abcam

Product datasheet

Anti-PD-L1 antibody [EPR19759] ab213524

KO 評価済 リコンビナント RabMAb

★★★★★ 1 Abreviews 74 References 画像数 17

製品の概要

製品名	Anti-PD-L1 antibody [EPR19759]
製品の詳細	Rabbit monoclonal [EPR19759] to PD-L1
由来種	Rabbit
アプリケーション	**適用あり:** Flow Cyt (Intra), WB, IHC-P, ICC/IF, IP **適用なし:** Flow Cyt
種交差性	**交差種:** Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	WB: Wild-type A549 treated with 100 ng/mL IFN gamma (**ab259377**) for 48 h cell lysate; 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 (intra): CHO-PDL1.
特記事項	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**.

製品の特性

製品の状態	Liquid
保存方法	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.
バッファー	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
精製度	Protein A purified
ポリ/モノ	モノクローナル

クローン名	EPR19759
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab213524の使用に適用されます

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		1/500.
WB	★★★★★ (1)	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 (**ab208572**).
ICC/IF		1/500.
IP		1/30.

追加情報 Is unsuitable for Flow Cyt.

ターゲット情報

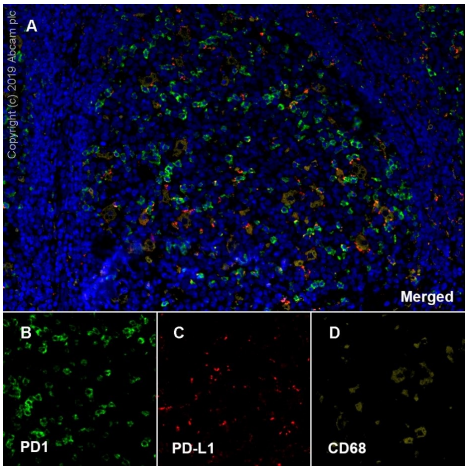
機能 Involved in the costimulatory signal, essential for T-cell proliferation and production of IL10 and IFNG, in an IL2-dependent and a PDCD1-independent manner. Interaction with PDCD1 inhibits T-cell proliferation and cytokine production.

組織特異性 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.

配列類似性 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.

細胞内局在 Cell membrane and Endomembrane system.

画像



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

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human spleen tissue labelling PDI with **ab243644** at 1.02 µg/mL (B), PD-L 1 with ab213524 at 1/100 dilution (C) and CD68 with **ab213363** at 1/300 dilution (D). Anti-Rabbit and Mouse Polymer HRP was used as a secondary antibody, and DAPI was used for a nuclear counter stain. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20mins. Heat mediated antigen retrieval (Leica ER2, PH9.0, 20 minutes) was used in between rounds of tyramide signal amplification to remove the antibodies from the previous round, to avoid any cross-reactivity.

Panel A: merged staining of anti- PD1 (green, Opal™520), anti-PD-L1 (red, Opal™570) and anti- CD68 (yellow, Opal™690).

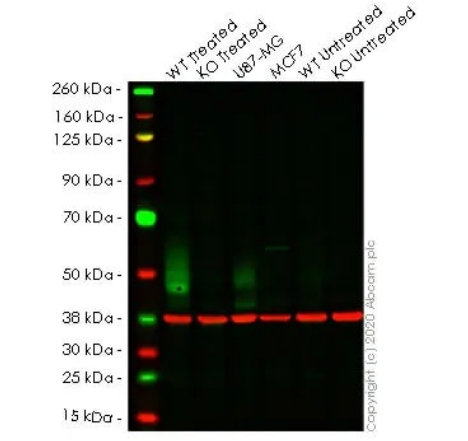
Panel B: Anti- PD1 stained on antigen-stimulated T cells.

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

Panel D: anti-CD68 stained on macrophages.

The section was incubated in three rounds of staining: in the order of **ab243644**, **ab213363** and ab213524 for 30 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.



