abcam

Product datasheet

Anti-PD-L1 antibody [SP142] - C-terminal ab228462

Recombinant RabMAb

*** * * 2 Abreviews 87 References 15 Images

Overview

Product name Anti-PD-L1 antibody [SP142] - C-terminal

Description Rabbit monoclonal [SP142] to PD-L1 - C-terminal

Host species Rabbit

Tested applications Suitable for: IHC-P, mIHC, ICC/IF

Species reactivity Reacts with: Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control IHC-P: Human placenta, tonsil, lung squamous cell carcinoma, cervical squamous cell carcinoma,

skin squamous cell carcinoma, Hodgkin's lymphoma, pancreatic adenocarcinoma and prostate

adenocarcinoma tissues. ICC/IF: CHO-PD-L1 cells. mlHC: Human tonsil

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**.

This product is FOR RESEARCH USE ONLY. For commercial use, please contact

partnerships@abcam.com.

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: 7.60

Preservative: 0.1% Sodium azide Constituents: PBS, 1% BSA

Purity Protein A/G purified

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Purification notes Purified from TCS by protein A/G.

ClonalityMonoclonalClone numberSP142

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab228462 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
IHC-P	★★★★★ (2)	1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. Primary antibody incubation for 10 minutes at room temperature.
mIHC		1/500.
ICC/IF		1/50.

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,

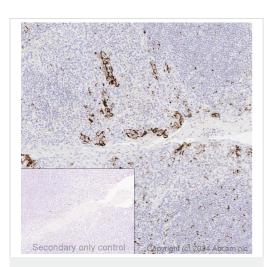
keratinocytes and monocytes.

Sequence similarities Belongs to the immunoglobulin superfamily. BTN/MOG family.

Contains 1 lg-like C2-type (immunoglobulin-like) domain.
Contains 1 lg-like V-type (immunoglobulin-like) domain.

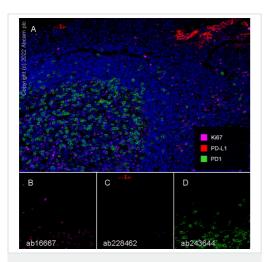
Cellular localization Cell membrane and Endomembrane system.

Images



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PD-L1 antibody [SP142] - C-terminal (ab228462)

Immunohistochemical analysis of formalin fixed paraffin (FFPE) embedded tonsil labelling PD-L1 with ab228462 at a dilution of 1/200. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with an OptiView DAB IHC Detection Kit followed by OptiView Amplification kit. Heat mediated antigen retrieval was conducted for 32min with DISCOVERY cell conditioning solution (CC1) 100°C, pH 8.5. ab228462 was incubated at 37°C for 16 min. Sections were counterstained is with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control



Multiplex immunohistochemistry - Anti-PD-L1 antibody [SP142] - C-terminal (ab228462)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human tonsil labelling PD1 with <u>ab243644</u> at 1/500 dilution (1.02 μ g/mL) (D), Ki67 with <u>ab16667</u> at 1/200 dilution (0.15 μ g/ml) (B) and PD-L1 with ab228462 at 1/100 dilution (0.52 μ g/ml) (C). Opal Polymer HRP Ms + Rb was used as a secondary antibody, and DAPI was used for a nuclear counter stain. Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins.

Panel A: merged staining of anti-Ki67 (magenta; Opal[™]690), anti-PD-L1 (red; Opal[™]570) and anti-PD1 (green; Opal[™]520) on human tonsil.

Panel B: anti-Ki67 stained on nucleus of proliferating cells.

Panel C: anti-PD-L1 stained on membrane of cells involved in T cell

Panel D: anti-PD1 stained on antigen-stimulated T cells.

inhibition.

The section was incubated in three rounds of staining: in the order of <u>ab16667</u> for 10 mins, <u>ab243644</u> for 30 mins and ab228462 for 10 mins at room temperature. Each round was followed by a

separate fluorescent tyramide signal amplification system.

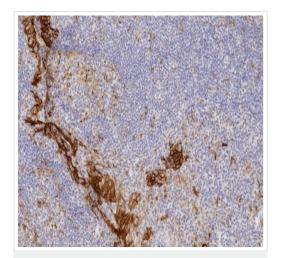
The immunostaining was performed on a Leica Biosystems
BOND® RX instrument with an Opal™ 4-color kit. Image acquisition
was performed with Leica SP8 confocal microscope.

