

## Product datasheet

# Anti-PD-L1 antibody [EPR19759] ab213524

Recombinant RabMAB

2 References 10 Images

### Overview

<b>Product name</b>	Anti-PD-L1 antibody [EPR19759]
<b>Description</b>	Rabbit monoclonal [EPR19759] to PD-L1
<b>Host species</b>	Rabbit
<b>Tested applications</b>	<b>Suitable for:</b> Flow Cyt, WB, IHC-P, ICC/IF, IP
<b>Species reactivity</b>	<b>Reacts with:</b> Human
<b>Immunogen</b>	Synthetic peptide (the amino acid sequence is considered to be commercially sensitive) within Human PD-L1 aa 250 to the C-terminus. The exact sequence is proprietary. Database link: <a href="#">Q9NZQ7</a>
<b>Positive control</b>	WB: Chinese hamster ovary cell lysate overexpressing PD-L1; NCI-H1975 whole cell lysate. IHC-P: Human tonsil, placenta and stomach cancer tissues. ICC/IF: CHO-PDL1 and NCI-H1975 cells. IP: NCI-H1975 whole cell lysate. Flow Cyt: CHO-PDL1.
<b>General notes</b>	Our RabMAB <sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMab<sup>®</sup> patents</a>  This product is a recombinant rabbit monoclonal antibody.

### 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>Purity</b>	Protein A purified
<b>Clonality</b>	Monoclonal
<b>Clone number</b>	EPR19759
<b>Isotype</b>	IgG

### Applications

Our [Abpromise guarantee](#) covers the use of **ab213524** 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
Flow Cyt		1/500.
WB		1/1000. Detects a band of approximately 40-60 kDa (predicted molecular weight: 33 kDa).
IHC-P		1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. Antigen retrieval: Universal HIER antigen retrieval reagent ( <a href="#">ab208572</a> ).
ICC/IF		1/500.
IP		1/30.

## Target

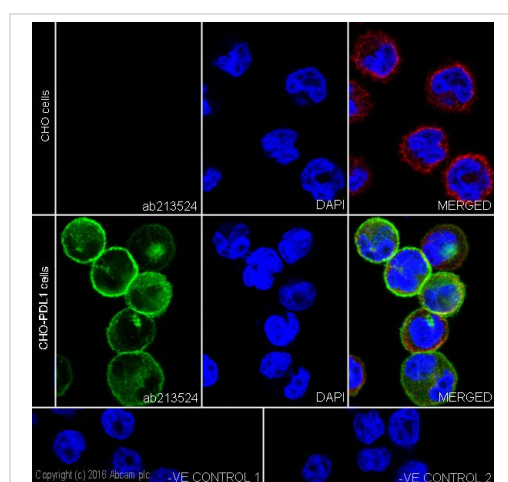
**Function** Involved in the costimulatory signal, essential for T-cell proliferation and production of IL10 and IFNG, in an IL2-dependent and a PDCD1-independent manner. Interaction with PDCD1 inhibits T-cell proliferation and cytokine production.

**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



Immunocytochemistry/ Immunofluorescence - Anti-PD-L1 antibody [EPR19759] (ab213524)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized CHO (Chinese hamster ovary cell line) cells labeling PD-L1 with ab213524 at 1/100 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor<sup>®</sup> 488) ([ab150077](#)) secondary antibody at 1/1000 dilution (green).

Confocal image showing membrane and cytoplasmic staining on CHO-PDL1 cells.

