abcam

Product datasheet

Anti-PD-L1 antibody [CAL10] - Mouse IgG2a (Chimeric) - BSA and Azide free ab279305

KO VALIDATED Recombinant

5 Images

Overview

Product name	Anti-PD-L1 antibody [CAL10] - Mouse IgG2a (Chimeric) - BSA and Azide free
Description	Mouse monoclonal [CAL10] to PD-L1 - Chimeric – BSA and Azide free
Host species	Mouse
Tested applications	**Suitable for:** WB, Flow Cyt (Intra), ICC, IHC-P
Species reactivity	**Reacts with:** Human, Recombinant fragment
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: PD-L1 stably expressed CHO cells. Flow Cyt (intra): PD-L1 stably expressed CHO cells. IHC-P: Human tonsil tissue.
General notes	ab279305 is the carrier free version of **ab279293**. This mouse 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. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	Constituent: 100% PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	CAL10
Isotype	IgG2a

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab279305 in the following tested applications.

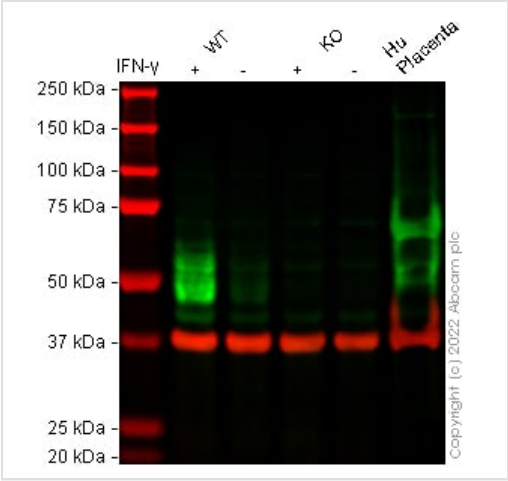
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration.
ICC		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 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 [CAL10] - Mouse IgG2a (Chimeric) - BSA and Azide free (ab279305)

All lanes : Anti-PD-L1 antibody [CAL10] - Mouse IgG2a (Chimeric) (**ab279293**) at 1/1000 dilution

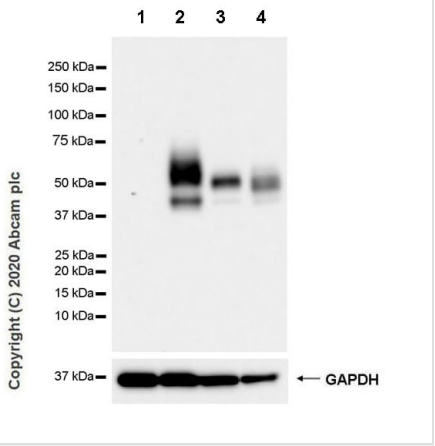
Lane 1 : Wild-type A549 Treated IFN-gamma (100 ng/mL, 48 h) cell lysate
Lane 2 : Wild-type A549 Vehicle Control IFN-gamma (0 ng/mL, 48 h) cell lysate
Lane 3 : CD274 knockout A549 Treated IFN-gamma (100 ng/mL, 48 h) cell lysate
Lane 4 : CD274 knockout A549 Vehicle Control IFN-gamma (0 ng/mL, 48 h) cell lysate
Lane 5 : Human Placenta cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 45-65 kDa

False colour image of Western blot: Anti-PD-L1 antibody [CAL10] - Mouse IgG2a staining at 1/1000 dilution, shown in green; Rabbit Anti-GAPDH antibody [EPR16891] (**ab181602**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab279293** was shown to bind specifically to PD-L1. A band was observed at 45-65 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 fluorescent western blot (TBS-based) blocking solution 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-Mouse IgG H&L 800CW and Goat anti-Rabbit IgG H&L 680RD at 1/20000 dilution.



Western blot - Anti-PD-L1 antibody [CAL10] - Mouse IgG2a (Chimeric) - BSA and Azide free (ab279305)

All lanes : Anti-PD-L1 antibody [CAL10] - Mouse IgG2a (Chimeric) (**ab279293**) 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

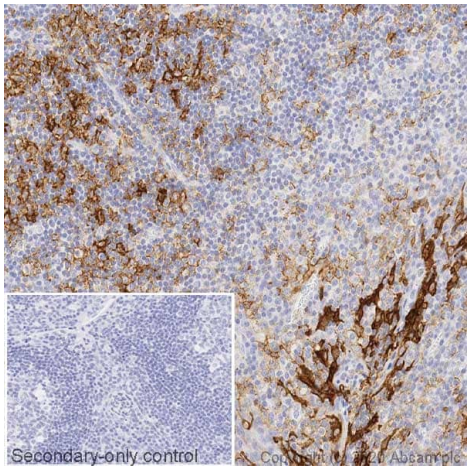
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Peroxidase-Conjugated Goat anti-Mouse IgG (H+L) at 1/5000 dilution

Observed band size: 40-60 kDa

This data was produced using **ab279293**, the same clone in a different formulation.

Blocking/Dilution buffer: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [CAL10] - Mouse IgG2a (Chimeric) - BSA and Azide free (ab279305)

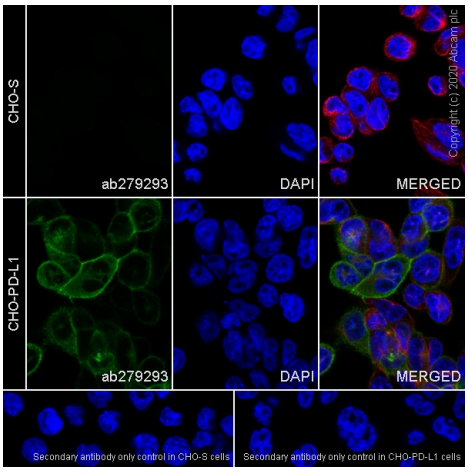
This data was produced using **ab279293**, the same clone in a different formulation.

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 **ab279293**, 1ug/ml, for 15 mins at room temperature. A rabbit anti-mouse IgG2a, 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



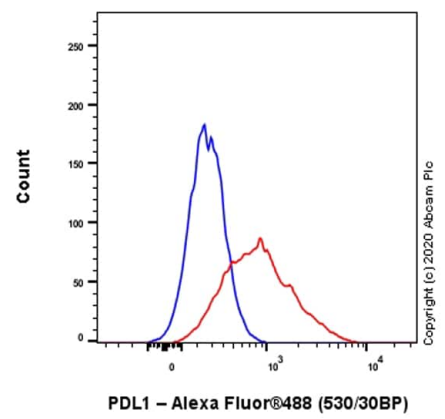
Immunocytochemistry - Anti-PD-L1 antibody [CAL10] - Mouse IgG2a (Chimeric) - BSA and Azide free (ab279305)

This data was produced using **ab279293**, the same clone in a different formulation.

Immunocytochemical analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100-fixed permeabilized CHO-PD-L1 cells labeling PD-L1 with **ab279293** at 1/100 dilution, followed by **ab150113** Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) antibody 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: ab279293 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] - Mouse IgG2a (Chimeric) - BSA and Azide free (ab279305)

This data was produced using **ab279293**, the same clone in a different formulation.

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 **ab279293** at 1/50 dilution (0.1µg).

Goat Anti-Mouse IgG (Alexa Fluor® 488, **ab150113**) at 1/2000 dilution was used as the secondary antibody.

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