**Overview**

<table>
<thead>
<tr>
<th>Product name</th>
<th>Anti-PD-L1 antibody [28-8]</th>
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<tbody>
<tr>
<td>Description</td>
<td>Rabbit monoclonal [28-8] to PD-L1</td>
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<tr>
<td>Host species</td>
<td>Rabbit</td>
</tr>
<tr>
<td>Tested applications</td>
<td>Suitable for: IHC-P, WB, Flow Cyt, ICC/IF</td>
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<tr>
<td>Species reactivity</td>
<td>Reacts with: Human</td>
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<tr>
<td>Immunogen</td>
<td>Recombinant full length protein corresponding to Human PD-L1 (extracellular). The immunogen contains the specific extracellular domain of huPD-L1 (Phe19-Thr239). See reference for more info - <a href="http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4561627/">www.ncbi.nlm.nih.gov/pmc/articles/PMC4561627/</a></td>
</tr>
<tr>
<td></td>
<td>Database link: Q9NZQ7</td>
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<tr>
<td>Positive control</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 here: 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></td>
</tr>
<tr>
<td>General notes</td>
<td><strong>Information on positive controls</strong> (Chinese version found here)</td>
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<tr>
<td></td>
<td><strong>Tissue:</strong> Tonsil- with hyperreactive changes</td>
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<td></td>
<td>Recommended to screen hyper-reactive tonsils to find those with highest expression of PD-L1 in crypt epithelium, macrophages homing the germinal centers and interfollicular mononuclear leukocytes.</td>
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<td><strong>Tumor tissues- prescreened for positive tumor and inflammatory infiltrates</strong> PD-L1 expression varies by tumor type so screening is recommended to find positive and negative tumor controls. The following publication is useful for suggesting suitable tumor types: <a href="http://www.immunotherapyofcancer.org/content/1/S1/P53">http://www.immunotherapyofcancer.org/content/1/S1/P53</a></td>
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<tr>
<td></td>
<td>Note: Look for specimens with high numbers of inflammatory macrophages and mononuclear leukocytes.</td>
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<tr>
<td></td>
<td><strong>Cell Lines:</strong> Positive controls: B-CPAP- high, ES-2- medium, HCC70 – low, Negative controls: COLO205, see ab95356.</td>
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<tr>
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<td><strong>Primary negative control, isotype control:</strong> RabMAb negative control antibody, ab172730.</td>
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</table>
**Recombinant protein:** Recombinant human PD-L1 protein, ab167713.

**Recommended protocols:**
For recommended Western Blotting (WB) protocol, Flow Cytometry (FC) protocol and Immunohistochemistry (IHC) protocol, please refer to the protocol section, here.

**IHC usage:**
For IHC usage on FFPE tissues, the following antigen solution and detection kit are recommended with clone 28-8 - Universal HIER antigen retrieval reagent (ab208572) and IHC detection kit HRP/DAB (ab209101).

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

**Alternative versions available:**
Conjugated versions of the antibody available (Alexa Fluor® 488, Alexa Fluor® 647, PE, HRP) - see list.

A low endotoxin, azide free version of the antibody is available: ab209889.

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

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

This product is a recombinant rabbit monoclonal antibody.

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**Properties**

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

**Applications**

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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<th>Application</th>
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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.

IHC-P

Use a concentration of 2 µg/ml. Perform heat mediated antigen retrieval via the microwave method before commencing with IHC staining protocol. Boil slides seven times for five minutes each in pH 6 citrate buffer.

For antigen buffer for FFPE tissue, it is recommended to use Universal HIER antigen retrieval reagent (ab208572).

WB

Use at an assay dependent concentration. Predicted molecular weight: 33 kDa.

Flow Cyt

Use at an assay dependent concentration.

ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

ICC/IF

Use a concentration of 2 µg/ml.

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
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).

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).
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, Exposure time 3 min.

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).

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).
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).
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).
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 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 on Ventana Ultra. Tumor cells and immune cells show PD-L1 positive 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.
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.

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

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 immunee 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.

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

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.
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 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 ab2205921 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.
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).

(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).

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