The nuclear counterstain is DAPI

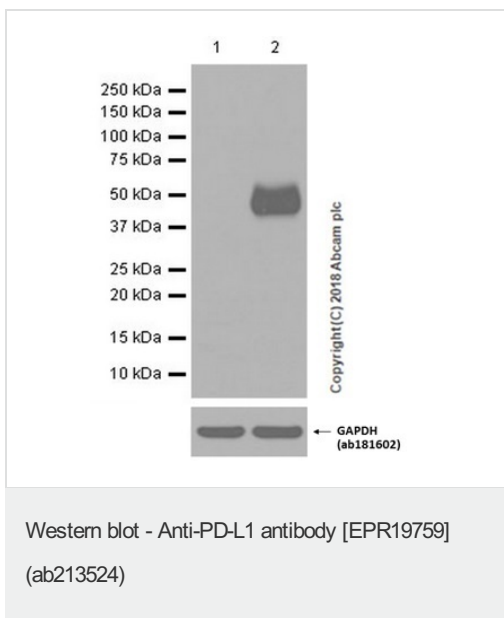
(blue).

Tubulin is detected with Anti-alpha Tubulin mouse MAb ([ab7291](#)) at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor<sup>®</sup> 594) ([ab150120](#)) secondary antibody at 1/1000 dilution (red).

The negative controls are as follows:-

-ve control 1: [ab213524](#) at 1/100 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor<sup>®</sup> 594) ([ab150120](#)) secondary antibody at 1/1000 dilution.

-ve control 2: Anti-alpha Tubulin mouse MAb ([ab7291](#)) at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) ([ab150077](#)) secondary antibody at 1/1000 dilution.



**All lanes :** Anti-PD-L1 antibody [EPR19759] ([ab213524](#)) at 0.5 µg/ml

**Lane 1 :** Untreated A549 (Human lung carcinoma epithelial cell) whole cell lysate

**Lane 2 :** A549 (Human lung carcinoma epithelial cell) treated with 100ng/ml Interferon gamma for 48 hours whole cell lysate

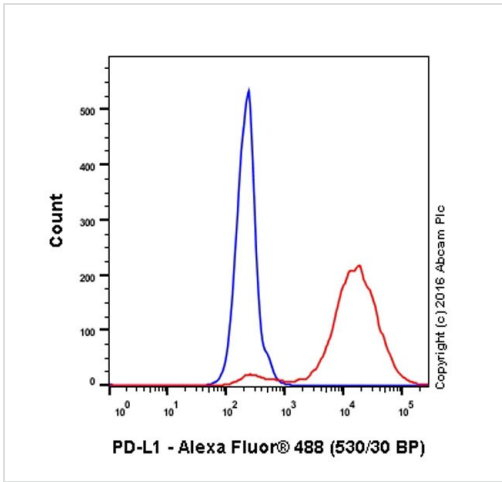
#### Secondary

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

**Predicted band size:** 33 kDa

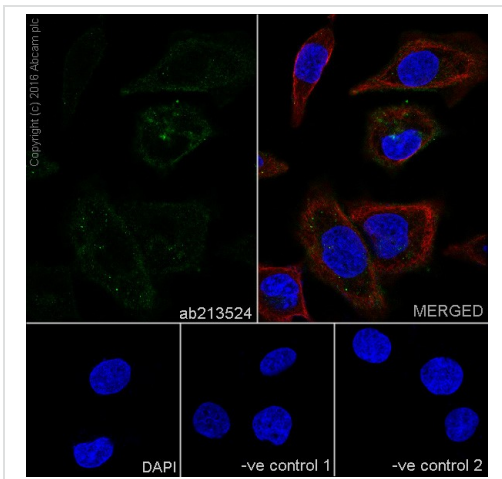
**Exposure time:** 3 seconds

Blocking and diluting buffer: 5% NFDN/TBST.



Flow Cytometry - Anti-PD-L1 antibody [EPR19759]  
(ab213524)

Flow Cytometry analysis of CHO-PD-L1 (red) and CHO-S (blue) cells labelling PD-L1 with ab213524 at 1/500. Cells were fixed with 4% paraformaldehyde, permeabilized with 0.1% tween-20-PBS and blocked with 10% goat serum. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/2000) was used as the secondary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-PD-L1 antibody [EPR19759] (ab213524)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NCI-H1975 (Human lung non small cell carcinoma cell line) cells labelling PD-L1 with ab213524 at 1/100 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green).

