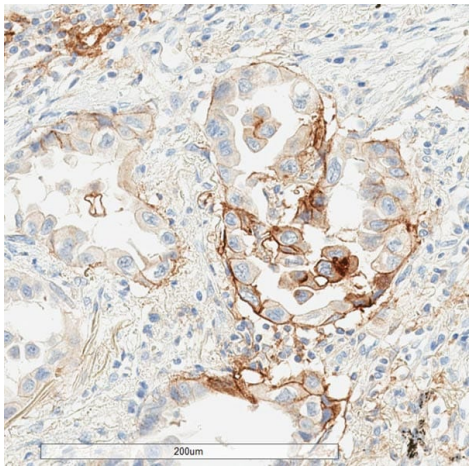


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [28-8] - Low endotoxin, Azide free (ab209889)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab205921**).

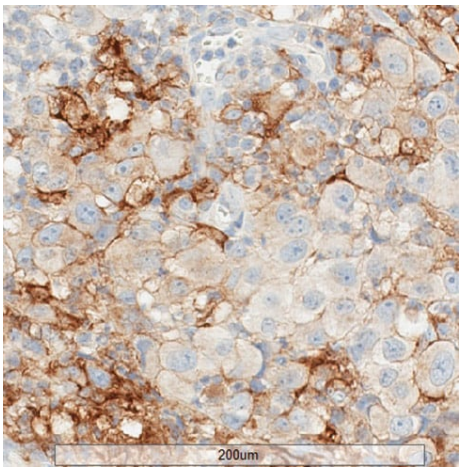


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [28-8] - Low endotoxin, Azide free (ab209889)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab205921**).

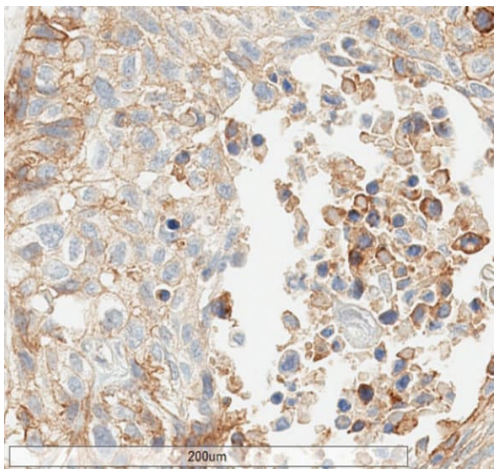


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [28-8] - Low endotoxin, Azide free (ab209889)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human melanoma tissue labelling PD-L1 with **ab205921** on Ventana Ultra. Tumor cells and immune cells show PD-L1 positive 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab205921**).

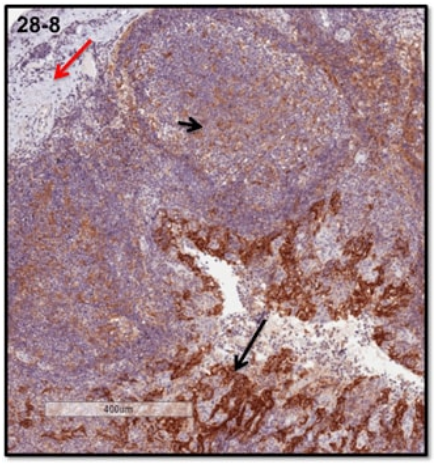


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [28-8] - Low endotoxin, Azide free (ab209889)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human non-small cell lung cancer tissue labelling PD-L1 with **ab205921**. Staining can be seen in tumor associated macrophages and tumor cells.

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab205921**).

