

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



★★★★★ 2 Abreviews | 16 References | 15 Images

## Overview

Product name	Anti-PD-L1 antibody [28-8]
Description	Rabbit monoclonal [28-8] to PD-L1
Tested applications	IHC-P, WB, Flow Cyt
Species reactivity	<b>Reacts with:</b> Human
Immunogen	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> Database link: <a href="#">Q9NZQ7</a>
Positive control	Tissue: Human tonsil and head and neck squamous cell carcinoma tissues; L2987 cell line. Cell Lines: Positives: B-CPAP- high, ES-2- medium, HCC70 - low 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>
General notes	Produced using Abcam's <a href="#">RabMAb® technology</a> . RabMAb® technology is covered by the following U.S. Patents, No. 5,675,063 and/or 7,429,487.

**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 Western Blotting (WB) protocol, please refer to the protocol book (line 3) in the protocol section.

For recommended Immunohistochemistry (IHC) protocol, please refer to the protocol book (line 2) 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 \(ab208572\)](#)

For recommended Flow Cytometry (Flow Cyt) protocol, please refer to the protocol book (line 1) in the protocol section and/or [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.

For colormetric detection of PDL1, it is recommended to use [Anti-PD-L1 antibody \(ab58810\)](#)

**Properties**

<b>Form</b>	Liquid
<b>Storage instructions</b>	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
<b>Storage buffer</b>	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
<b>Clonality</b>	Monoclonal
<b>Clone number</b>	28-8
<b>Isotype</b>	IgG

**Applications**

Our [Abpromise guarantee](#) covers the use of **ab205921** in the following tested applications.

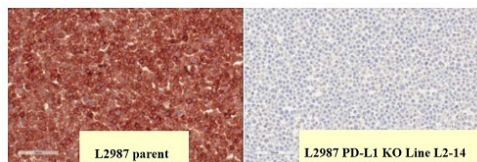
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
<b>IHC-P</b>	★★★★★	Use a concentration of 2 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.  For antigen buffer for FFPE tissue, it is recommended to use <a href="#">Universal HIER antigen retrieval reagent (ab208572)</a>
<b>WB</b>		Use at an assay dependent concentration. Predicted molecular weight: 33 kDa.
<b>Flow Cyt</b>		Use at an assay dependent concentration.  <a href="#">ab172730 - Rabbit monoclonal IgG</a> , is suitable for use as an isotype control with this antibody.

**Target**

<b>Function</b>	Involved in the costimulatory signal, essential for T-cell proliferation and production of IL10 and IFNG, in an IL2-dependent and a PD1-independent manner. Interaction with PD1 inhibits T-cell proliferation and cytokine production.
<b>Tissue specificity</b>	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.
<b>Sequence similarities</b>	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.
<b>Cellular localization</b>	Cell membrane and Endomembrane system.

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

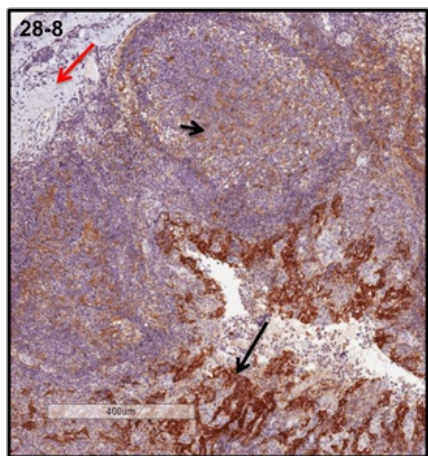


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [28-8] (ab205921)

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 an L2987 colorectal cell line. 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 (line 2) in the protocol section and/or [here \(downloadable copy\)](#)



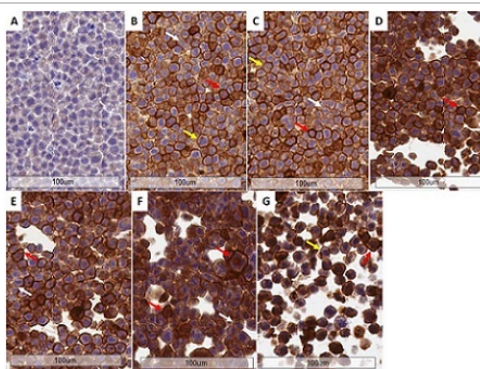
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [28-8] (ab205921)

Immunohistochemical analysis of 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 (line 2) 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 CHO PD-L1 cells with ab205921 at 2 µg/ml.