Confocal image showing weakly membrane and cytoplasmic staining on NCI-H1975 cells.

The nuclear counterstain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin mouse MAb (ab7291) at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (ab150120) secondary antibody at 1/1000 dilution (red).

The negative controls are as follows:-

-ve control 1: ab213524 at 1/100 dilution followed by Goat Anti-Mouse IgG H&L (Alexa Fluor®

594) ([ab150120](#)) secondary antibody at 1/1000 dilution.

-ve control 2: Anti-alpha Tubulin mouse MAb ([ab7291](#)) at 1/1000 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) ([ab150077](#))

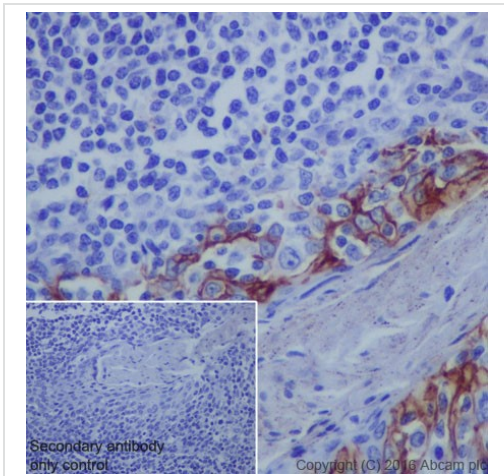
secondary antibody at 1/1000 dilution.

Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling PD-L1 with [ab213524](#) at 1/250 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB ([ab209101](#)).

Membrane staining on the human tonsil crypt epithelium is observed.

Counter stained with Hematoxylin.

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



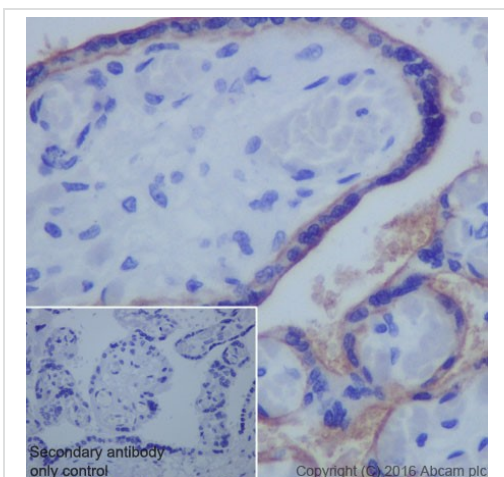
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [EPR19759] ([ab213524](#))

Immunohistochemical analysis of paraffin-embedded human placenta tissue labeling PD-L1 with [ab213524](#) at 1/250 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB ([ab209101](#)).

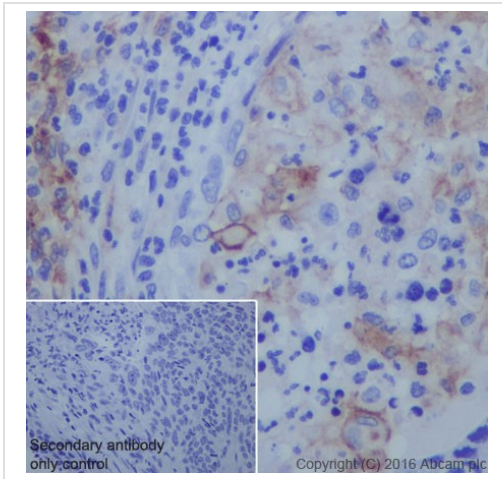
Membrane staining on the human placenta is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is rabbit specific IHC polymer detection kit HRP/DAB ([ab209101](#))



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [EPR19759] ([ab213524](#))