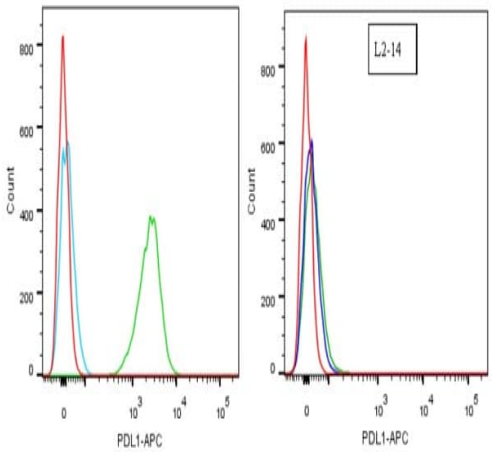


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [28-8] - Low endotoxin, Azide free (ab209889)

Immunohistochemical analysis of Human Tonsil tissue with **ab205921** at 2 µg/ml.
PD-L1 positive expression of the crypt epithelium (large black arrow) and cells localized within the germinal centers (small black arrow)
Note negative staining of the stroma (red arrow), additionally, stainings of follicles and some interfollicular cells

For recommended Immunohistochemistry (IHC) protocol, please refer to the protocol book in the protocol section and/or **here (downloadable copy).**

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab205921**).

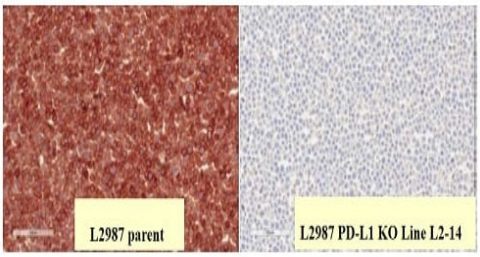


Flow Cytometry - Anti-PD-L1 antibody [28-8] - Low endotoxin, Azide free (ab209889)

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 K.O. cell line.

For recommended Flow Cytometry (Flow Cyt) protocol, please refer to the protocol book in the protocol section and/or **here (downloadable copy).**

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab205921**).



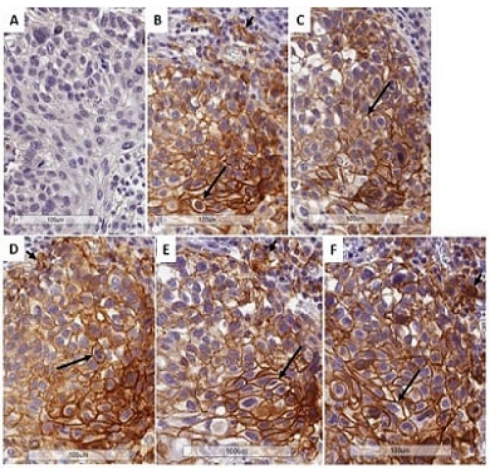
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [28-8] - Low endotoxin, Azide free (ab209889)

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 protocol section and/or **here (downloadable copy).**

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab205921**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [28-8] - Low endotoxin, Azide free (ab209889)

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)

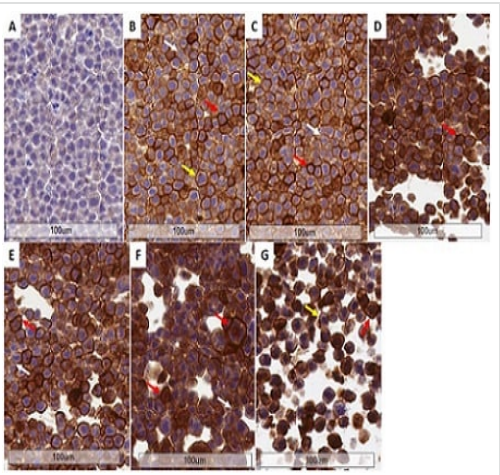
All batches/lots (1,3,4,5,6) showed consistent results.