IHC image of ab228462 staining PD-L1 in human tonsil formalin fixed paraffin embedded tissue sections*, performed on a Leica BOND RX (standard Protocol F, Polymer Refine kit). The section was pre-treated using heat mediated antigen retrieval with EDTA buffer (pH9, epitope retrieval solution 2) for 30 mins at 98°C. The section was then incubated with ab228462, 1/400 working dilution, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system for 8 minutes at room temperature. DAB was used as the chromogen for 10 minutes at room temperature. The section was then counterstained with hematoxylin, blued, dehydrated, cleared and mounted with DPX.

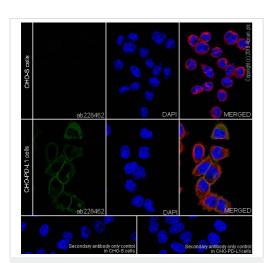
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre

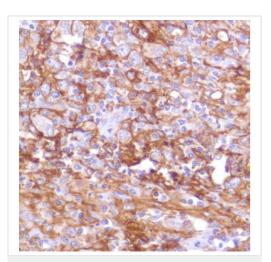
Immunocytochemistry/ Immunofluorescence analysis of CHO-PD-L1 (PD-L1 stably expessed Chinese hamster ovary epithelial cell) cells labeling PD-L1 with purified ab228462 at 1/50 (2 μ g/ml). Cells were fixed in 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. Cells were counterstained with **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.5 μ g/ml). Goat anti rabbit lgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1/1000 (2 μ g/ml) dilution. DAPI was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PD-L1 antibody [SP142] - C-terminal (ab228462)



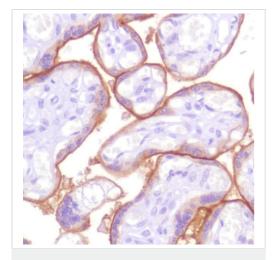
Immunocytochemistry/ Immunofluorescence - Anti-PD-L1 antibody [SP142] - C-terminal (ab228462)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PD-L1 antibody [SP142] - C-terminal (ab228462)

Formalin-fixed, paraffin-embedded human Hodgkin's lymphoma tissue stained for PD-L1 using ab228462 at 1/100 dilution in immunohistochemical analysis.

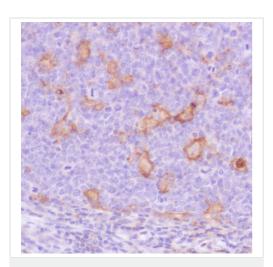
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PD-L1 antibody [SP142] - C-terminal (ab228462)

Formalin-fixed, paraffin-embedded human placenta tissue stained for PD-L1 using ab228462 at 1/100 dilution in immunohistochemical analysis.

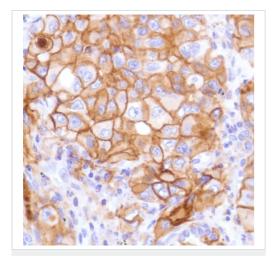
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PD-L1 antibody [SP142] - C-terminal (ab228462)

Formalin-fixed, paraffin-embedded human tonsil tissue stained for PD-L1 using ab228462 at 1/100 dilution in immunohistochemical analysis.

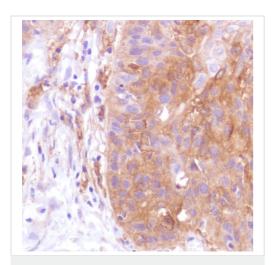
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PD-L1 antibody [SP142] - C-terminal (ab228462)

Formalin-fixed, paraffin-embedded human lung squamous cell carcinoma tissue stained for PD-L1 using ab228462 at 1/100 dilution in immunohistochemical analysis.

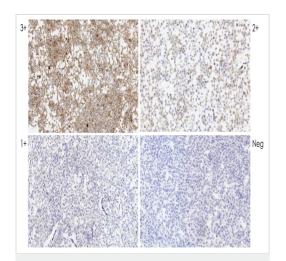
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PD-L1 antibody [SP142] - C-terminal (ab228462)

Formalin-fixed, paraffin-embedded human cervical squamous cell carcinoma tissue stained for PD-L1 using ab228462 at 1/100 dilution in immunohistochemical analysis.