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

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

Lane 1 : Wild-type A549 treated with 100 ng/ml IFN gamma (**ab259377**) for 48 h cell lysate
Lanes 2 & 6 : CD274 knockout A549 treated with 100 ng/ml IFN gamma (**ab259377**) for 48 h cell lysate
Lane 3 : U-87 MG cell lysate
Lane 4 : MCF7 cell lysate
Lane 5 : Wild-type A549 untreated cell lysate

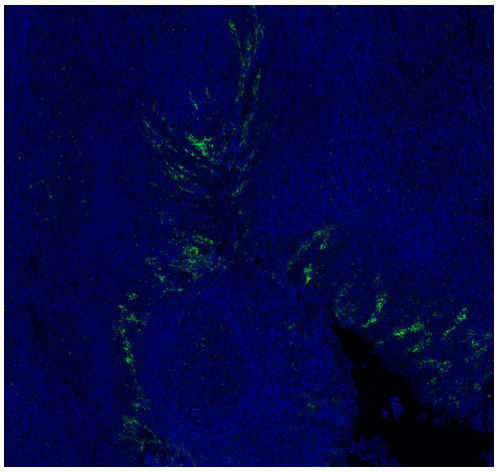
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 33 kDa
Observed band size: 50 kDa

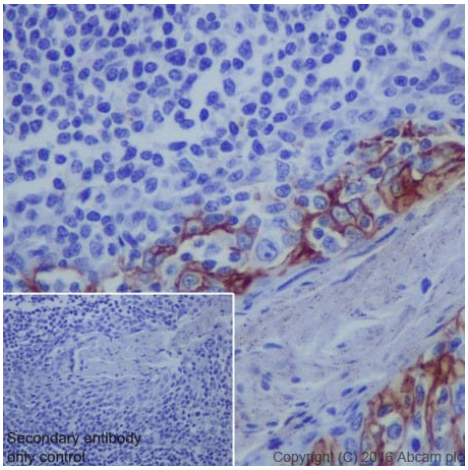
Lanes 1 - 6: Merged signal (red and green). Green - ab213524 observed at 50 kDa. Red - loading control, **ab8245** observed at 37 kDa.

ab213524 Recombinant Anti-PD-L1 antibody [EPR19759] was shown to specifically react with PD-L1 in wild-type A549 treated with 100 ng/mL IFN gamma for 48 h cells in western blot. Loss of signal was observed when both treated and untreated knockout cell line **ab267054** (treated and untreated knockout cell lysates **ab256831**) were used. Wild-type and PD-L1 knockout samples were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab213524 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 1000 and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



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

Anti-PD-L1 antibody [EPR19759] (ab213524) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human tonsil tissue labelling PD-L1 with ab213524 at a dilution of 1:250. Heat mediated antigen retrieval was performed using AR9 antigen retrieval solution, and microwave treatment for 15 min at 20% power. Anti-Rabbit/Mouse HRP polymer (PerkinElmer Opal Polymer HRP Ms Plus Rb) was used as secondary antibody. Opal tyramide amplification was performed using Opal 520 fluorophore. Counterstained with DAPI stain. Image scanned with Vectra 3.0 and analyzed via Phenochart software. This image was courteously provided by Dr. Houssein Abdul Sater, Georgia Cancer Center.



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

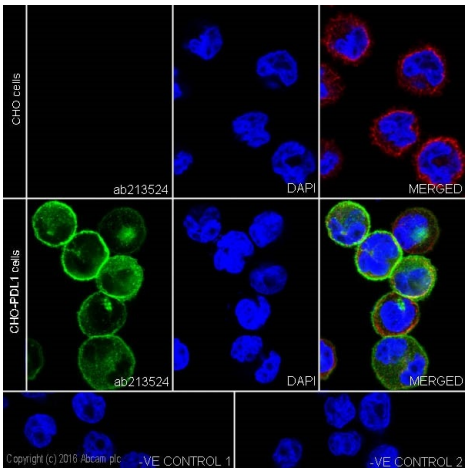
Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling PD-L1 with ab213524 at 1/250 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

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**).

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



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® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

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

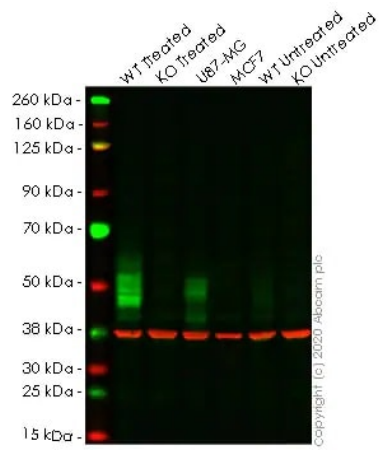
The nuclear counterstain is DAPI (blue).

