abcam

Product datasheet

Anti-PD-L1 antibody [RM1012] ab282458

KO VALIDATED Recombinant RabMAb

1 References 11 Images

Overview

Product name	Anti-PD-L1 antibody [RM1012]
Description	Rabbit recombinant multiclonal [RM1012] to PD-L1
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt, IP, Indirect ELISA, ICC/IF, WB, IHC-P
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: NCI-H1975 and MDA-MB-231 whole cell lysates; Human placenta tissue lysate, Human lung carcinoma epithelial cell) cell lysate, CD274 (PD-L1) KO A549, wild-type A549 cell lysate, Wild-type A549, MDA-MB-231 (Human breast adenocarcinoma epithelial cell) cell lysate, NCI-H1975 (Human adenocarcinoma lung epithelial cell) cell lysate IHC-P: Human breast cancer, lung cancer, placenta and tonsil tissue. ICC/IF & Flow Cyt: CHO-PDL1 cells. IP: NCI-H1975 whole cell lysates.
General notes	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

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59.94% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Recombinant Multiclonal

Clone number	RM1012
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab282458 in the following tested applications.

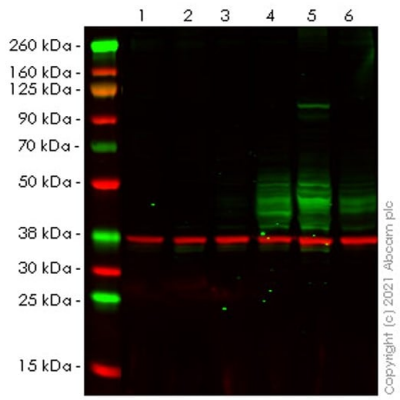
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt		1/50.
IP		1/30.
Indirect ELISA		Use a concentration of 0.25 µg/ml.
ICC/IF		1/1000.
WB		1/1000. Detects a band of approximately 40-60 kDa (predicted molecular weight: 33 kDa).
IHC-P		1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

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



Western blot - Anti-PD-L1 antibody [RM1012] (ab282458)

All lanes : Anti-PD-L1 antibody [RM1012] (ab282458) at 1/10000 dilution

Lane 1 : Untreated CD274 (PD-L1) KO A549 (Human lung carcinoma epithelial cell) cell lysate with Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS
Lane 2 : CD274 (PD-L1) KO A549 treated with 100 ng/ml human IFN gamma for 48 hours cell lysate with Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS
Lane 3 : Untreated wild-type A549 cell lysate with Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS
Lane 4 : Wild-type A549 treated with 100ng/ml human IFN gamma for 48 hours cell lysate with Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS
Lane 5 : MDA-MB-231 (Human breast adenocarcinoma epithelial cell) cell lysate with Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS
Lane 6 : NCI-H1975 (Human adenocarcinoma lung epithelial cell) cell lysate with Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/100000 dilution

Performed under reducing conditions.

Predicted band size: 33 kDa
Observed band size: 40-60 kDa

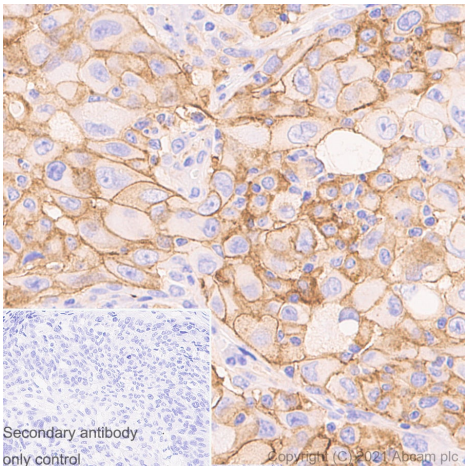
Exposure time: 3 minutes

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

False colour image of Western blot: Anti-PD-L1 antibody [RM1012] (ab282458) staining at 1/10, 000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red.

In Western blot, ab282458 was shown to bind specifically to PD-L1. Target band was observed at 40-60 kDa in wild-type A549 treated with IFN gamma cell lysates with no signal observed at this size in PD-L1 knockout cell line **ab267054** (knockout cell lysate **ab256831**). To generate this image, wild-type and CD274 (PD-L1) knockout A549 cell lysates were analyzed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.