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 protocol section and/or **here**

(downloadable copy).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab205921**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [28-8] - Low endotoxin, Azide free (ab209889)

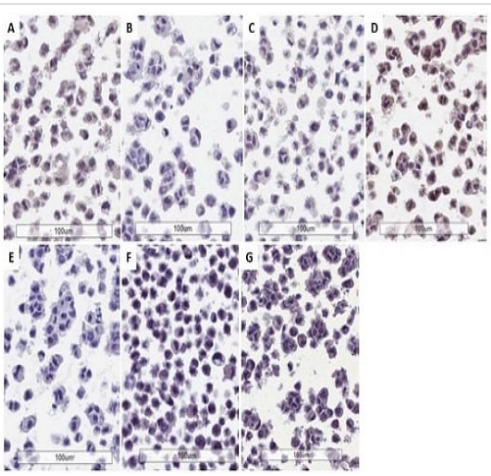
Immunohistochemical analysis of CHO PD-L1 cells with **ab205921** at 2 µg/ml.
High power view
A) Rabbit IgG, 5 µg/mL. No staining
B) Anti PD-L1, 2 µg/mL (**ab205921** batches 1)
C) Anti PD-L1, 2 µg/mL (**ab205921** batches 3)
D) Anti PD-L1, 2 µg/mL (**ab205921** batches 4)
E) Anti PD-L1, 2 µg/mL (**ab205921**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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab205921**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [28-8] - Low endotoxin, Azide free (ab209889)

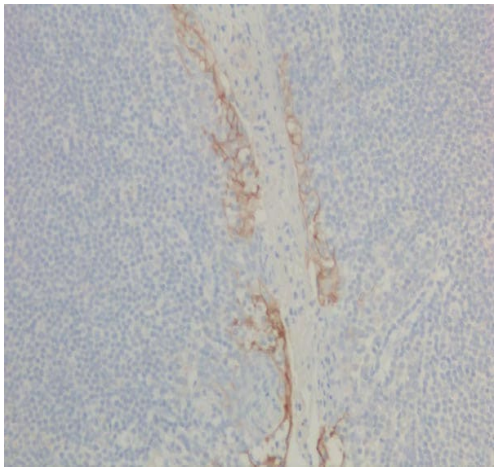
Immunohistochemical analysis of CHO Parental cells with **ab205921** at 2 µg/ml.
High power view
A) Rabbit IgG, 5 µg/mL. No staining
B) Anti PD-L1, 2 µg/mL (**ab205921** batches 1)
C) Anti PD-L1, 2 µg/mL (**ab205921** batches 3)
D) Anti PD-L1, 2 µg/mL (**ab205921** batches 4)
E) Anti PD-L1, 2 µg/mL (**ab205921** 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 (1,3,4,5,6,7) 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 protocol section and/or **here (downloadable copy).**

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab205921**).

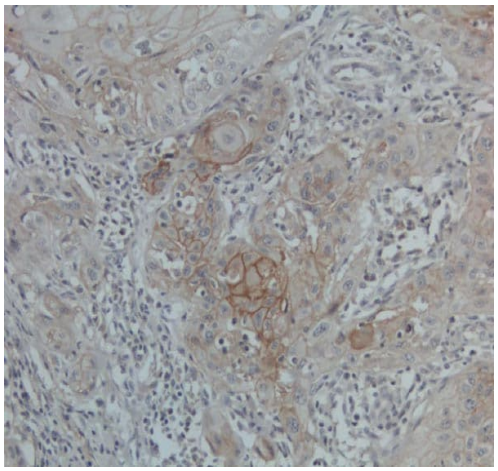


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [28-8] - Low endotoxin, Azide free (ab209889)

Immunohistochemical analysis of formalin-fixed, paraffin-embedded Human tonsil 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab205921**).

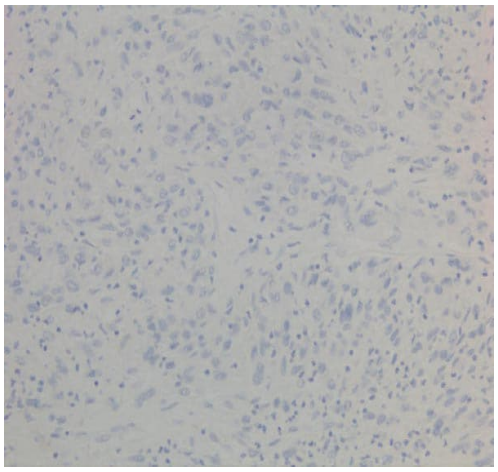


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [28-8] - Low endotoxin, Azide free (ab209889)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab205921**).