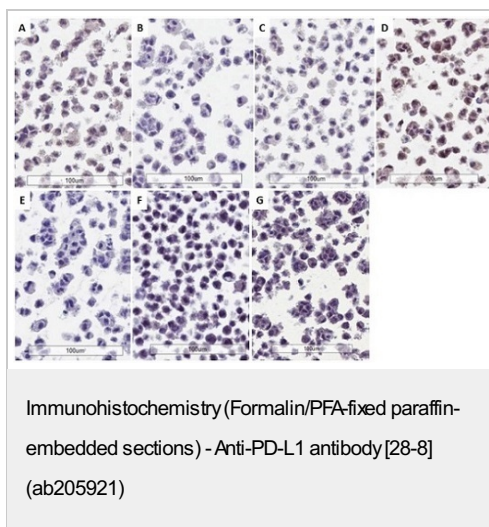
High power view

- 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 (line 2) 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)

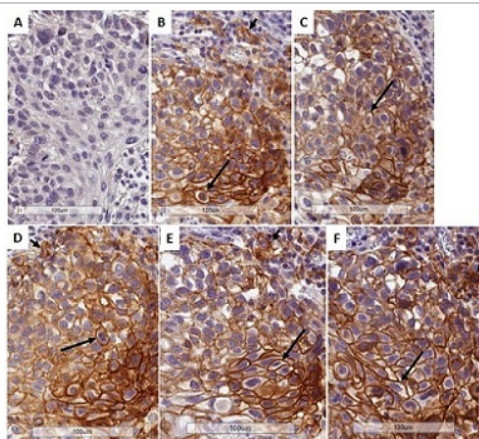
F) Anti PD-L1, 2 µg/mL (ab205921 batches 6)

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 (line 2) 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 Human Lung NSCLC with ab205921 at 2 µg/ml.

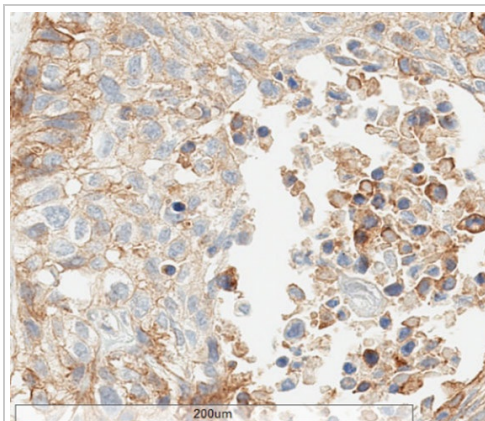
High power view

- 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 (line 2) in the protocol section and/or [here \(downloadable copy\)](#)



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

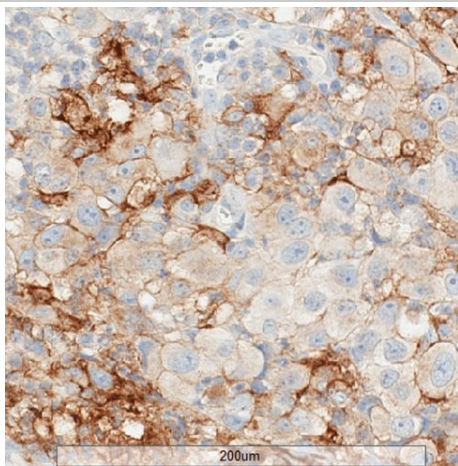
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human non-small cell lung cancer tissue labelling PD-L1 with ab205921.

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.



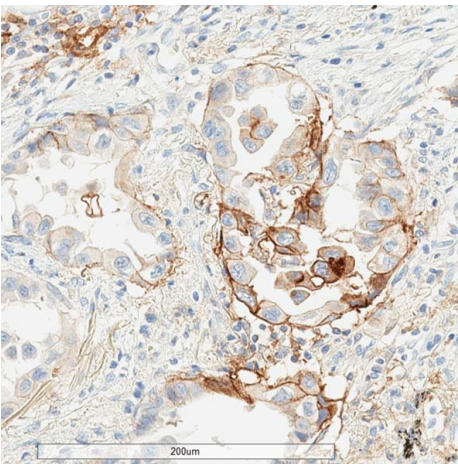


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [28-8] (ab205921)

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.

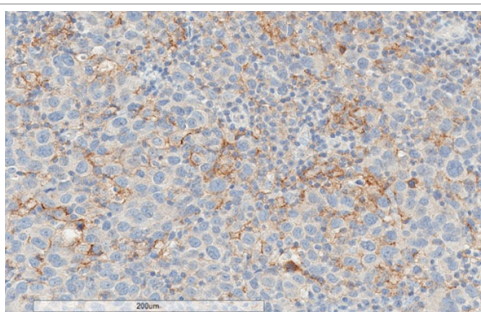


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [28-8] (ab205921)

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.