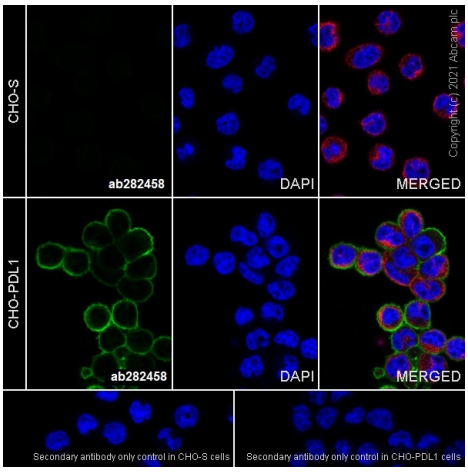


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [RM1012] (ab282458)

Immunohistochemical analysis of paraffin-embedded Human lung cancer tissue labelling PD-L1 with ab282458 at 1/500 (0.876 ug/ml) dilution followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Positive staining on human lung cancer. The section was incubated with ab282458 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins

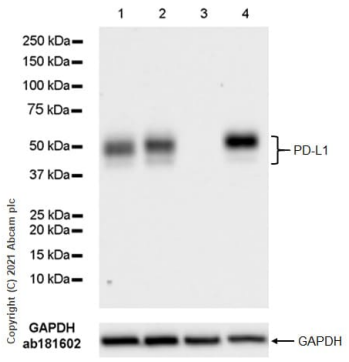


Immunocytochemistry/ Immunofluorescence - Anti-PD-L1 antibody [RM1012] (ab282458)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized CHO-PDL1 cells labelling PD-L1 with ab282458 at 1/1000 (0.438 ug/ml) dilution, followed by **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green). Confocal image showing membranous staining in CHO-PDL1 cells and no staining in CHO-S cells.

ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 2.5ug/ml dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 dilution.



Western blot - Anti-PD-L1 antibody [RM1012] (ab282458)

All lanes : Anti-PD-L1 antibody [RM1012] (ab282458) at 1/1000 dilution

Lane 1 : NCI-H1975 (Human adenocarcinoma lung epithelial cell) whole cell lysate
Lane 2 : MDA-MB-231 (Human breast adenocarcinoma epithelial cell) whole cell lysate
Lane 3 : MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate
Lane 4 : Human placenta lysate

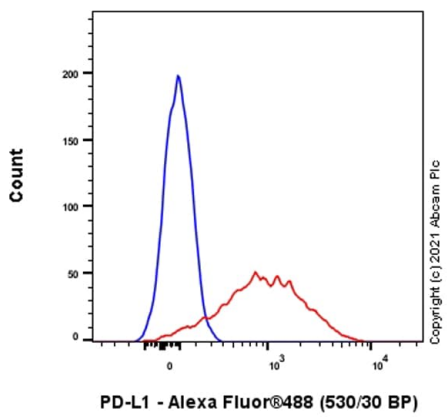
Lysates/proteins at 20 µg per lane.

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

Predicted band size: 33 kDa
Observed band size: 40-60 kDa

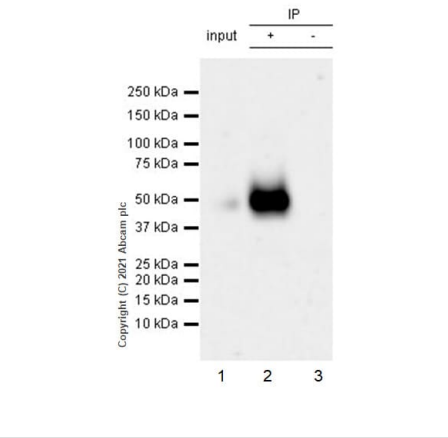
Blocking and Diluting buffer and concentration: 5% NFDM/TBST.

Low expression control: MCF7 (PMID: 28184013, 31741201).



Flow Cytometry - Anti-PD-L1 antibody [RM1012] (ab282458)

Flow cytometric analysis of CHO-S (Chinese hamster ovary epithelial cell (Blue)) / CHO-PDL1 (PD-L1 stably expessed Chinese hamster ovary epithelial cell) (Red)) cells labelling PD-L1 with ab282458 at 1/50 dilution (1ug). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody. Gated on viable cells.



Immunoprecipitation - Anti-PD-L1 antibody [RM1012] (ab282458)

PD-L1 was immunoprecipitated from 0.35 mg NCI-H1975 (Human adenocarcinoma lung epithelial cell) whole cell lysate with ab282458 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab282458 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP) (**ab131366**) was used at 1/5000 dilution.