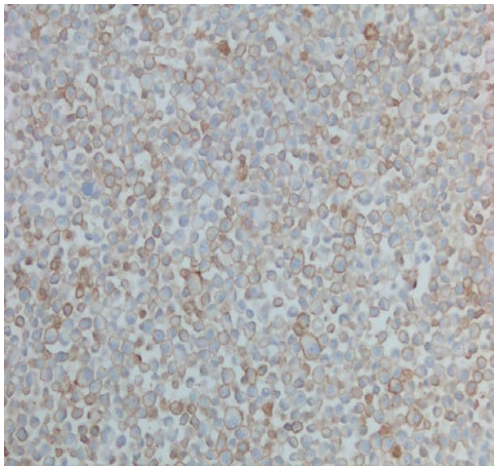


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [28-8] - Low endotoxin, Azide free (ab209889)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab205921**).

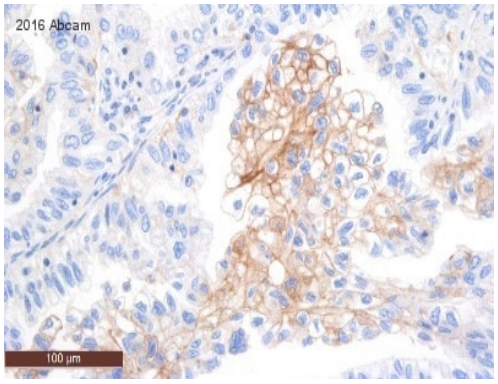


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [28-8] - Low endotoxin, Azide free (ab209889)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab205921**).

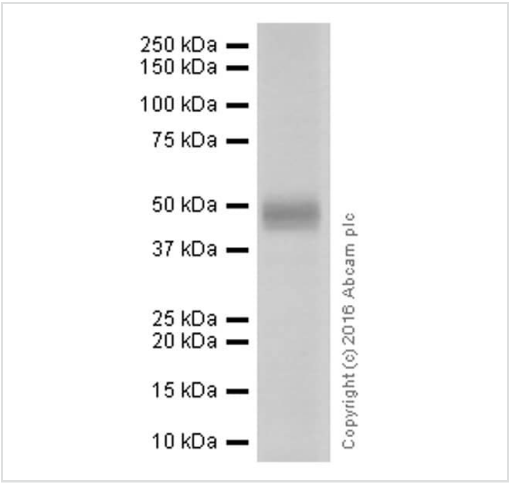


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [28-8] - Low endotoxin, Azide free (ab209889)

This IHC data was generated using the same anti-PDL1 antibody clone, 28-8, in a different buffer formulation (cat# **ab205921**).

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 antibody [28-8] - Low endotoxin, Azide free (ab209889)

Anti-PD-L1 antibody [28-8] - Low endotoxin, Azide free (ab209889) + NCI-H1975 (human non-small cell lung cancer) whole cell lysate at 15 µg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**)

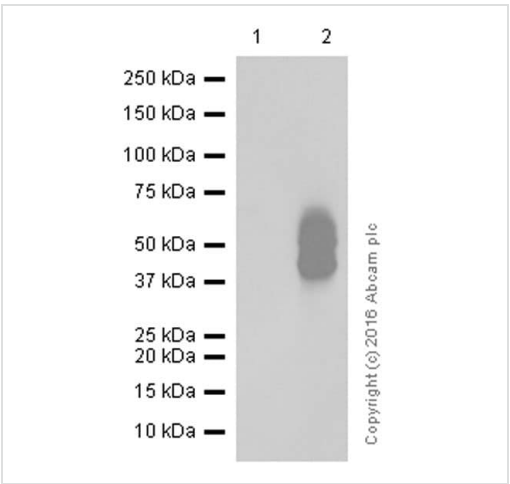
Predicted band size: 33 kDa

Observed band size: 40-60 kDa

Exposure time: 3 minutes

Blocking buffer and concentration: 5% NFDM/TBST

Diluting buffer and concentration: 5% NFDM/TBST



Western blot - Anti-PD-L1 antibody [28-8] - Low endotoxin, Azide free (ab209889)