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



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

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

Lane 1 : Wild-type A549 treated with 100 ng/mL IFN gamma for 48 h cell lysate
Lane 2 : CD274 knockout A549 treated with 100 ng/mL IFN gamma for 48 h cell lysate
Lane 3 : U-87 MG cell lysate
Lane 4 : MCF7 cell lysate
Lane 5 : Wild-type A549 untreated cell lysate
Lane 6 : CD274 knockout A549 untreated cell lysate

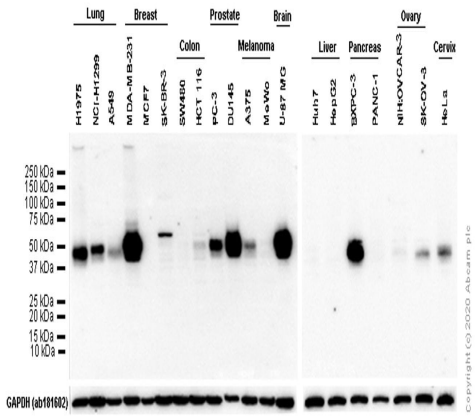
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 33 kDa
Observed band size: 50 kDa

Lanes 1 - 6: Merged signal (red and green). Green - ab213524 observed at 50 kDa. Red - loading control, **ab8245** observed at 37 kDa.

ab213524 Recombinant Anti-PD-L1 antibody [EPR19759] was shown to specifically react with PD-L1 in wild-type A549 treated with 100 ng/mL IFN gamma for 48 h cells in western blot. Loss of signal was observed when both treated and untreated knockout cell line **ab267055** (treated and untreated knockout cell lysates **ab256866**) were used. Wild-type and PD-L1 knockout samples were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab213524 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 1000 and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



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

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

Lane 1 : H1975 (Human non-small cell lung cancer epithelial cell) whole cell lysate

Lane 2 : NCI-H1299 (Human lung carcinoma epithelial cell) whole cell lysate

Lane 3 : A549 (Human lung carcinoma epithelial cell) whole cell lysate

Lane 4 : MDA-MB-231 (Human breast adenocarcinoma epithelial cell) whole cell lysate

Lane 5 : MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate

Lane 6 : SK-BR-3 (Human breast adenocarcinoma epithelial cell) whole cell lysate

Lane 7 : SW480 (Human colorectal adenocarcinoma epithelial cell) whole cell lysate

Lane 8 : HCT 116 (Human colorectal carcinoma epithelial cell) whole cell lysate

Lane 9 : PC-3 (Human prostate adenocarcinoma epithelial cell) whole cell lysate

Lane 10 : DU 145 (Human prostate carcinoma epithelial cell) whole cell lysate

Lane 11 : A375 (Human malignant melanoma epithelial cell) whole cell lysate

Lane 12 : MeWo (Human malignant melanoma fibroblast) whole cell lysate

Lane 13 : U-87 MG (Human glioblastoma-astrocytoma epithelial cell) whole cell lysate

Lane 14 : Huh7 (Human hepatocellular carcinoma epithelial cell) whole cell lysate

Lane 15 : HepG2(Human hepatocellular carcinoma epithelial cell) whole cell lysate

Lane 16 : BXPC-3 (Human pancreas adenocarcinoma epithelial cell) whole cell lysate

Lane 17 : PANC-1 (Human pancreatic epithelioid carcinoma epithelial cell) whole cell lysate

Lane 18 : NIH:OVCAR-3 (Human ovary adenocarcinoma epithelial cell) whole cell lysate

Lane 19 : SK-OV-3 (Human ovarian cancer epithelial cell) whole cell lysate

Lane 20 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/20000 dilution

Developed using the ECL technique.

