**Product datasheet**

**Anti-PD-L1 antibody [28-8] ab205921**

*KO VALIDATED*  *Recombinant*  *RabMab*

⭐⭐⭐⭐☆здесь 15 Abreviews  418 References  23 Images

### Overview

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<tr>
<th><strong>Product name</strong></th>
<th>Anti-PD-L1 antibody [28-8]</th>
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<tbody>
<tr>
<td><strong>Description</strong></td>
<td>Rabbit monoclonal [28-8] to PD-L1</td>
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<tr>
<td><strong>Host species</strong></td>
<td>Rabbit</td>
</tr>
<tr>
<td><strong>Tested applications</strong></td>
<td>Suitable for: ICC/IF, IHC-P, WB, Flow Cyt, IHC-Fr</td>
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<tr>
<td><strong>Species reactivity</strong></td>
<td>Reacts with: Human</td>
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<td><strong>Immunogen</strong></td>
<td>Fusion protein. This information is proprietary to Abcam and/or its suppliers.</td>
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<tr>
<td><strong>Positive control</strong></td>
<td>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 - <a href="http://www.immunotherapyofcancer.org/content/1/S1/P53">http://www.immunotherapyofcancer.org/content/1/S1/P53</a>. IHC-Fr: Frozen human tonsil tissue sections</td>
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</table>

### General notes

**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](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](http://www.abcam.com/ab172730).

**Recombinant protein positive control:** Recombinant human PD-L1 protein, [ab167713](http://www.abcam.com/ab167713).

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

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

<table>
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<tr>
<th>Form</th>
<th>Liquid</th>
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| Storage buffer| pH: 7.2
Preservative: 0.01% Sodium azide
 Constituents: 59% PBS, 40% Glycerol, 0.05% BSA |
| Purity        | Protein A purified |
| Clonality     | Monoclonal        |
| Clone number  | 28-8              |
| Isotype       | IgG               |

Applications

The Abpromise guarantee
Our Abpromise guarantee covers the use of ab205921 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
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<tr>
<th>Application</th>
<th>Abreviews</th>
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<tr>
<td>ICC/IF</td>
<td>⭐⭐⭐⭐⭐⭐ (1)</td>
<td>Use a concentration of 2 µg/ml.</td>
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| IHC-P       | ⭐⭐⭐⭐⭐ (7)  | Use a concentration of 2 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
Please refer to protocol section here.
For antigen buffer for FFPE tissue, it is recommended to use Universal HIER antigen retrieval reagent (ab208572). |
| WB          | ⭐⭐⭐⭐⭐♀ (5) | Use at an assay dependent concentration. Detects a band of approximately 40-60 kDa (predicted molecular weight: 33 kDa).
Please refer to protocol section here. |
**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.

<table>
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</tr>
</thead>
<tbody>
<tr>
<td>Flow Cyt</td>
<td></td>
<td>Use at an assay dependent concentration. Please refer to protocol section <a href="#">here</a>. ab172730 - Rabbit monoclonal IgG is suitable for use as an isotype control with this antibody.</td>
</tr>
<tr>
<td>IHC-Fr</td>
<td>★★★★☆☆☆☆☆ (1)</td>
<td>Use a concentration of 1 µg/ml.</td>
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**Target**

**Images**

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
Immunocytochemistry analysis of CHO-PDL1 (PD-L1 stably expressed 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 transfected cells without the primary 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 and/or here (downloadable copy).

Alexa Fluor® 488 (ab209959) and Alexa Fluor® 647 (ab209960) conjugated versions are available for this clone.
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 section and/or [here (downloadable copy)](https://www.abcam.com/).

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.

**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, 1ug/ml, 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

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

**All lanes**: Anti-PD-L1 antibody [28-8] (ab205921) at 1/100 dilution

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

**Lane 2**: NCI-H1299 (Human lung carcinoma epithelial cell) whole cell lysate

**Lane 3**: A549 (Human lung carcinoma epithelial cell) whole cell lysate

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

**Lane 5**: MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate

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

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

Expression of PD-L1 varied widely among the tumor cell lines.

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.


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

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.

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.
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).

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

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.
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).

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

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

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

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 retrieval 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) analysis of human melanoma tissue labelling PD-L1 with ab205921. Tumor cells show weak and partial positive PD-L1 expression in the plasma membrane. PD-L1 positive tumor associated immuno cells are also stained.

For antigen retrieval buffer, Universal HIER antigen retrieval reagent (ab208572) was used.

For IHC detection kit, Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) is recommended.
Western blot - Anti-PD-L1 antibody [28-8] (ab205921)

(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 (Lane 2). 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 (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [28-8] (ab205921)

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 retrieval buffer, Universal HIER antigen retrieval reagent (ab208572) was used.

For IHC detection kit, Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) is recommended.
Paraformaldehyde-fixed, paraffin-embedded human placenta tissue stained for PD-L1 using ab205921 at 1/100 dilution in immunohistochemical analysis.

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 retrieval buffer, Universal HIER antigen retrieval reagent (ab208572) was used.

For IHC detection kit, Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) is recommended.
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 HER 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.
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