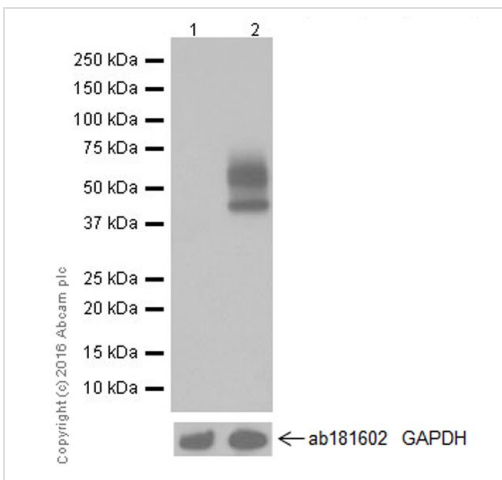
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [EPR19759] (ab213524)

Immunohistochemical analysis of paraffin-embedded human stomach cancer tissue labeling PD-L1 with ab213524 at 1/250 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Membrane staining on the human stomach cancer is observed.

Counter stained with Hematoxylin.

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



Western blot - Anti-PD-L1 antibody [EPR19759] (ab213524)

**All lanes** : Anti-PD-L1 antibody [EPR19759] (ab213524) at 1/1000 dilution

**Lane 1** : Chinese hamster ovary cell lysate

**Lane 2** : Chinese hamster ovary cell lysate overexpressing PD-L1

Lysates/proteins at 10 µg per lane.

**Secondary**

**All lanes** : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

**Predicted band size:** 33 kDa

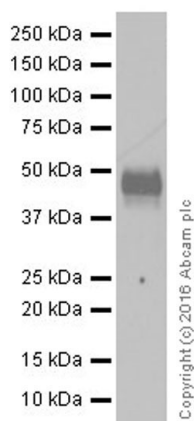
**Observed band size:** 40-60 kDa

why is the actual band size different from the predicted?

**Exposure time:** 3 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

The lower band is predicted to be isoform 2.



Western blot - Anti-PD-L1 antibody [EPR19759]  
(ab213524)

Anti-PD-L1 antibody [EPR19759] (ab213524) at 1/1000 dilution + NCI-H1975 (Human non-small cell lung cancer cell line) whole cell lysate at 10  $\mu$ g

### Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

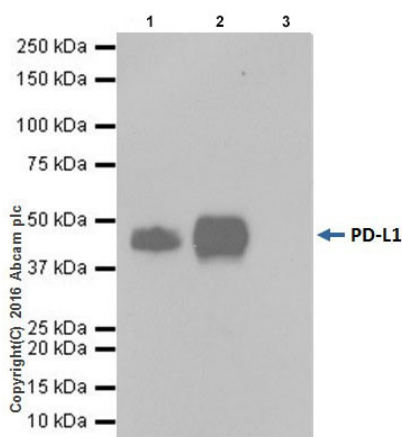
**Predicted band size:** 33 kDa

**Observed band size:** 40-60 kDa [why is the actual band size different from the predicted?](#)

**Exposure time:** 3 minutes

Blocking/Dilution buffer: 5% NFDm/TBST.

The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID: 26546452).



Immunoprecipitation - Anti-PD-L1 antibody  
[EPR19759] (ab213524)

PD-L1 was immunoprecipitated from 0.35 mg of NCI-H1975 (Human non-small cell lung cancer cell line) whole cell lysate with ab213524 at 1/30 dilution.

Western blot was performed from the immunoprecipitate using ab213524 at 1/1000 dilution.

VeriBlot for IP secondary antibody (HRP) (ab131366), was used as secondary antibody at 1/10000 dilution.

Lane 1: NCI-H1975 whole cell lysate 10  $\mu$ g (Input).

Lane 2: ab213524 IP in NCI-H1975 whole cell lysate.

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab213524 in NCI-H1975 whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDm/TBST.

Exposure time: 3 minutes.

**Please note:** All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE"

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