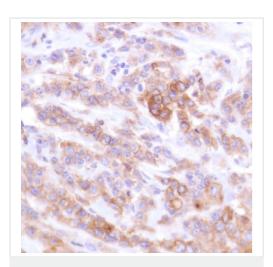
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PD-L1 antibody [SP142] - C-terminal (ab228462)

IHC image of ab228462 staining PD-L1 in PD-L1 Dynamic Range Analyte Control formalin fixed paraffin embedded human cell lines (HistoCyte Laboratories), performed on a Leica BOND RX (standard Protocol F, Polymer Refine kit). The section was pretreated using heat mediated antigen retrieval with EDTA buffer (pH9, epitope retrieval solution 2) for 30 mins at 98°C. The section was then incubated with ab228462, 1/400 working dilution, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system for 8 minutes at room temperature. DAB was used as the chromogen for 10 minutes at room temperature. The section was then counterstained with hematoxylin, blued, dehydrated, cleared and mounted with DPX.

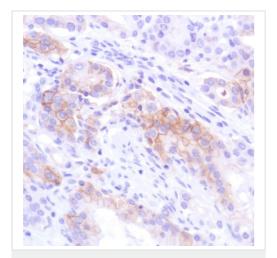
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PD-L1 antibody [SP142] - C-terminal (ab228462)

Formalin-fixed, paraffin-embedded human pancreatic adenocarcinoma tissue stained for PD-L1 using ab228462 at 1/100 dilution in immunohistochemical analysis.

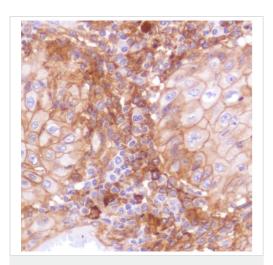
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PD-L1 antibody [SP142] - C-terminal (ab228462)

Formalin-fixed, paraffin-embedded human prostate adenocarcinoma tissue stained for PD-L1 using ab228462 at 1/100 dilution in immunohistochemical analysis.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PD-L1 antibody [SP142] - C-terminal (ab228462)

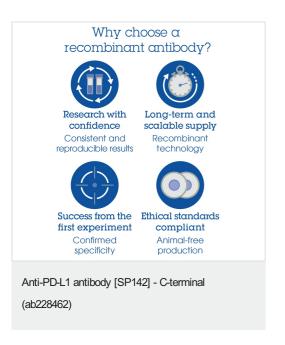
Formalin-fixed, paraffin-embedded human skin squamous cell carcinoma tissue stained for PD-L1 using ab228462 at 1/100 dilution in immunohistochemical analysis.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Normal fissue samples				Malignant tissue samples			
Human cardiac muscle	x	Human placenta	✓	Clear cell carcinoma of human kidney	х	Human gastric adenocarcinoma	x
Human cerebrum	x	Human skeletal muscle	×	Human astrocytoma	×	Human hepatocellular carcinoma	x
Human colon	x (mmune cells √)	Human skin	×	Human bladder cancer	x	Human lung carcinoma	✓
Human endometrium	x	Human spleen	×	Human breast carcinoma	x [immune cells √]	Human ovarian corcinoma	x
Human kidney	x	Human stomach	×	Human cervical carcinoma	x [immune cels √]	Human pancreatic carcinoma	x
Human liver	x	Human testis	×	Human colon carcinoma	¥ [immune cels √]	Human prostatic hyperplasia	x
Human lung	x	Human thyroid	×	Human endometrial carcinoma	x	Human thyroid corcinoma	x
Human mammary gland	x	Human tonsil	✓				
Human pancreas	x						

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PD-L1 antibody [SP142] - C-terminal (ab228462)

Tissue Microarrays stained for "Anti-PD-L1 antibody [SP142] - C-terminal" using "ab228462" in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negative (cross mark) staining per sample type tested. The sections were pre-treated using Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) for 20 minutes. The sections were incubated with ab228462 for 10 mins at room temperature followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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