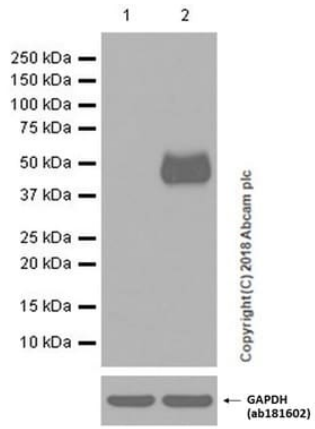
Predicted band size: 33 kDa
Observed band size: 40-60 kDa

Exposure time: 180 seconds

Expression of PD-L1 varied widely among the tumor cell lines

Blocking buffer and concentration : 5% NFDM/TBST

Diluting buffer and concentration : 5% NFDM/TBST



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

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

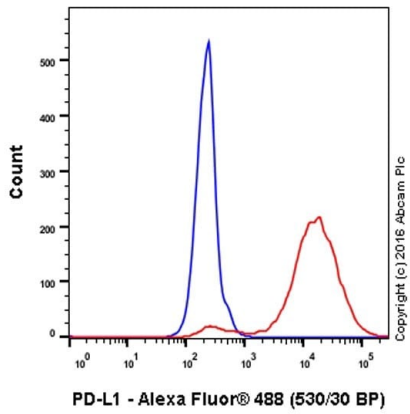
Lane 1 : Untreated A549 (Human lung carcinoma epithelial cell) whole cell lysate
Lane 2 : A549 (Human lung carcinoma epithelial cell) treated with 100ng/ml Interferon gamma for 48 hours whole cell lysate

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

Predicted band size: 33 kDa

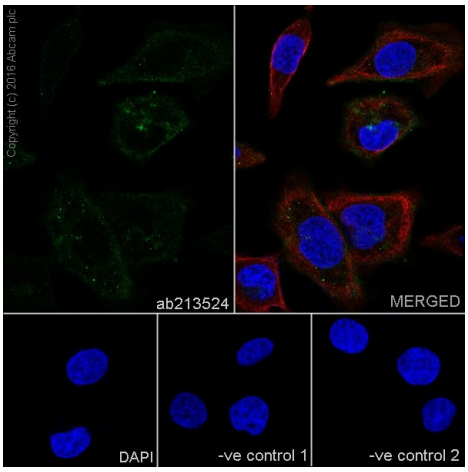
Exposure time: 3 seconds

Blocking and diluting buffer: 5% NFDM/TBST.



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

Intracellular 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 labeling 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.

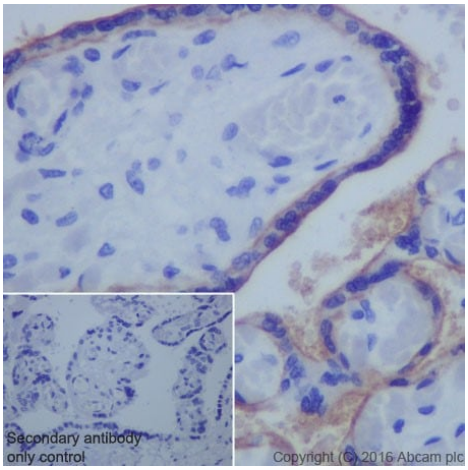
The nuclear counterstain is DAPI (blue).

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.



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

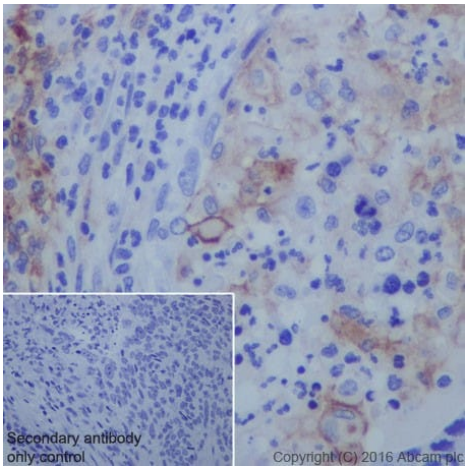
Immunohistochemical analysis of paraffin-embedded human placenta tissue labeling PD-L1 with ab213524 at 1/250 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

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**)

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



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

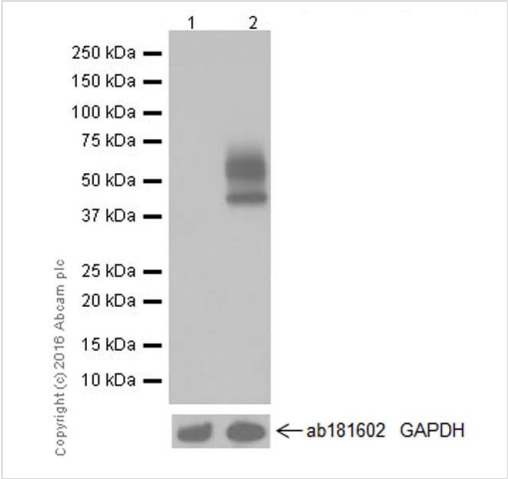
Immunohistochemical analysis of paraffin-embedded human stomach cancer tissue labeling PD-L1 with ab213524 at 1/250 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

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**).