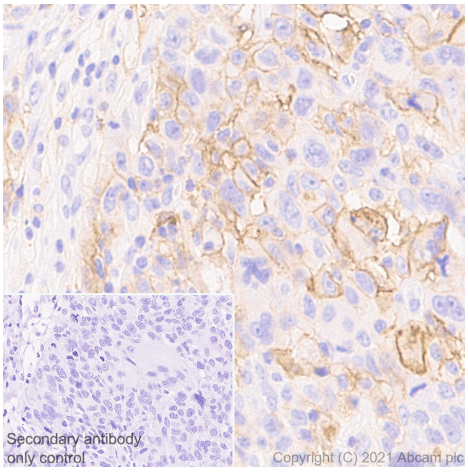
Lane 1: NCI-H1975 (Human adenocarcinoma lung epithelial cell) whole cell lysate 10 ug

Lane 2: ab282458 IP in NCI-H1975 whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab282458 in NCI-H1975 whole cell lysate

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

Exposure time: 32 seconds

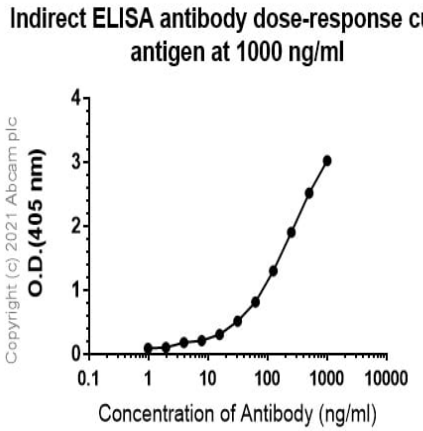


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [RM1012] (ab282458)

Immunohistochemical analysis of paraffin-embedded Human breast cancer tissue labelling PD-L1 with ab282458 at 1/500 (0.876 ug/ml) dilution followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Positive staining on human breast cancer. The section was incubated with ab282458 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

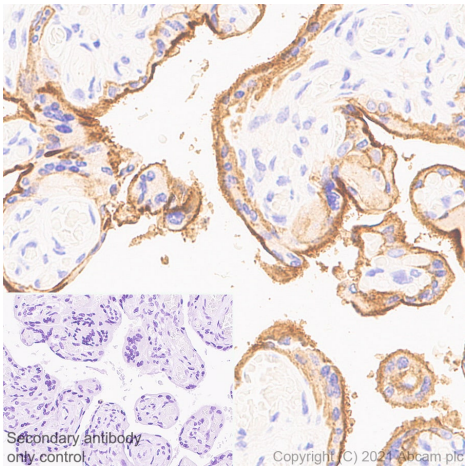
Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Indirect ELISA - Anti-PD-L1 antibody [RM1012] (ab282458)

ELISA using ab282458 at varying antibody concentrations and antigen concentration at 1000 ng/ml. An Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) (1/2500) was used as the secondary antibody.

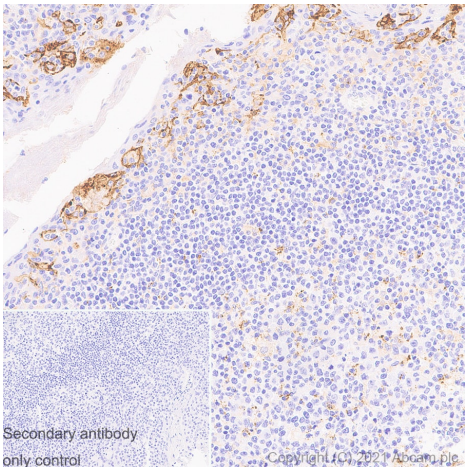


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [RM1012] (ab282458)

Immunohistochemical analysis of paraffin-embedded Human placenta tissue labelling PD-L1 with ab282458 at 1/500 (0.876 ug/ml) dilution followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Positive staining on human placenta. The section was incubated with ab282458 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins

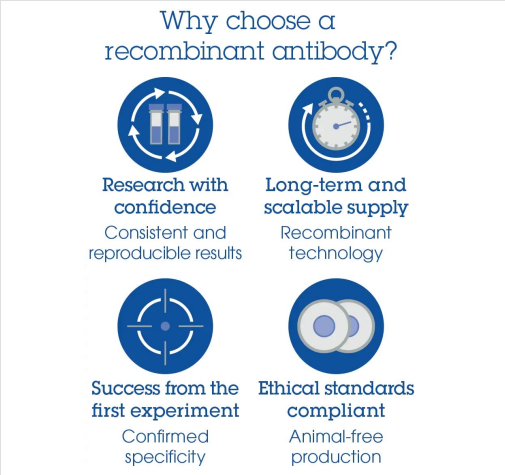


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [RM1012] (ab282458)

Immunohistochemical analysis of paraffin-embedded Human tonsil tissue labelling PD-L1 with ab282458 at 1/500 (0.876 ug/ml) dilution followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Positive staining on human tonsil. The section was incubated with ab282458 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Anti-PD-L1 antibody [RM1012] (ab282458)

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