abcam

Product datasheet

Anti-PD-L1 antibody [CAL10] - Rat IgG2a (Chimeric) ab279294

KO VALIDATED Recombinant

5 Images

Overview

Product name	Anti-PD-L1 antibody [CAL10] - Rat IgG2a (Chimeric)
Description	Rat monoclonal [CAL10] to PD-L1 - Rat IgG2a
Host species	Rat
Tested applications	**Suitable for:** Flow Cyt (Intra), IHC-P, ICC/IF, WB
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: PD-L1 stably expressed CHO whole cell lysate. Human placenta tissue lysate. NCI-H1299 whole cell lysate. ICC/IF: PD-L1 stably expressed CHO cells. Flow Cyt (intra): PD-L1 stably expressed CHO cells. IHC-P: Human tonsil tissue.
General notes	This rat monoclonal chimeric antibody has been engineered from a RabMAb parent antibody (**ab237726**). By necessity, some rabbit sequence is retained as part of the variable domain. When multiplexing with other rabbit-derived antibodies, using cross absorbed Fc-reactive secondary antibodies are recommended.

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 long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	CAL10
Isotype	IgG2a

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab279294 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 (Intra)		1/50.
IHC-P		Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF		1/100.
WB		1/1000.

Target

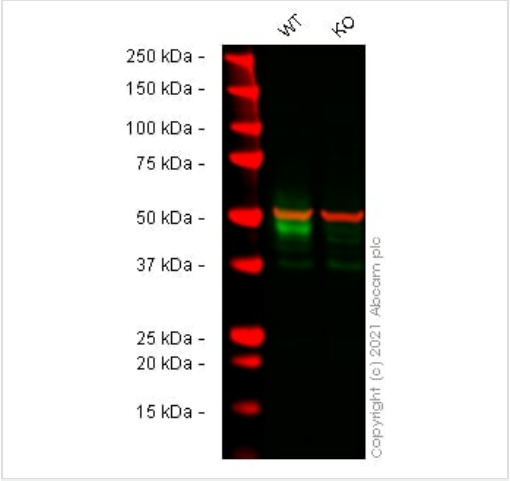
Function Involved in the costimulatory signal, essential for T-cell proliferation and production of IL10 and IFNG, in an IL2-dependent and a PDCD1-independent manner. Interaction with PDCD1 inhibits T-cell proliferation and cytokine production.

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 [CAL10] - Rat IgG2a (Chimeric) (ab279294)

All lanes : Anti-PD-L1 antibody [CAL10] - Rat IgG2a (Chimeric) (ab279294) at 1/1000 dilution

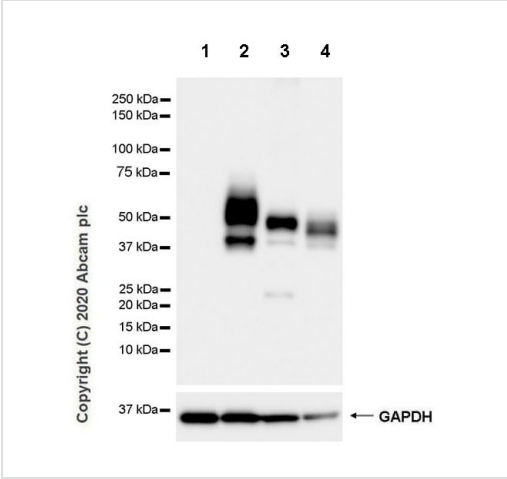
Lane 1 : Wild-type A549 Treated IFN-gamma (100 ng/ml) for 48 hours cell lysate
Lane 2 : CD274 knockout A549 Treated IFN-gamma (100 ng/ml) for 48 hours cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 48 kDa

False colour image of Western blot: Anti-PD-L1 antibody [CAL10] – Rat IgG2a (Chimeric) staining at 1/1000 dilution, shown in green; Rabbit anti-alpha Tubulin antibody [EP1332Y] (**ab52866**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab279294 was shown to bind specifically to PD-L1. A band was observed at 48 kDa in treated wild-type A549 cell lysates with no signal observed at this size in Cd274 knockout cell line **ab267054** (knockout cell lysate **ab256831**). To generate this image, wild-type and Cd274 knockout A549 cell lysates were analysed. 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-Rat IgG H&L (IRDye® 800CW) preabsorbed (**ab253031**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (**ab216777**) at 1/20000 dilution.