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



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.

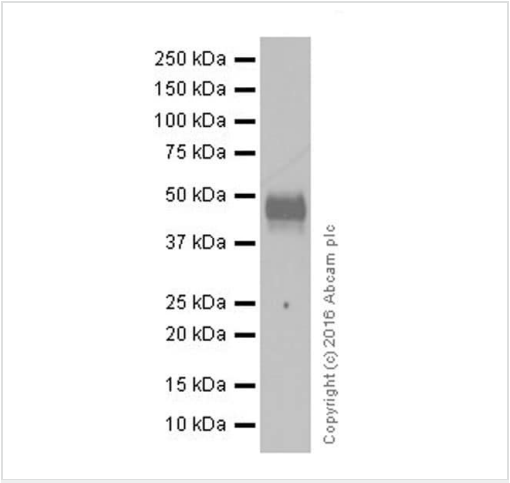
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

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

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 µg

Secondary

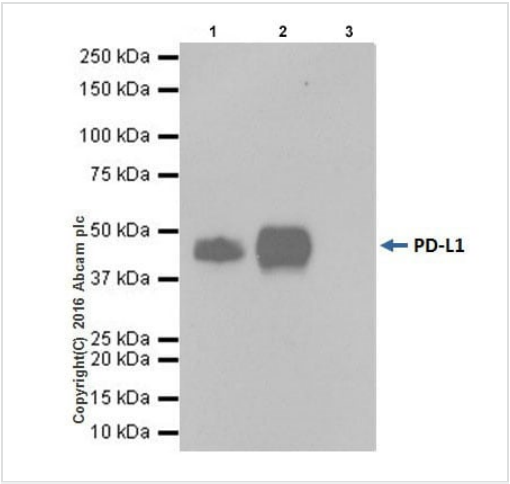
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

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

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 Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10,000 dilution.

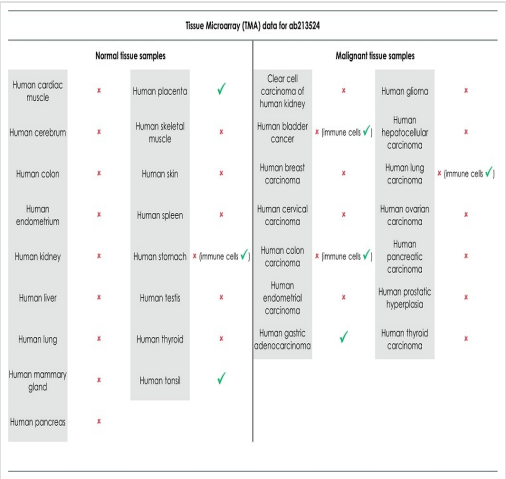
Lane 1: NCI-H1975 whole cell lysate 10µg (Input).

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.

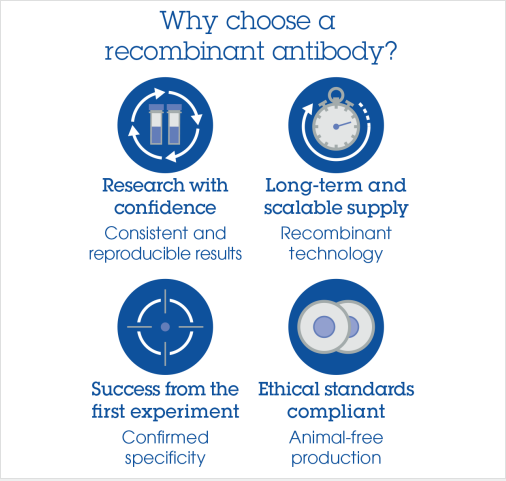
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 minutes.



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

Tissue Microarrays stained for "Anti-PD-L1 antibody [EPR19759]" using "ab213524" 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 perform heat mediated antigen retrieval before commencing with IHC staining protocol. The sections were incubated with ab213524 at +4°C overnight. The secondary antibody is rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).



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

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