All lanes : Anti-PD-L1 antibody [28-8] - Low endotoxin, Azide free (ab209889)

Lane 1 : CHO-S cell lysate

Lane 2 : Human PD-L1 transfected CHO-S cell lysate

Lysates/proteins at 15 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**)

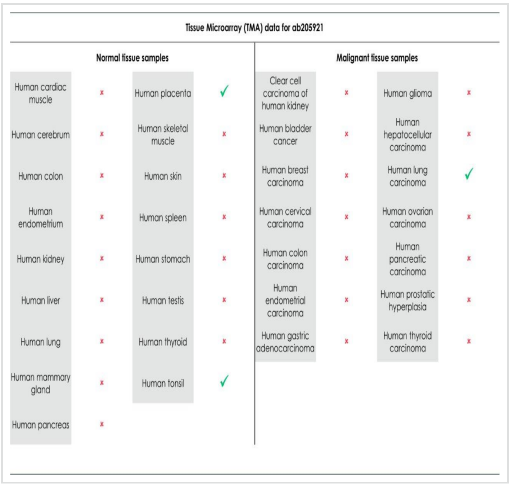
Predicted band size: 33 kDa

Observed band size: 40-60 kDa

Exposure time: 1 minute

Blocking buffer and concentration: 5% NFDM/TBST

Diluting buffer and concentration: 5% NFDM/TBST

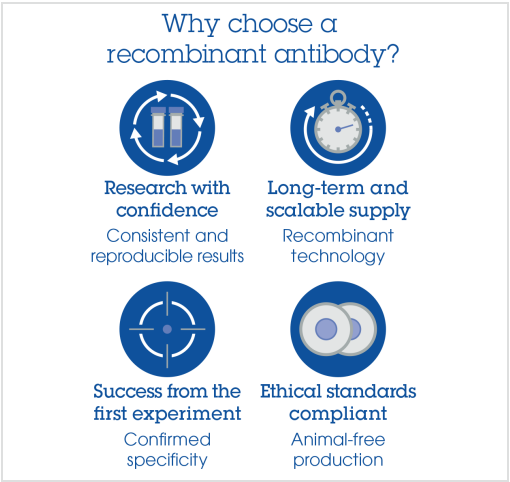


Tissue Microarray (TMA) data for ab205921

Normal tissue samples				Malignant tissue samples			
Human cardiac muscle	x	Human placenta	✓	Clear cell carcinoma of human kidney	x	Human glioma	x
Human cerebrum	x	Human skeletal muscle	x	Human bladder cancer	x	Human hepatocellular carcinoma	x
Human colon	x	Human skin	x	Human breast carcinoma	x	Human lung carcinoma	✓
Human endometrium	x	Human spleen	x	Human cervical carcinoma	x	Human ovarian carcinoma	x
Human kidney	x	Human stomach	x	Human colon carcinoma	x	Human pancreatic carcinoma	x
Human liver	x	Human testis	x	Human endometrial carcinoma	x	Human prostatic hyperplasia	x
Human lung	x	Human thyroid	x	Human gastric adenocarcinoma	x	Human thyroid carcinoma	x
Human mammary gland	x	Human tonsil	✓				
Human pancreas	x						

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [28-8] - Low endotoxin, Azide free (ab209889)

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.



Anti-PD-L1 antibody [28-8] - Low endotoxin, Azide free (ab209889)

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