Western blot - Anti-PD-L1 antibody [CAL10] - Rat IgG2a (Chimeric) (ab279294)

All lanes : Anti-PD-L1 antibody [CAL10] - Rat IgG2a (Chimeric) (ab279294) at 1/1000 dilution

Lane 1 : CHO-S (Chinese hamster ovary epithelial cell) whole cell lysate
Lane 2 : CHO-PD-L1 (PD-L1 stably expressed Chinese hamster ovary epithelial cell) whole cell lysate
Lane 3 : Human placenta tissue lysate
Lane 4 : NCI-H1299 (human lung carcinoma epithelial cell), whole cell lysate

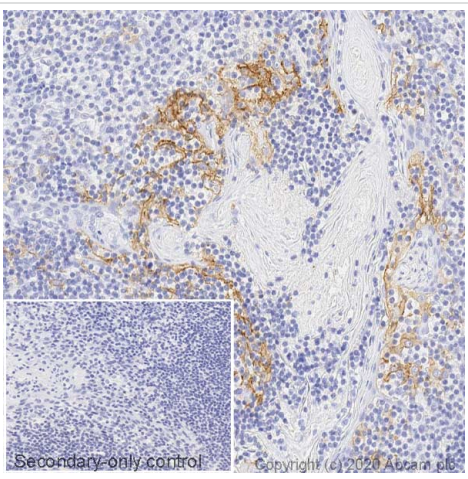
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rat IgG H&L (HRP) (**ab205720**) at 1/5000 dilution

Observed band size: 40-60 kDa

Exposure time: 8 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

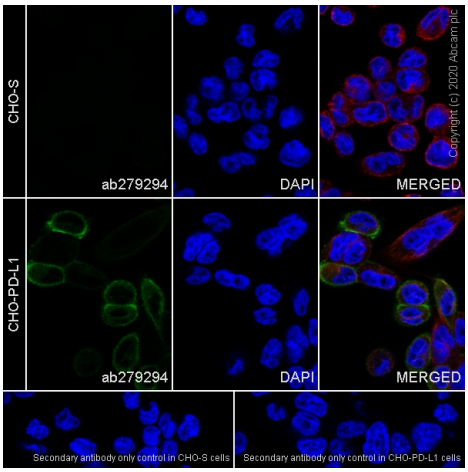


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [CAL10] - Rat IgG2a (Chimeric) (ab279294)

IHC image of PD-L1 staining in a section of formalin-fixed paraffin-embedded normal human tonsil* performed on a Leica BOND™ system using the standard protocol F.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab279294, 1ug/ml, for 15 mins at room temperature. A rabbit anti-rat IgG2a, **ab102248**, was added for 8 mins at room temperature and detected using an HRP conjugated goat anti-rabbit 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

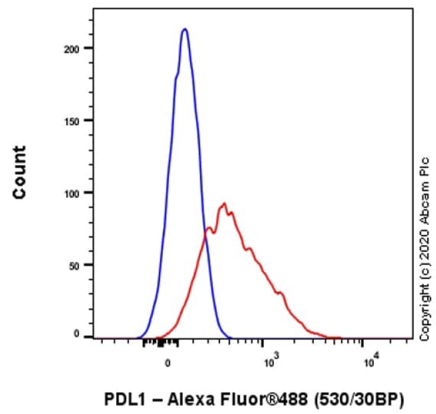


Immunocytochemistry/ Immunofluorescence - Anti-PD-L1 antibody [CAL10] - Rat IgG2a (Chimeric) (ab279294)

Immunocytochemical analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100-fixed permeabilized CHO-PD-L1 cells labeling PD-L1 with ab279294 at 1/100 dilution, followed by **ab150157** Goat Anti-Rat IgG H&L (Alexa Fluor® 488) at 1/1000 dilution (Green). **ab179513** Anti-beta Tubulin rabbit monoclonal antibody was used to counterstain tubulin at 1/200 dilution, followed by **ab150080** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) at a 1/500 dilution (Red). The nuclear counterstain was DAPI (Blue). Confocal image showing membranous and cytoplasmic staining in CHO-PD-L1 cells.

Negative control 1: ab279294 at a 1/100 dilution followed by **ab150080** at a 1/200 dilution.

Negative control 2: **ab179513** at a 1/200 dilution followed by **ab150157** at a 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-PD-L1 antibody [CAL10] - Rat IgG2a (Chimeric) (ab279294)

Flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized CHO-s (Chinese hamster ovary epithelial cell, Blue) / CHO-PDL1 (PD-L1 stably expessed Chinese hamster ovary epithelial cell, Red) labelling PD-L1 with ab279294 at 1/50 dilution (0.1µg).

Goat F(ab)2 Anti-Rat IgG Fc (Alexa Fluor® 488, **ab150161**) at 1/2000 dilution was used as the secondary antibody.

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