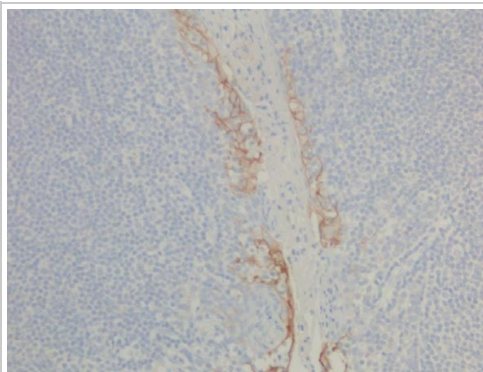


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [28-8] (ab205921)

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 expresseion in the plasma membrane. PD-L1 positive tumor associated immunoe 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.

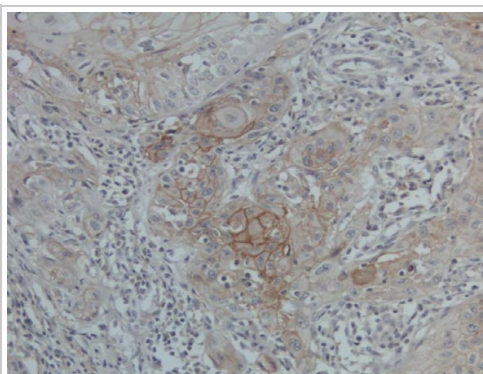


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [28-8] (ab205921)

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.

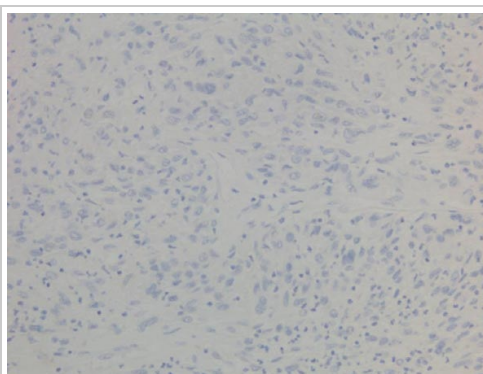


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [28-8] (ab205921)

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.



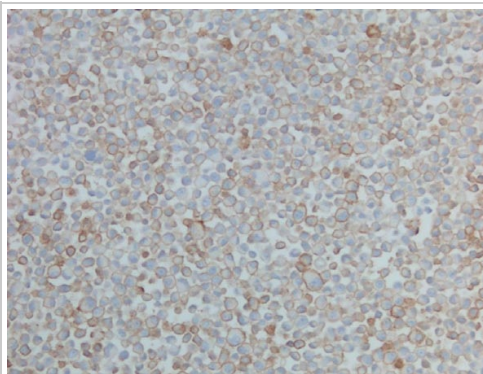
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [28-8] (ab205921)

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.



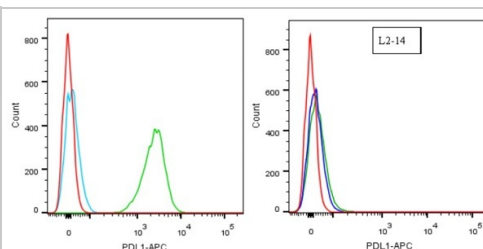


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [28-8] (ab205921)

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.



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

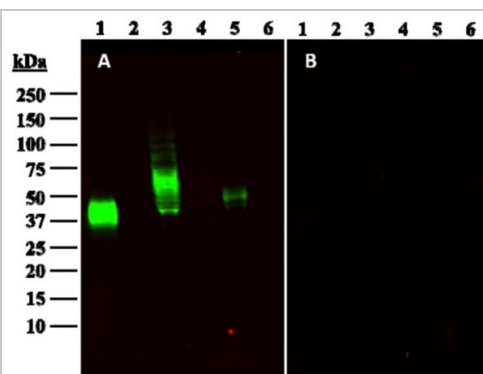
Ab205921 specificity testing by Flow Cytometry (KO testing): Loss of detection on KO Cells

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 (line 1) in the protocol section and/or [here \(downloadable copy\)](#)



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

**Predicted band size : 33 kDa**

Ab205921 specificity testing by Western Blot (KO testing):

Loss of detection on KO Cells

(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.

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